ZooKeys 672: 1–34 (2017)
doi: 10.3897/zookeys.672.10212
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

New species of semi-aquatic freshwater earthworm genus *Glyphidrilus* Horst, 1889 from Thailand and Laos (Oligochaeta, Almidae)

Ratmanee Chanabun[1,2], Khamla Inkavilay[3], Somsak Panha[2]

1 *Program in Animal Science, Faculty of Agriculture Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon 47000, Thailand* **2** *Animal Systematics Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand* **3** *Department of Biology, Faculty of Science, National University of Laos, Vientiane, Laos P.D.R.*

Corresponding author: *Somsak Panha* (somsak.pan@chula.ac.th)

Academic editor: *S. James* | Received 17 August 2016 | Accepted 31 March 2017 | Published 3 May 2017

http://zoobank.org/48810F76-97B9-40B2-9A75-DB0F98C5311F

Citation: Chanabun R, Inkavilay K, Panha S (2017) New species of semi-aquatic freshwater earthworm genus *Glyphidrilus* Horst, 1889 from Thailand and Laos (Oligochaeta, Almidae). ZooKeys 672: 1–34. https://doi.org/10.3897/zookeys.672.10212

Abstract

Seven new species of semi-aquatic freshwater earthworms belonging to the genus *Glyphidrilus* Horst, 1889 are described from Thailand and Laos, *Glyphidrilus nanensis* Chanabun & Panha **sp. n.**, *G. satunensis* Chanabun & Panha **sp. n.**, *G. chiangraiensis* Chanabun & Panha **sp. n.**, *G. namphao* Chanabun & Panha **sp. n.**, *G. sekongensis* Chanabun & Panha **sp. n.**, *G. namdonensis* Chanabun & Panha **sp. n.**, and *G. champasakensis* Chanabun & Panha **sp. n.** The species are characterized by their external and internal morphological characteristics, as well as body sizes. Other morphological character differences between these seven species were also compared, and an identification key is provided. The relationships of the new species to congeners are discussed.

Keywords

Almidae, earthworms, *Glyphidrilus*, Laos, southeast Asia, Thailand

Copyright *Ratmanee Chanabun et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

The semi-aquatic freshwater earthworm genus *Glyphidrilus* Horst, 1889 has been known since the nineteenth century. The unique morphological characters of the expanded epidermis at approximately the clitellum position, called "wings", and the absence of prostate glands, the rounded body with a posterior quadrangular shape and the long slender banana-like cocoons are prominent in *Glyphidrilus*. The semi-aquatic habitat between terrestrial and freshwater ecosystems of rivers, streams, canals, ponds, swamps or even in paddy rice systems are also consistent (Horst 1889, 1893, Jamieson 1968, Michaelsen 1896, 1897, 1900, 1902, 1910, 1918, 1922, Rao 1922, Shen and Yeo 2005, Chanabun et al. 2013). Up to now, the records of *Glyphidrilus* are only from Africa and Asia; however, most described species are from Asia and especially from Southeast Asia. The worms are now becoming threatened because of the modification, pollution, and destruction of their habitats, for example the contamination by chemical agriculture, and the dam constructions in the upper Mekong River (pers. obs.).

Most previous species were described with some illustrations in a format which was frequently poorly interpreted and insufficient in some species. However, in the recent descriptions and redescriptions of several species are mostly from Thailand, and some from Malaysia, Singapore, and Laos, these deficiencies have been corrected. The color images of both animals and habitats together with anatomical illustration details have made improvements in a new description format. The 19 newly described species reported bring the total number to 40 recognized *Glyphidrilus* species (Chanabun et al. 2011, Chanabun et al. 2012a, Chanabun et al. 2012b, Chanabun et al. 2013, Chanabun and Panha 2015, Jirapatrasilp et al. 2016).

The behavior of animals has been observed, and it was noted that the worms leave their tail tips exposed near surface of their muddy habitats. While submerging they produce casts as do most earthworms (Chanabun et al. 2013). It was also found that north of 12 degrees latitude in Thailand *Glyphidrilus* appears as mostly adults in the dry to early rainy seasons (March to July), while in the rainy season they mainly appeared as juveniles, especially in the upper parts of Thailand. However, south of 12 degrees latitude, the adults seem to be present all year round (pers. obs.).

Chanabun et al. (2013) interpreted the phylogeography of *Glyphidrilus* in light of recent hypotheses regarding ancient river drainage patterns, especially the Mekong River and other main basins, plus various habitat types, using morphological characters and genetic data. Enzyme electrophoresis has proved that the closely related species *G. mekongensis* Panha & Chanabun, 2012 and *G. vangviengensis* Panha & Chanabun, 2011 occurring along the lower Mekong River basin are definitely separate biological species; some possible cryptic species are also suggested in the paper (Jirapatrasilp et al. 2015). The present paper provides additional new species with careful morphological descriptions but no additional genetic data.

Materials and methods

The systematic and faunistic surveys of *Glyphidrilus* were conducted in the lower Mekong River basin both in Thailand and Laos (Fig. 1), and some other river systems in Thailand (Fig. 2) from June 2012 to April 2014. The collections were made by carefully digging up the topsoil near casts on the shore and in the water using hand sorting and sieving the soil from river banks. Adults, juveniles, and cocoons were collected and killed in 30% (v/v) ethanol, transferred to 5% (w/v) formalin for fixation in approximately 12 hours, and then transferred to 75% (v/v) ethanol for standard preservation and subsequent morphological studies. Duplicate specimens and/or tissue samples were preserved in 95% ethanol for further molecular and DNA barcoding analyses.

The type series are deposited in the Chulalongkorn University, Museum of Zoology, Bangkok, Thailand (**CUMZ**). Additional paratypes will be deposited in the Natural History Museum (**NHMUK**), London, Raffles Museum of Biodiversity Research, National University of Singapore, Singapore (**ZRC**) and at the Biozentrum Grindel und Zoologisches Museum, University of Hamburg (**ZMH**).

The descriptions were made from observations under an Olympus SZX16 stereoscopic light microscope. The following external and internal morphological characters were investigated and recorded: body length and segment number; the positions of clitellum and clitellar wings, genital markings, intestinal origin, gizzard, spermathecae, hearts and seminal vesicles. Illustrations were made for the whole body segments and the external and internal characters. The body width and length were measured in both full adults and juveniles, and are presented as the range (min-max) and mean±one standard deviation (SD).

Comparative studies of *Glyphidrilus* type specimens were made at four natural history museums:

CUMZ Chulalongkorn University, Museum of Zoology.
ZRC Raffles Museum of Biodiversity Research, National University of Singapore, Singapore.
NHMUK The Natural History Museum, London.
ZMH Biozentrum Grindel und Zoologisches Museum, University of Hamburg, Germany.

Anatomical abbreviations are as follows (Chanabun et al. 2013):

gm genital markings
he hearts
np nephridia
sc spermathecae
sv seminal vesicles
wi wings

Figure 1. Distribution map of the new *Glyphidrilus* species from Thailand and Laos. Numbers in the circles refer to localities of the new species. **1** *Glyphidrilus nanensis* sp. n. **2** *Glyphidrilus chiangraiensis* sp. n. **3** *Glyphidrilus namphao* sp. n. **4** *Glyphidrilus sekongensis* sp. n. **5** *Glyphidrilus namdonensis* sp. n. and **6** *Glyphidrilus champasakensis* sp. n.

Systematics

Family ALMIDAE Duboscq, 1902

Genus *Glyphidrilus* Horst, 1889

Diagnosis. Prostomium zygolobous. Body shape nearly circular in cross section in anterior part, and becoming quadrangular in posterior part or after clitellum. Anus

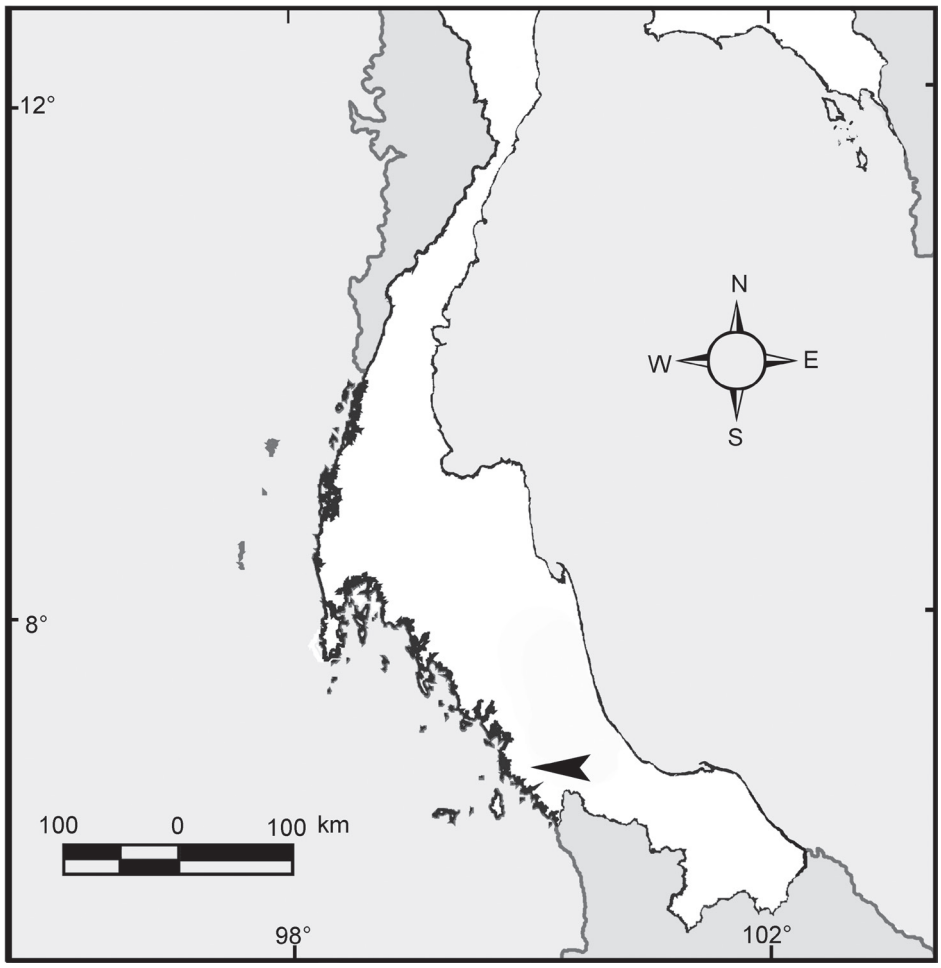


Figure 2. Map showing type locality of *Glyphidrilus satunensis* sp. n. (arrow head) located on the shore of the Nong Prakpraya at Mueang, Satun, south Thailand.

dorsal or dorso-terminal. A longitudinal lamellar ridge at maturity from body wall on each side in bc, through several of the clitellar segments, which are called wings. Dorsal pores absent. Setae four pairs per segment. Clitellum annular. Genital apertures, all minute and superficial. Male pores inter-or intraclitellar. Spermathecal pores usually all behind the testis segments. Gizzard in VII or VIII sometimes extending into an adjacent segment. Calciferous glands absent. Seminal vesicles usually short, usually four pairs in IX–XII. Holonephridia. Nephrostomes single ducts avesiculate and without sphincters or caeca. Testis and funnels free in X and XI. Male ducts intramural. Ovaries fan shaped and with several egg strings. Ovisacs present or absent. Prostate glands absent and spermathecae without diverticulum (Chanabun et al. 2013, Gates 1972).

Type species. *Glyphidrilus weberi* Horst, 1889. Type species by original designation in Horst (1889: 77).

***Glyphidrilus nanensis* Chanabun & Panha, sp. n.**
http://zoobank.org/E3CF3E43-7045-4C0F-9946-73AAB425425A
Figs 1, 3, 4, Table 1

Type material. Holotype: One adult (CUMZ 3403) in a rice field near Nan River at Saklek, Phichit, north Thailand (16°30'28.4"N, 100°31'15.0"E), 49 meters elevation on 16 June 2012. **Paratypes:** 42 adults and 10 juveniles (CUMZ 3404), 2 adults (ZMH 14579), 2 adults (NHMUK), and 2 adults (ZRC), all same collection data as holotype.

Diagnosis. A small sized earthworm with the clitellar wings on the lateral side of the body in XXIV, XXV, XXVI– XXVII, XXVIII, XXIX. Clitellum in XVII, XVI-II–XXXIII, XXXIV. Female pores, male pores, and spermathecal pores not visible. Genital markings: paired or asymmetrical on aa in X, XI, XII, XIII, XIV and XXVIII, XXIX, XXX; paired or asymmetrical on bc in XV, XVI, XVII–XXIII, XXIV, XXV. Four pairs of seminal vesicles in IX–XII. Intestinal origin in XV. Ovaries in XIII–XIV. Spermathecae between 13/14–17/18.

Description of holotype. Dimensions: body length 78 mm, diameter 2.6 mm in segment VIII, 4.7 mm before the clitellar wing in segment XXIII, 4.2 mm after wing in segment XXX in clitellar region; body cylindrical in anterior part, quadrangular in transverse section behind clitellum. 241 segments. Body color pale brown with variations from red to pink at adjacent tissues of wing portion in different individuals of newly collected specimens. At posterior end dorsal surface considerably broader than the ventral. Clitellar wing on ventro-lateral part of clitellum in XXVI–XXIX, 3.6 mm in height, and 0.3 mm in width on both sides. Prostomium zygolobous. Dorsal pores absent. Clitellum annular in XVII–XXXIII. Four pairs of setae per segment from II, setal formula aa:ab:bc:cd:dd = 1.0:0.6:1.3:0.6:1.4 in segment VIII and 1.0:0.6:1.5:0.6:1.5 in postclitellar segments. Female pores, male pores, and spermathecal pores not visible. Genital markings: paired on aa in XII–XIII, laterally paired or asymmetrical on bc in XVII–XXIV.

Septa 5/6–8/9 thicker than between other segments, 9/10–14/15 thick and 15/16 to the last segment thin. Gizzard small, globular in 7/8. Intestine enlarged from XV. Hearts, five pairs in VII–XI, first in VII and last in XI. A pair of holonephridia in each segment from segment XIII onwards. Seminal vesicles, four pairs in IX, X, XI, XII. Ovaries, two pairs in XIII–XIV. Testes free in X and XI. Prostate and accessory glands absent. Spermathecae sessile and diverticula absent, elongated oval or globular between 13/14–17/18, about 0.2–0.3 mm in diameter, two to ten on each side per segment.

Variations. Body lengths of adult (n = 49) and juvenile paratypes (n = 10). Adults ranged from 72–99 mm (85.2±19.0), with 186–257 segments. Juveniles ranged from 73–93 mm (82.3±7.5), with 186–251 segments. Wings in XXIV, XXV, XXVI–XX-VII, XXVIII, XXIX, clitellum in XVII, XVIII –XXXIII, XXXIV. Genital markings: paired or asymmetrical on aa in X, XI, XII, XIII, XIV and XXVIII, XXIX, XXX; paired or asymmetrical on bc in XV, XVI, XVII–XXIII, XXIV, XXV.

Distribution. The new species was found in the river banks of Nan River at Saklek, Phichit, north Thailand, which was covered with worm casts.

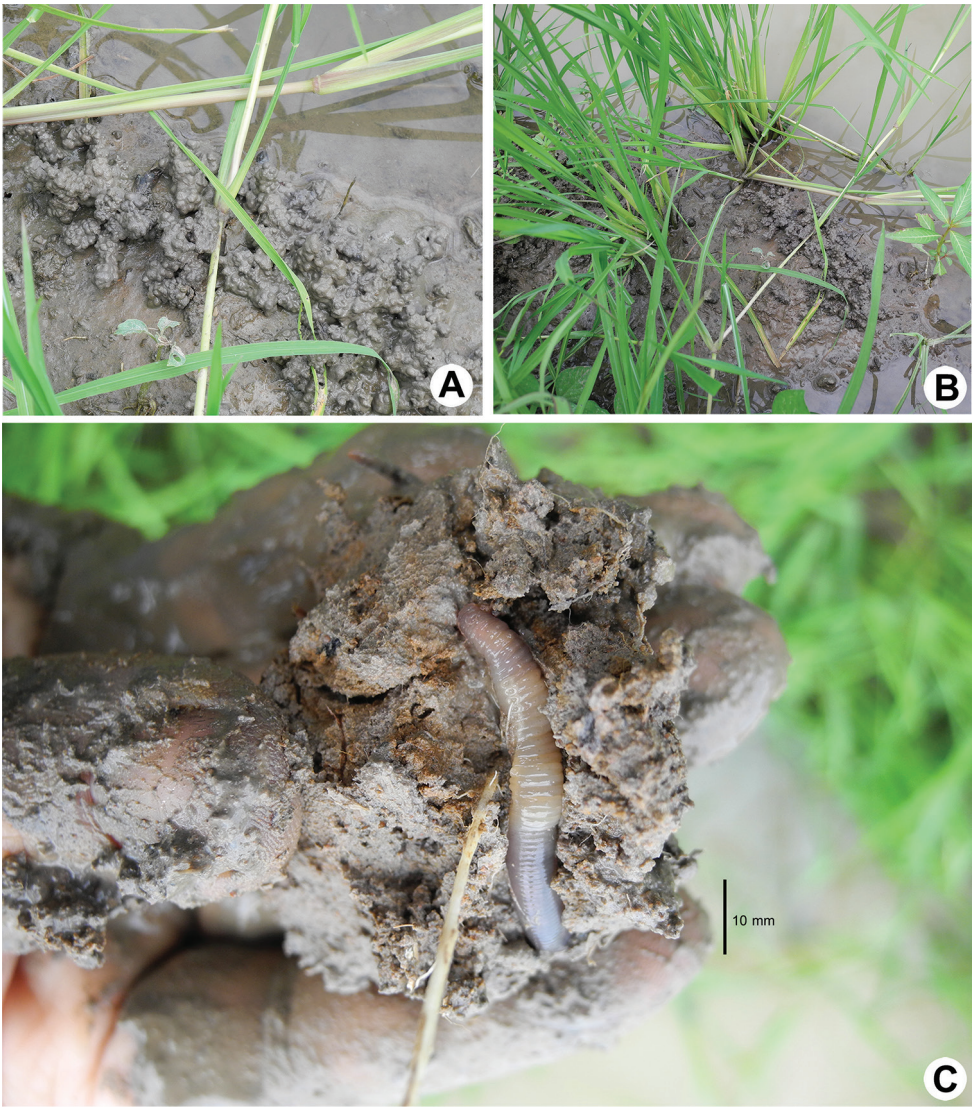


Figure 3. Photographs showing the **A** *Glyphidrilus nanensis* sp. n. casts **B** type locality of *G. nanensis* sp. n. in the rice field near Nan River at Saklek, Phichit, north Thailand **C** coloration of living paratype (CUMZ 3404).

Etymology. The species was named after the locality on the banks of the Nan River. This was the first time that the worm genus was ever recorded from near this river.

Remarks. For a summary of the following comparisons please see Table 1. The new species differs from *G. yunnanensis* Chen & Xu, 1977 reported from China by the latter having longer wings in XXII–XXXII, the clitellum in XVIII–XXXVIII, paired genital markings on bc in XVII–XXI and XXXII–XXXIV, lacking a spermathecae. It differs from *G. vangviengensis* Chanabun & Panha, 2011 from Laos by *G. vangviengensis*

Table 1. The comparison of characters among *Glyphidrilus* species from Thailand and Laos. The morphological characters of the species described are from type specimens and original description.

Species	Length (mm)	Segments	Cli.	Wi.	Gm.			He.	In.	Gi.	Sc.	Type locality
					Paired on bc	Paired on aa	Un paired on aa					
G. mekongensis	125–224	223–382	XIX–XXXVII, XXXVIII	XXIV–½XXXIII, XXXIII, XXX-IV, ½XXXV	XXIII	absent	absent	VII–XI	XV	VIII	absent	Khong Chiam, Ubon Ratchathani, Thailand
G. vangviengensis	104–160	145–229	XIX, XX–XXXV, XXXVI, XXXVII	XXIV, XXV–XXXI, XXXII	XVIII, XIX, XX, XXI–XXIV, XXXIII, XXXIV	XII–XIV, XV	absent	VII–XI	XVI	VIII	absent	Song River Veintiane, Laos
G. yunnanensis	123	139	XVIII–XXXVIII	XXII–XXXII	XVII–XXI, XXXII–XXXIV	absent	absent	VII–XI	XVI	VIII	absent	Yunnan, China
G. borealis	66–90	180–284	XIV, XVI, XVII–XXXI, XXXII, XXXIII, XXXIV, XXXV, XXXVI	XXI, XXII–XXVII, XX-VIII, XXIX	XIII, XIV, XVI, XVII, XVIII–XXII, XXIII, XXVII, XXVIII, XXIX, XXX	absent	absent	VII–XI	XIII	7/8	14/15–18/19	Maeklang waterfall, Doi Inthanon National Park, Chiangmai, Thailand
G. vangthongensis	62–195	150–358	XII, XIII, XIV, XV, XVI– XL, XLI, XLII	XXIV, XXV, XXVI–XXXI, XXXII	XIII, XIV–XXIV, XXV, XXVI, XXXI, XXXII, XXXIII	XII, XIII, XIV, XXX, XXXII, XXXIII, XXX-IV, XXXV, XXXVI	absent	VII–XI	XIV	7/8	12/13–18/19	Sakulnothayan waterfall, Vangthong, Phitsanu lok, Thailand
G. chaophraya	113–138	325–414	XX–XLIII, XLIV, XLV	XXIV, XXV–XXXII, XXXIII	XVI, XIX, XX–XXIII, XXXII, XXXIII	XII, XIII, XIV, XXXIV, XXXV, XXX-VII, XXXVIII	absent	VII–XI	XV	VIII	16/17–22/23	Chao phraya River, Payuha kiri, Nakhonsawan, Thailand
G. chiensis	61–193	122–386	XVII, XVIII–XXXIII, XXXIV, XXXV, XXXVI, XXXVII, XXXVIII	XXIII, XXIV, XXV, XXVI–XXIX, XXX, XXXI, XXXII	XV, XVI, XVII, XVIII, XIX–XX, XXI, XXII, XXIII, XXIV, XXX, XXXI, XXXIII	XI, XII, XIII, XIV, XXX, XXXII, XXXIII, XXX-IV, XXXV, XXXVI	absent	VII–XI	XV	VIII	12/13–18/19	Rice filed at Thatoom, Mueang, Mahasa rakham, Thailand

Species	Length (mm)	Segments	Cli.	Wi.	Gm.			He.	In.	Gi.	Sc.	Type locality
					Paired on bc	Paired on aa	Un paired on aa					
G. quadratus	54–156	186–378	XV, XVI, XVII, XVIII–XXXI, XXXII, XXXIII, XXXIV, XXXV, XXXVI	XXIII, XXIV–XXVIII, XXIX, XXX, XXXI	XIII, XV, XVI, XVII, XVIII, XIX–XXI, XXII, XXIII, XXX, XXXI	XI, XII, XIII, XIV, XXXI, XXXII, XXXIII, XXXIV	absent	VII–XI	XV	VIII	12/13–17/18	Kang Sapue, Phibonmang sa-han, Ubon Ratcha-thani, Thailand
G. huailuangensis	50–91	131–228	XII, XIII, XVI–XXXII, XXXIII	XXV, XXVI–XXX, XXXI	XVI–XXIV	XXXI	absent	VIII–XI	XIII	7/8	absent	Huailung waterfall, Najahlauy, Ubon Ratchathani, Thailand
G. trangensis	11⁺–63⁺	41⁺–153⁺	XVII, XVIII–XXX	XXII, XXIII–XXVII, XX-VIII	absent	absent	XVIII–XXI	VIII–XI	XVI	8/9	XVIII–XXI	Trang River, Nayong, Trang
G. wararamensis	18⁺–120	46⁺–279	XI, XII, XIII–XXXIII, XXXIV, XXXV	XX, XXI–XXVI, XXVII	XIV, XV, XVII–XIX, XX, XXVII	absent	XI–XIII, XIV, XV, XVII, XVIII–XIX, XX, XXVIII, XXIX–XXX	VIII–XI	XIV	6/8	13/14–17/18	Stream near Wattham Wararam, Phanom, Suratthani
G. kratuensis	48–93	221–282	XVIII–XXX, XXXI, XXXII	XXIII, XXIV–XXVIII, XXIX, XXX	XIV, XV, XVI, XVII, XVIII, XIX, XXII, XXIII, XXIV, XXIX, XXX	absent	XVII, XIX–XX, XXI, XXII, XXIII, XXX–XXXI, XXXII–XXXIV	VIII–XI	XIV	VIII	14/15–17/18	Kratu waterfall, Kratu, Phuket
G. nanensis sp. n.	72–99	186–257	XVII, XVIII–XXXIII, XXXIV	XXIV, XXV, XXVI–XXVII, XXVIII, XXIX	XV, XVI, XVII–XXIII, XXIV, XXV	X, XI, XII, XIII, XIV, XX-VIII, XXIX, XXX	absent	VII –XI	XV	7/8	13/14–17/18	Rice field near Nan River, Saklek, Phichit, Thailand
G. satunensis sp. n.	60⁺–131	156⁺–326	XVII, XVIII–XXXII, XXXIII, XXXIV, XXXV	XXIV, XXV, XXVI–XXIX, XXX, XXXI	XVII, XVIII, XIX	absent	XVI, XVII, XVIII–XXII, XXIII, XXIV	VIII–XI	XVI	VII	13/14–15/16	Nong Prakpraya, Mueang, Satun, Thailand

Species	Length (mm)	Segments	Cli.	Wi.	Gm.			He.	In.	Gi.	Sc.	Type locality
					Paired on bc	Paired on aa	Un paired on aa					
G. chiangraiensis sp. n.	94–340	89–394	XVII, XVIII, XIX, XX, XXI–XXXVI, XXXVII, XXXVIII, XXXIX	XXIII, XXIV–XXVI, ½XXVII, XXVII, ½XXVIII, XXVIII	XXI, XXII, XXIII, XXIV, XXVII, XXVIII, XXIX–XXXV	XII–XIV, XV, XVI	absent	VII–XI	XVI	VIII	15/16–20/21	Mekong River at Wat Hatkai, Chiang khong, Chiangrai, Thailand
G. namphao sp. n.	64–122	190–320	XVII–XXVI, XXIX	XVIII–XXIV	XII–XV, XXV, XXVI	XVII	absent	VII–XI	XVI	VIII	13/14–16/17	Phao River Kamkerd, Bolikhamxai, Laos
G. sekongensis sp. n.	90–134	237–337	XVI, XVII–XXXVI, XXXVII	XXV–XXXI	XVI, XVII–XXIV, XXXII	absent	absent	VIII–XI	XVI	VIII	12/13–15/16	Stream at Ban Kiang kong, Lamarm, Sekong, Laos
G. namdonensis sp. n.	90–139	183–259	XIX, XX–XXXVI, XXXVII, XXXVIII	XXIV, XXV–XXVIII, XXX	XXII, XXIII, XXIV, XXVII, XXVIII–XXXIII	XIII, XIV	absent	VII–XI	XVI	VIII	14/15–18/19	Done River, Thakhek, Kham mouan, Laos
G. champasakensis sp. n.	167–301	248–424	XIX, XX– XLIX, L, LI, LII	XXIII, XXIV–½XXXII, XXXII, XXXIII	XVII, XVIII, XIX–XXII, XXIII, XXIV, XXXII, XXXIII, XXXIV, XXXV	XII, XIII, XXXIII, XXXIV	absent	VII–XI	XVI	VIII	14/15–19/20	Mekong River at Ban Khonkhen, Champa sak, Laos

Abbreviations: Cli.: clitellum; Wi.: wings; Gm.: genital markings; He.: hearts; In.: intestinal caeca; Gi.: gizzard; Sc.: spermathecae.

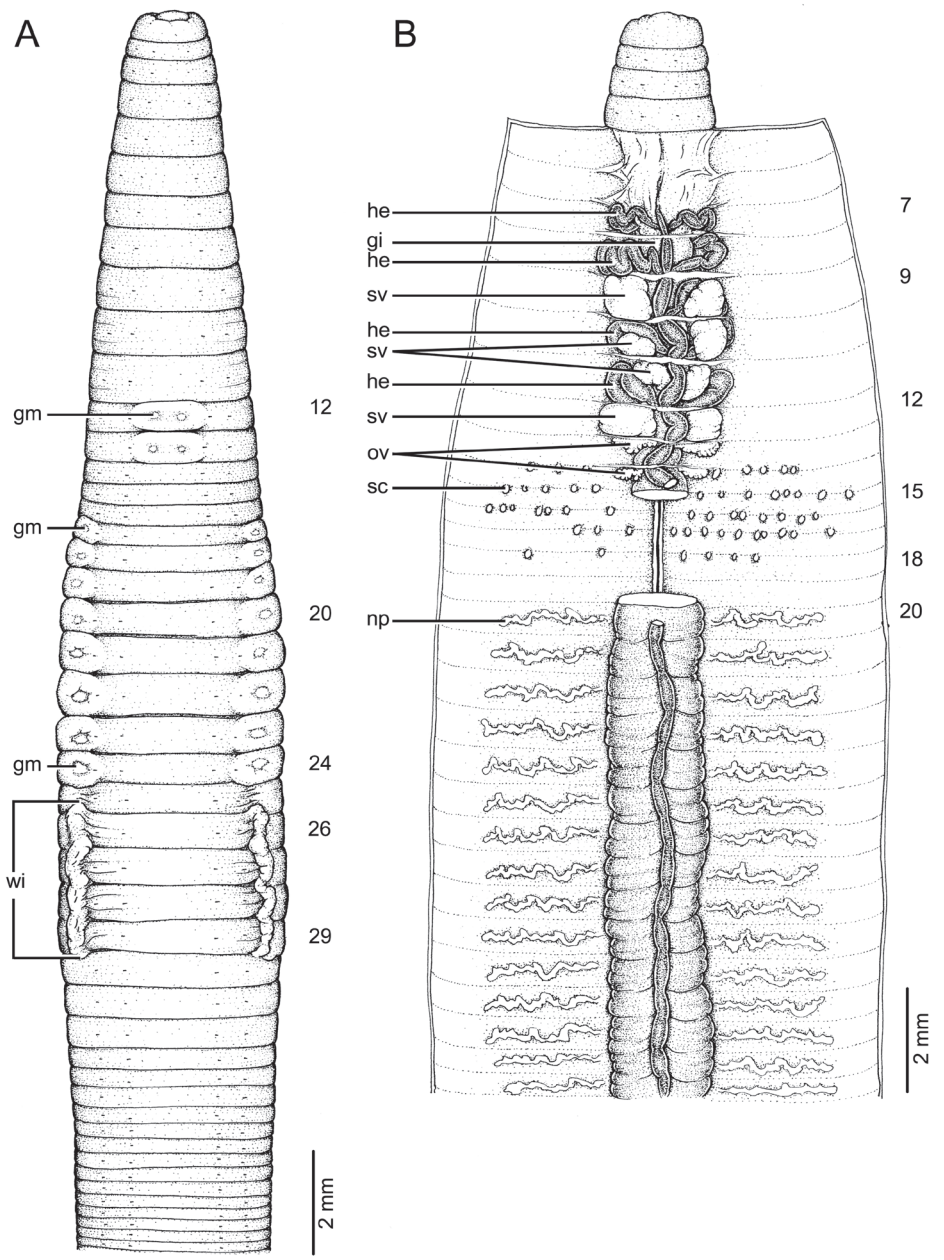


Figure 4. Morphology of holotype (CUMZ 3403) of *Glyphidrilus nanensis* sp. n. **A** external ventral view, annular clitellum in XVII–XXXIII **B** internal dorsal view.

having longer wings in XXIV, XXV–XXXI, XXXII, the clitellum in XIX, XX–XXXV, XXXVI, XXXVII, and lacking spermathecae. It differs from the species recorded as *G. mekongensis* Panha & Chanabun, 2012 from the Mekong River, Ubon Ratchathani,

northeast Thailand by the latter species having longer wings in XXIV–½XXXIII, XXXIII, XXXIV, ½XXXV, the clitellum in XIX–XXXVII, XXXVIII, and lacking spermathecae. It differs from *G. borealis* Chanabun & Panha, 2013 from Chiangmai, north Thailand by the latter having longer wings in XXI, XXII–XXVII, XXVIII, XXIX, a longer clitellum in XIV, XVI, XVII–XXXI, XXXII, XXXIII, XXXIV, XXXV, XXXVI, and the intestine enlarged from XIII. It differs from *G. vangthongensis* Chanabun & Panha, 2013 reported from Phitsanulok, north Thailand by *G. vangthongensis* having longer wings in XXIV, XXV, XXVI–XXXI, XXXII, a longer clitellum in XII, XIII, XIV, XV, XVI–XL, XLI, XLII, an intestine enlarged from XIV, and spermathecae between 12/13–18/19. It differs from *G. chaophraya* Chanabun & Panha, 2013 from Chaophraya River, Nakhonsawan, central Thailand by the latter having longer wings in XXIV, XXV–XXXII, XXXIII, a longer clitellum in XX–XLIII, XLIV, XLV, and spermathecae between 16/17–22/23. It differs from *G. chiensis* Chanabun & Panha, 2013 from Mahasarakham, northeast Thailand by *G. chiensis* having wings in XXIII, XXIV, XXV, XXVI–XXIX, XXX, XXXI, XXXII, the clitellum in XVII, XVIII–XXXIII, XXXIV, XXXV, XXXVI, XXXVII, XXXVIII, and spermathecae between 12/13–18/19. It differs from *G. quadratus* Chanabun & Panha, 2013 reported from the Mun River drainage area, northeast Thailand by *G. quadratus* having wings in XXIII, XXIV–XXVIII, XXIX, XXX, XXXI, the clitellum in XV, XVI, XVII, XVIII–XXXI, XXXII, XXXIII, XXXIV, XXXV, XXXVI, and spermathecae between 12/13–17/18. It differs from *G. huailuangensis* Chanabun & Panha, 2013 recorded from Najahlauy National Park, Ubon Ratchathani, northeast Thailand which has wings in XXV, XXVI–XXX, XXXI, a clitellum in XII, XIII, XVI–XXXII, XXXIII, and lacking spermathecae. It differs from *G. sekongensis* sp. n. by *G. sekongensis* sp. n. having longer wings in XXV–XXXI, a longer clitellum in XVI, XVII–XXXVI, XXXVII, and spermathecae between 12/13–15/16 (see Table 1).

Glyphidrilus satunensis Chanabun & Panha, sp. n.

http://zoobank.org/16A9B4B7-9D5F-409E-B438-059A0FC21BB0
Figs 2, 5, Table 1

Material examined. Holotype: One adult (CUMZ 3405), in Nong Prakpraya, Mueang, Satun, Thailand (06°44'34.0"N, 100°02'23.0"E), 27 meters elevation on 16 January 2014. **Paratypes:** 15 adults and 21 juveniles (CUMZ 3406), 2 adults (ZMH 14580), 2 adults (NHMUK), and 2 adults (ZRC), all same collection data as holotype.

Diagnosis. *Glyphidrilus satunensis* sp. n. has the clitellar wings on the lateral side of the body in XXIV, XXV, XXVI–XXIX, XXX, XXXI. Clitellum in XVII, XVIII–XXXII, XXXIII, XXXIV, XXXV. Female pores, male pores and spermathecal pores not visible. Genital markings: unpaired on aa in XVI, XVII, XVIII–XXII, XXIII, XXIV; paired or asymmetrical on bc in XVII, XVIII, XIX. Four pairs of seminal vesicles in IX–XII. Intestinal origin in XVI. Ovaries in XIII–XIV. Spermathecae between 13/14–15/16.

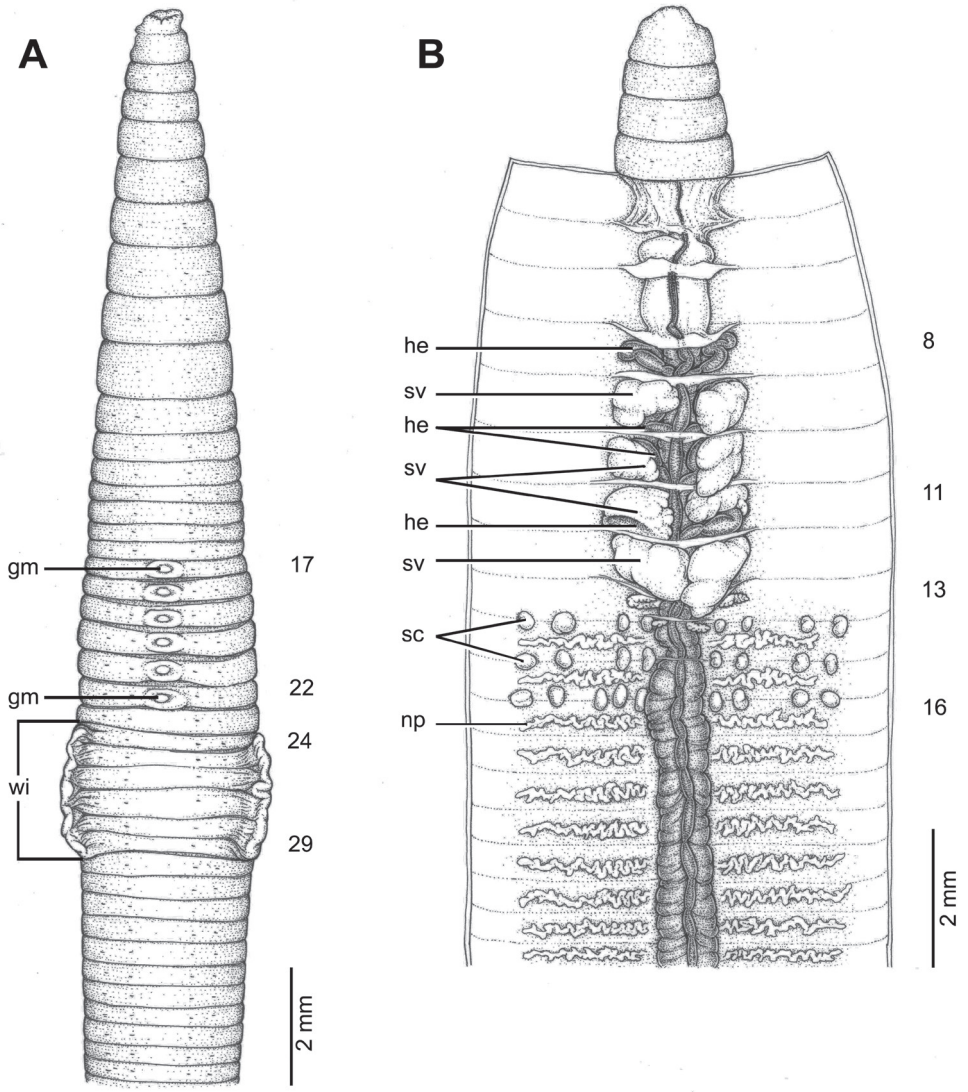


Figure 5. Morphology of holotype (CUMZ 3405) of *Glyphidrilus satunensis* sp. n. **A** external ventral view, annular clitellum in XVII–XXXIII **B** internal dorsal view.

Description of holotype. Dimensions: body length 131 mm, diameter 2.2 mm in segment VIII, 2.3 mm before the clitellar wing in segment XXIII, 2.4 mm after wing in segment XXX in clitellar region; body cylindrical in anterior part, quadrangular in transverse section behind clitellum. 326 segments. Body color pale brown with variations from red to pink at adjacent tissues of wing portion in different individuals of newly collected specimens. At posterior end dorsal surface considerably broader than the ventral. Clitellar wing on ventro-lateral part of clitellum in XXIV–XXIX, 2.5 mm in height, and 0.5 mm in width on both sides. Prostomium zygolobous.

Dorsal pores absent. Clitellum annular in XVII–XXXIII. Four pairs of setae per segment from II, setal formula aa:ab:bc:cd:dd = 1.0:0.5:1.5:1.0:1.5 in segment VIII and 1.0:0.5:1.5:0.5:2.0 in postclitellar segments. Female pores, male pores, and spermathecal pores not visible. Genital markings: unpaired on aa in XVII–XXII.

Septa 5/6–7/8 thicker than between other segments, 8/9–9/10 thick and 10/11 to the last segment thin. Gizzard small, globular in VII. Intestine enlarged from XVI. Hearts, four pairs in VIII–XI. A pair of holonephridia in each segment from segment XIII onwards. Seminal vesicles, four pairs in IX–XII. Ovaries, two pairs in XIII–XIV. Testes free in X and XI. Prostate and accessory glands absent. Spermathecae sessile and diverticula absent, elongated oval or globular between 13/14–15/16, about 0.2–0.3 mm in diameter, four to five on each side per segment.

Variations. Body lengths of adult (n = 22) and juvenile paratypes (n = 21). Adults ranged from 60⁺–131 mm (95.8 ± 24.5), with 156⁺–326 segments. Juveniles ranged from 62–129 mm (82.5 ± 22.1), with 166–323 segments. Wings in XXIV, XXV, XXVI–XXIX, XXX, XXXI, clitellum in XVII, XVIII–XXXII, XXXIII, XXXIV, XXXV. Genital markings: unpaired on aa in XVI, XVII, XVIII–XXII, XXIII, XXIV; paired or asymmetrical on bc in XVII, XVIII, XIX.

Distribution. The new species was found at a pond in Satun, south Thailand.

Etymology. The name "satunensis" is given in reference to the type locality, Satun.

Remarks. See Table 1 for a summary of these comparisons. *Glyphidrilus satunensis* sp. n. is quite similar to *G. kratuensis* Chanabun & Panha, 2013 from Kratu waterfall, Kratu, Phuket, south Thailand in the location of wings but *G. kratuensis* has a smaller size, a shorter clitellum in XVIII–XXX, XXXI, XXXII, the intestine begins from XIV, and spermathecae are between 14/15–17/18. It differs from *G. trangensis* Chanabun & Panha, 2013 from Trang River, Nayong, Trang, south Thailand by the latter having wings in XXII, XXIII–XXVII, XXVIII, the clitellum in XVII, XVIII–XXX, and spermathecae in XVIII–XXI. It differs from *G. wararamensis* Chanabun & Panha, 2013 from stream near Wattham Wararam, Phanom, Suratthani, south Thailand by *G. wararamensis* having wings in XX, XXI–XXVI, XXVII, a clitellum in XI, XII, XIII–XXXIII, XXXIV, XXXV, and spermathecae between 13/14–17/18. It differs from *G. nanensis* sp. n. by *G. nanensis* having a smaller size, slightly shorter wings in XXIV, XXV, XXVI–XXVII, XXVIII, XXIX, unpaired genital markings absent, five pairs of hearts in VII–XI, and spermathecae between 13/14–17/18 (see Table 1).

Glyphidrilus chiangraiensis Chanabun & Panha, sp. n.

http://zoobank.org/733E54E7-AE95-451F-998F-BC79E71A4155
Figs 1, 6, 7, Table 1

Type material. Holotype: One adult (CUMZ 3407) in the river banks of Mekong River at Wat Hatkai, Chiangkhong, Chiangrai, north Thailand (20°15'8.5"N, 100°24'46.8"E), 384 meters elevation on 14 March 2014. **Paratypes:** 9 adults (CUMZ

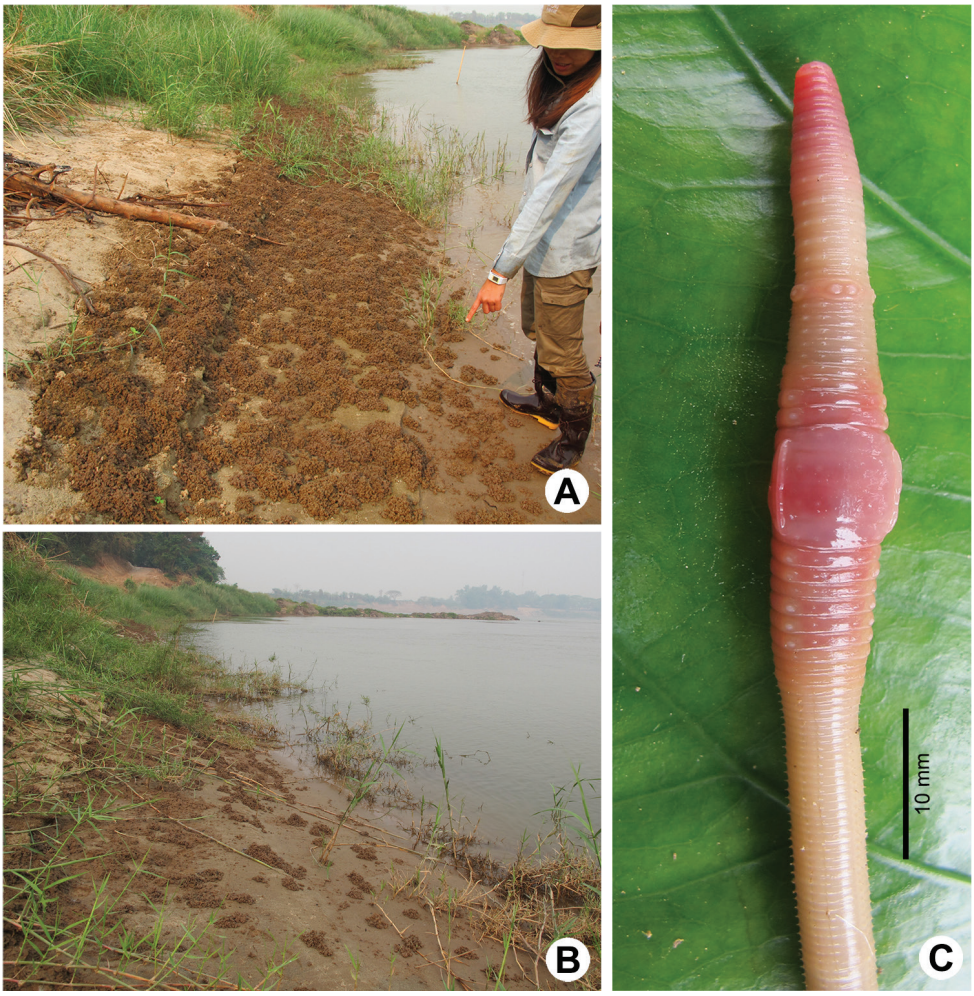


Figure 6. Photographs showing the **A** *Glyphidrilus chiangraiensis* sp. n. and other earthworms casts **B** type locality of *G. chiangraiensis* sp. n. in the river banks of Mekong River at Wat Hatkai, Chiangkhong, Chiangrai, north Thailand, and **C** coloration of newly collected paratype (CUMZ 3408) after the first preservation step in 30% (v/v) ethanol.

3408), 2 adults (ZMH 14581), 2 adults (NHMUK), and 2 adults (ZRC), all specimens collected only from the type locality.

Other material examined. 43 adults (CUMZ 3409), in the river banks of Mekong River at Mueang, Buengkan, northeast Thailand (18°22'2.4"N, 103°38'58.0"E), 144 meters elevation on 6 December 2013. 33 adults (CUMZ 3410), in the river banks of Mekong River at Kang Kudku, Chiangkhan, Loei, northeast Thailand (17°54'24.5"N, 101°42'7.5"E), 195 meters elevation on 8 December 2013. 25 adults (CUMZ 3411), in the river banks of Mekong River at Wat Srisomsanook, Chiangkhan, Loei, northeast Thailand (17°59'25.4"N, 101°44'51.3"E), 212 meters elevation on 7 December

2013. 11 adults (CUMZ 3412), in the river banks of Mekong River at Wat Hadphatum, Srichiangmai, Nongkhai, northeast Thailand (17°57'32.2"N, 102°35'26.8"E), 174 meters elevation on 7 December 2013. 38 adults (CUMZ 3413), in the river banks of Mekong River at Wat Jomnang, Phonphisai, Nongkhai, northeast Thailand (18°01'53.6"N, 103°4'47.4"E), 165 meters elevation on 6 December 2013. 30 adults (CUMZ 3414), in the river banks of Mekong River at Wat Prayanakmai, Wiangkaen, Chiangrai, north Thailand (20°11'45.2"N, 100°27'32.0"E), 359 meters elevation on 15 March 2014. 19 adults (CUMZ 3415), in the river banks of Mekong River at Wat Bansaw, Chiangsan, Chiangrai, north Thailand (20°15'19.4"N, 100°10'44.9"E), 385 meters elevation on 14 March 2014. 26 adults (CUMZ 3416), in river banks of Mekong River at Ban Rimkhong, Pakchom, Loei, northeast Thailand (18°12'48.9"N, 102°04'52.2"E), 181 meters elevation on 8 December 2013. 3 adults (CUMZ 3417), in river banks of Mekong River, Mueng Paksay, Chaiyaburi, Laos (18°12'40.0"N, 101°24'28.1"E), 214 meters elevation on 15 April 2014. 15 adults (CUMZ 3418) in river banks of Mekong River, Bandon, Luangprabang, Laos (19°55'27.6"N, 102°10'49.7"E), 304 meters elevation on 14 April 2014. 24 adults (CUMZ 3419) in river banks of Mekong River, between Sanakham to Vientiane, Laos (17°57'39.7"N, 101°43'53.8"E), 224 meters elevation on 15 April 2014.

Diagnosis. *Glyphidrilus chiangraiensis* sp. n. has clitellar wings on the lateral side of the body in XXIII, XXIV–XXVI, ½XXVII, XXVII, ½XXVIII, XXVIII. Clitellum in XVII, XVIII, XIX, XX, XXI–XXXVI, XXXVII, XXXVIII, XXXIX. Female pores, male pores and spermathecal pores not visible. Genital markings: paired or asymmetrical on aa in XII–XIV, XV, XVI, paired or asymmetrical on bc in XXI, XXII, XXIII, XXIV and XXVII, XXVIII, XXIX–XXXV. Four pairs of seminal vesicles in IX–XII. Intestinal origin in XVI. Ovaries in XIII–XIV. Spermathecae between 15/16–20/21.

Description of holotype. Dimensions: body length 158 mm, diameter 3.0 mm in segment VIII, 4.0 mm before the clitellar wing in segment XXIII, 4.0 mm after wing in segment XXIX in clitellar region; body cylindrical in anterior part, quadrangular in transverse section behind clitellum. 282 segments. Body color pale brown with variations from red to pink at adjacent tissues of wing portion in different individuals of newly collected specimens. Clitellar wing on ventro-lateral part of clitellum in XXIV–XXVII, ½XXVIII, 3.7 mm and 4.0 mm in height, and 0.5 mm in width on both sides. Prostomium zygolobous. Dorsal pores absent. Clitellum annular in XVII–XXXVIII. Four pairs of setae per segment from II, setal formula aa:ab:bc:cd:dd=2.0:1.0:2.0:1.0:2.0 in segment VIII and 2.0:1.0:2.0:1.0:2.5 in postclitellar segments. Female pores, male pores, and spermathecal pores not visible. Genital markings: paired on aa in XIV, laterally paired or asymmetrical on bc in XXIII and XXIX–XXXV.

Septa 5/6–8/9 thicker than between other segments, 9/10–13/14 thick and 14/15 to the last segment thin. Gizzard small, globular in VIII. Intestine enlarged from XVI. Hearts, five pairs in VII–XI, first in VII and last in XI. A pair of holonephridia in each segment from segment XIII onwards. Seminal vesicles, four pairs in IX, X, XI, XII. Ovaries, two pairs in XIII and XIV. Testis free in X and XI. Prostate and accessory

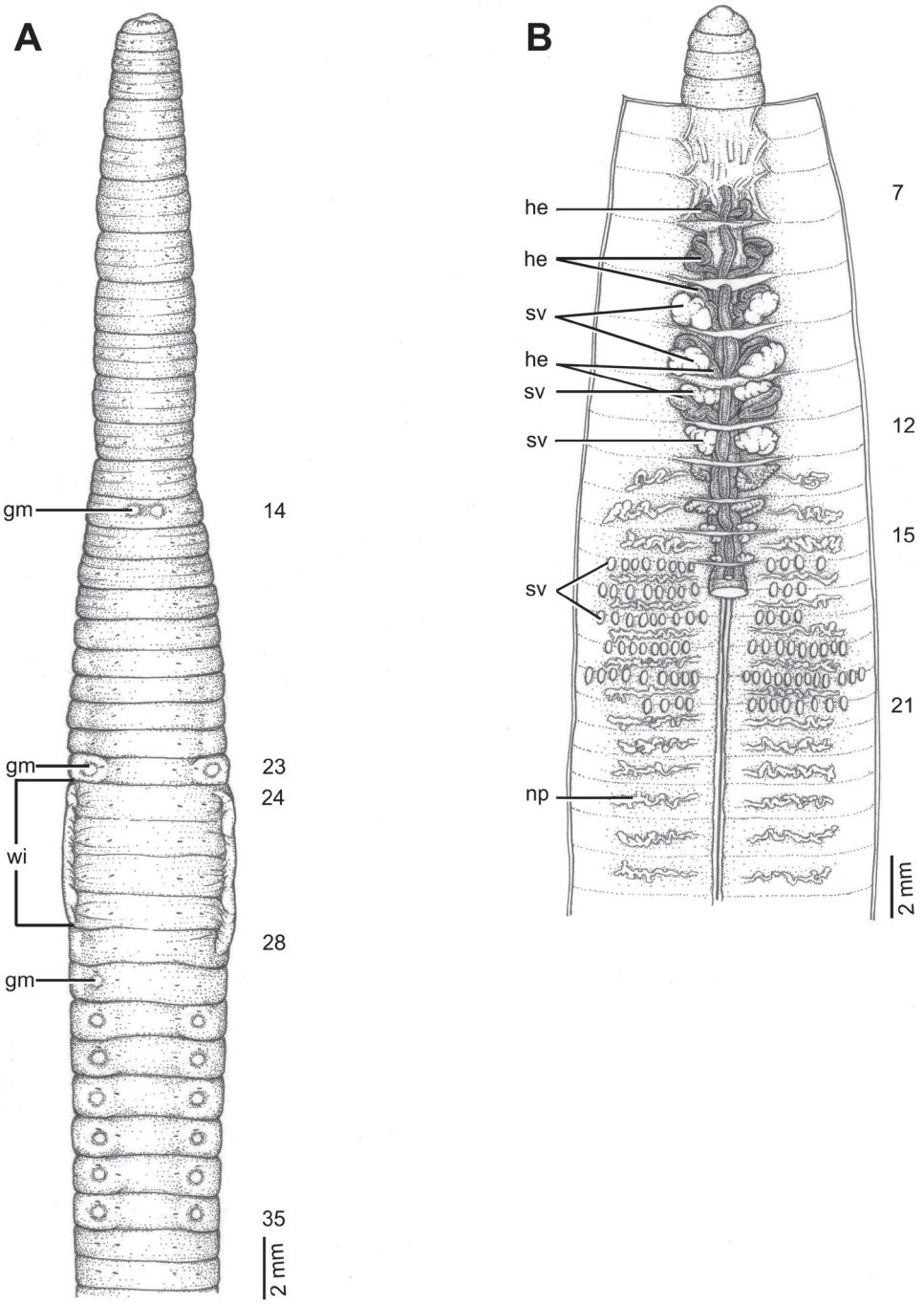


Figure 7. Morphology of holotype (CUMZ 3407) of *G. chiangraiensis* sp. n. **A** external ventral view, annular clitellum in XVII–XXXVIII **B** internal dorsal view.

glands absent. Spermathecae sessile and diverticula absent, small elongated oval or globular between 15/16–20/21, about 0.1–0.2 mm in diameter, three to twelve per segment on each side per segment.

Variations. Body lengths of adults (n = 283) ranged from 94–340 mm (155.42 ± 54.93), with 89–394 segments. Wings in XXIII, XXXIV–XXVI, ½XXVII, XXVII, ½XXVIII, XXVIII, clitellum in XVII, XVIII, XIX, XX, XXI–XXXVI, XXXVII, XXXVIII, XXXIX. Genital markings: paired or asymmetrical on aa in XII–XIV, XV, XVI, paired or asymmetrical on bc in XXI, XXII, XXIII, XXIV and XXVII, XXVIII, XXIX–XXXV.

Distribution. The new species is known from the type locality in the river banks of Mekong River at Wat Hatkai, Chiangkhong, Chiangrai, north Thailand, and was found in several locations along the Mekong River and its tributaries in the northeast and north of Thailand at Buengkan, Loei, Nongkhai, and Chiangrai and in Chaiyaburi, Luangprabang, and Sanakham to Vientiane of Laos.

Etymology. The species was named after Chiangrai, the locality name.

Remarks. (see Table 1) *Glyphidrilus chiangraiensis* sp. n. differs from *G. vangviengensis* Chanabun & Panha, 2011 from Song River, Vieintiane, Laos by the latter having longer wings in XXIV, XXV–XXXI, XXXII, the genital markings widely paired in bc XVIII, XIX, XX, XXI–XXIV and XXXIII, XXXIV, paired on aa in XII–XIV, XV and lacking spermathecae. It differs from *G. yunnanensis* Chen & Xu, 1977 reported from China by *G. yunnanensis* having longer wings in XXII–XXXII, clitellum in XVIII–XXXVIII, and lacking spermathecae. It differs from the species recorded as *G. mekongensis* Panha & Chanabun, 2012 from Mekong River, Thailand by *G. mekongensis* having longer wings in XXIV–½XXXIII, XXXIII, XXXIV, ½XXXV, and lacking spermathecae. It differs from *G. chiensis* Chanabun & Panha, 2013 from Chi River, Mahasarakham, northeast Thailand by *G. chiensis* having longer wings in XXIII, XXIV, XXV, XXVI–XXIX, XXX, XXXI, XXXII, and spermathecae between 12/13–18/19. It differs from *G. quadratus* Chanabun & Panha, 2013 reported from the Mun River by *G. quadratus* having longer wings in XXIII, XXIV–XXVIII, XXIX, XXX, XXXI, a bit longer clitellum in XV, XVI, XVII, XVIII–XXXI, XXXII, XXXIII, XXXIV, XXXV, XXXVI, and spermathecae between 12/13–17/18. It differs from *G. huailuangensis* Chanabun & Panha, 2013 recorded from Najahlauy National Park, Ubon Ratchathani, northeast Thailand by the latter having longer wings in XXV, XXVI–XXX, XXXI, clitellum in XII, XIII, XVI–XXXII, XXXIII, and lacks spermathecae. *Glyphidrilus chiangraiensis* sp. n. differs from *G. namphao* sp. n. by the latter having wings in XVIII–XXIV, clitellum in XVII–XXVI, XXIX, genital markings: paired on aa in XVII; paired or asymmetrical on bc in XII–XV, XXV, XXVI, and spermathecae between 13/14–16/17. It differs from *G. sekongensis* sp. n. by the latter having wings in XXV–XXXI, clitellum in XVI, XVII–XXXVI, XXXVII, and spermathecae between 12/13–15/16. It differs from *G. champasakensis* sp. n. from Mekong River at Ban Khonkhen, Champasak, Laos by the latter having longer wings in XXIII, XXIV–½XXXII, XXXII, XXXIII, longer clitellum in XIX, XX–XLIX, L, LI, LII, and spermathecae between 14/15–19/20.

***Glyphidrilus namphao* Chanabun & Panha, sp. n.**
http://zoobank.org/D559CDC2-5A05-4D38-93C8-681FB80F3415
Figs 1, 8, Table 1

Type material. Holotype: One adult (CUMZ 3420) in a river banks of Phao River between Ban Lak 7 and Ban Lak 5, Kamkerd, Bolikhamxai, Laos (18°16'27.7"N, 105°2'44.0"E), 525 meters elevation on 7 December 2013. **Paratypes:** 2 adults and 8 juveniles (CUMZ 3421), 1 adult (ZMH 14582), all specimens collected from only the type locality.

Diagnosis. *Glyphidrilus namphao* sp. n. is a small worm with the clitellar wings on the lateral side of the body in XVIII–XXIV. Clitellum in XVII–XXVI, XXIX. Female pores, male pores and spermathecal pores not visible. Genital markings: paired or asymmetrical on aa in XVII; paired or asymmetrical on bc in XII–XV and XXV, XXVI. Four pairs of seminal vesicles in IX–XII. Intestinal origin in XVI. Ovaries in XIII–XIV. Spermathecae between 13/14–16/17.

Description of holotype. Dimensions: body length 92 mm, diameter 3.1 mm in segment VIII, 4.2 mm before the clitellar wing in segment XVII, 4.2 mm after wing in segment XXV in clitellar region; body cylindrical in anterior part, quadrangular in transverse section behind clitellum. 269 segments. Body color pale brown with variations from red to pink at adjacent tissues of wing portion in different individuals of newly collected specimens. At posterior end dorsal surface considerably broader than the ventral. Clitellar wing on ventro-lateral part of clitellum in XVIII–XXIV, 3.5 mm in height, and 0.25 mm in width on both sides. Prostomium zygolobous. Dorsal pores absent. Clitellum annular in XVII–XXIX. Four pairs of setae per segment from II, setal formula aa:ab:bc:cd:dd =1.5:0.5:1.5:0.5:2.0 in segment VIII and 1.5:0.5:1.5:0.5:2.5 in postclitellar segments. Female pores, male pores, and spermathecal pores not visible. Genital markings: paired on aa in XVII, laterally paired or asymmetrical on bc in XII–XV and XXV.

Septa 4/5–6/7 thicker than between other segments, 7/8–11/12 thick and 12/13 to the last segment thin. Gizzard small, globular in VIII. Intestine enlarged from XVI. Hearts, five pairs in VII–XI, first in VII and last in XI. A pair of holonephridia in each segment from segment XIII onwards. Seminal vesicles, four pairs in IX, X, XI, XII. Ovaries, two pairs in XIII and XIV. Testis free in X and XI. Prostate and accessory glands absent. Spermathecae sessile and diverticula absent, elongated oval or globular between 13/14–16/17, about 0.3–0.4 mm in diameter, two to seven on each side per segment.

Variations. Body lengths of adult (n = 4) and juvenile paratypes (n = 8). Adults ranged from 64–122 mm (89.7±20.8), with 190–320 segments. Juveniles ranged from 64–120 mm (98.3±17.6), with 193–311 segments. Wings in XVIII–XXIV, clitellum in XVII–XXVI, XXIX. Genital markings: paired or asymmetrical on aa in XVII; paired or asymmetrical on bc in XII–XV and XXV, XXVI.

Distribution. The new species was found in the river banks of Phao River which was covered with worm casts.

Etymology. The species was named after the Phao River, the type locality.

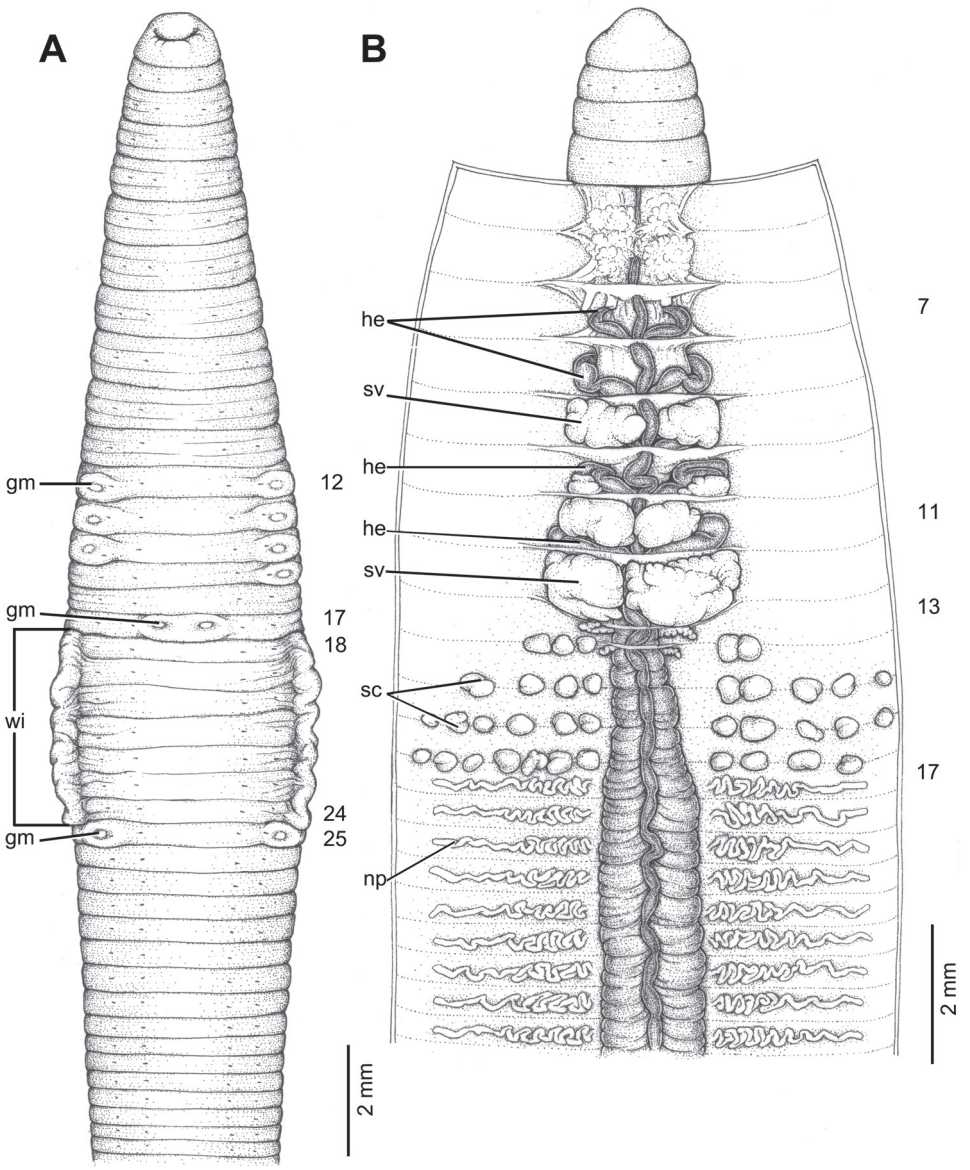


Figure 8. Morphology of holotype (CUMZ 3420) of *Glyphidrilus namphao* sp. n. **A** external ventral view, annular clitellum in XVII–XXIX **B** internal dorsal view.

Remarks. *Glyphidrilus namphao* sp. n. is different from *G. vangviengensis* Chanabun & Panha, 2011 reported from Song River Veintiane, Laos in different locations of wings in XXIV, XXV–XXXI, XXXII, longer clitellum in XIX, XX–XXXV, XXXVI, XXXVII and lacking spermathecae. It differs from *G. nanensis* sp. n. from Nan River at Saklek, Phichit, north Thailand by *G. nanensis* sp. n. having wings in XXIV, XXV,

XXVI–XXVII, XXVIII, XXIX, a longer clitellum in XVII, XVIII–XXXIII, XXXIV, and spermathecae between 13/14–17/18. It is different from *G. sekongensis* sp. n. from Ban Kiangkong, Lamarm, Sekong, Laos in the locations of wings in XXV–XXXI, a longer clitellum in XVI, XVII–XXXVI, XXXVII, the genital markings paired or asymmetrical on bc in XVI, XVII–XXIV, XXXII, and spermathecae between 12/13–15/16. *Glyphidrilus namphao* sp. n. differs from *G. chiangraiensis* sp. n. from Mekong River at Wat Hatkai, Chiangkhong, Chiangrai, north Thailand in the locations of wings in XXIII, XXIV–XXVI, ½XXVII, XXVII, ½XXVIII, XXVIII, a longer clitellum in XVII, XVIII, XIX, XX, XXI–XXXVI, XXXVII, XXXVIII, XXXIX, and spermathecae between 15/16–20/21 (see Table 1).

Glyphidrilus sekongensis **Chanabun & Panha, sp. n.**
http://zoobank.org/F9F57BA6-C6DC-46EB-8D14-9427E9ADD3E7
Figs 1, 9, Table 1

Type material. Holotype: One adult (CUMZ 3422) in a stream at Ban Kiangkong, Lamarm, Sekong, Laos (15°33'30.6"N, 106°19'19.4"E), 472 meters elevation on 17 October 2013. **Paratypes:** 2 adults and 11 juveniles (CUMZ 3423) all same collection data as holotype.

Diagnosis. *Glyphidrilus sekongensis* sp. n. is a small sized earthworm with the clitellar wings on the lateral side of the body in XXV–XXXI. Clitellum in XVI, XVII–XXXVI, XXXVII. Female pores, male pores and spermathecal pores not visible. Genital markings: paired or asymmetrical on bc in XVI, XVII–XXIV and XXXII. Four pairs of seminal vesicles in IX–XII. Intestinal origin in XVI. Ovaries in XIII–XIV. Spermathecae between 12/13–15/16.

Description of holotype. Dimensions: body length 134 mm, diameter 2.4 mm in segment VIII, 2.3 mm before the clitellar wing in segment XXIV, 2.4 mm after wing in segment XXXII in clitellar region; body cylindrical in anterior part, quadrangular in transverse section behind clitellum. 329 segments. Body color pale brown with variations from red to pink on adjacent tissues of wing portions in different individuals of newly collected specimens. The posterior end on the dorsal surface is considerably broader than the ventral. Clitellar wing on ventro-lateral part of clitellum in XXV–XXXI, 4.0 mm in height, and 0.5 mm in width on both sides. Prostomium zygolobous. Dorsal pores absent. Clitellum annular in XVI–XXXVII. Four pairs of setae per segment from II, setal formula aa:ab:bc:cd:dd = 1.0:0.3:1.0:0.5:1.3 in segment VIII and 1.8:0.8:1.3:0.6:1.5 in postclitellar segments. Female pores, male pores, and spermathecal pores not visible. Genital markings: laterally paired or asymmetrical on bc in XVI–XXIV, XXXII.

Septa 5/6–8/9 thicker than between other segments, 9/10–14/15 thick and 15/16 to the last others segment, 9/10–14/15 thick and 15/16 to the last segment thin. Gizzard small, globular in VIII. Intestine enlarged from XVI. Hearts, four pairs in VIII–XI, first in VIII and last in XI. A pair of holonephridia in each segment from segment

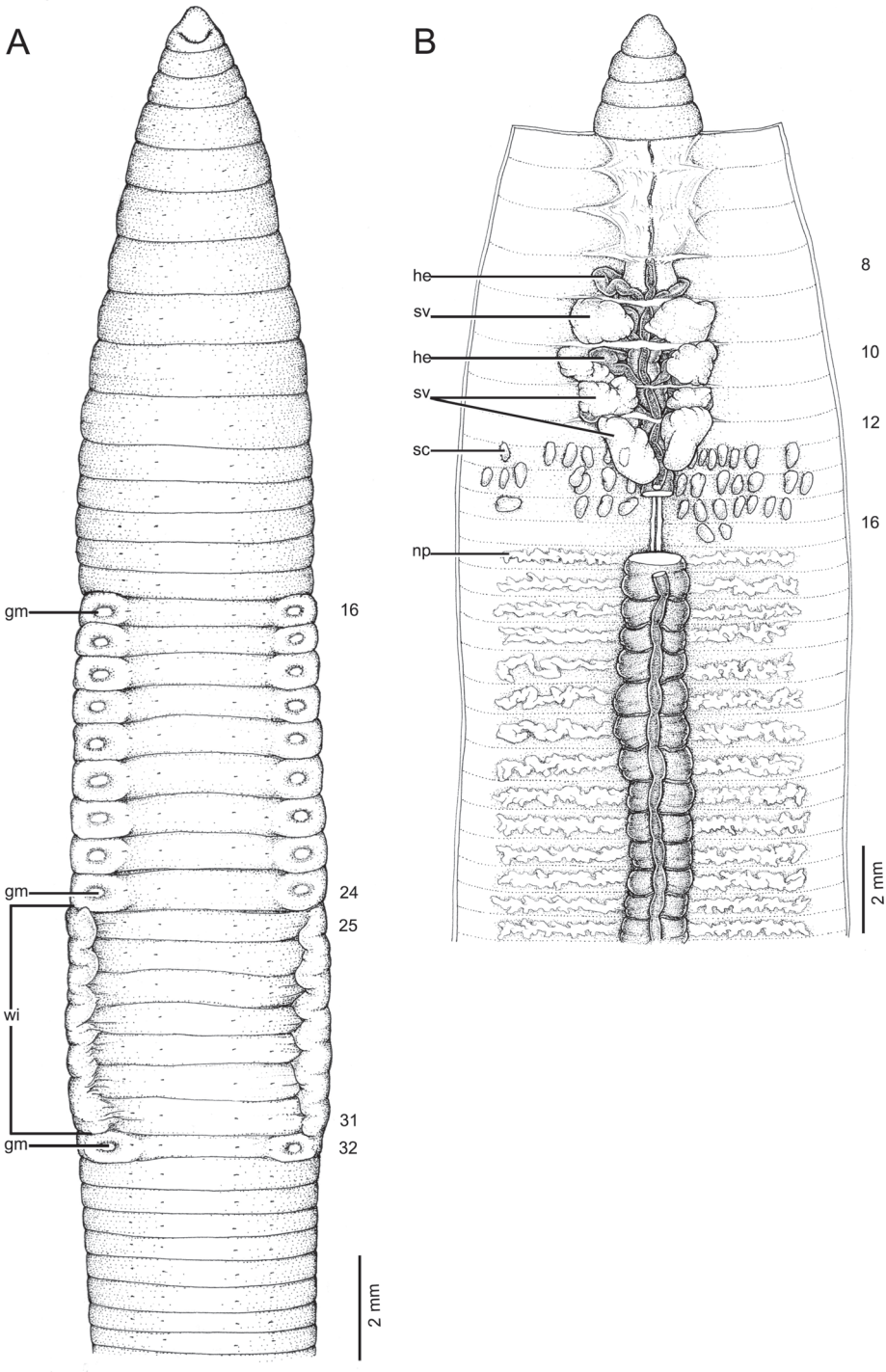


Figure 9. Morphology of holotype (CUMZ 3422) of *Glyphidrilus sekongensis* sp. n. **A** external ventral view, annular clitellum in XVI–XXXVII **B** internal dorsal view.

XIII onwards. Seminal vesicles, four pairs in IX, X, XI, XII. Ovaries, two pairs in XIII and XIV. Testis free in X and XI. Prostate and accessory glands absent. Spermathecae sessile and diverticula absent, small elongated oval or globular between 12/13–15/16, about 0.1–0.3 mm in diameter, two to seven on each side per segment.

Variations. Body lengths of adult (n = 3) and juvenile paratypes (n = 11). Adults ranged from 90–134 mm (109.6 ± 12.2), with 237–337 segments. Juveniles ranged from 86⁺–130 mm (104.2 ± 14.0), with 209⁺–329 segments. Wings in XXV–XXXI, clitellum in XVI, XVII–XXXVI, XXXVII. Genital markings: laterally paired or asymmetrical on bc in XVI, XVII–XXIV, XXXII.

Distribution. The new species was found in a stream at Ban Kiangkong, Lamarm, Sekong, Laos. Soils are slightly sandy mixed with black organic matter.

Etymology. The species was named after Sekong, the type locality in Laos.

Remarks. (see Table 1). *Glyphidrilus sekongensis* sp. n. differs from other species by the locations of external and internal characteristics such as wings, clitellum, the arrangement of genital markings, and spermathecae. The new species differs from *G. vangviengensis* Chanabun & Panha, 2011 from Song River, Vientiane, Laos by the latter having a shorter clitellum in XIX, XX–XXXV, XXXVI, XXXVII, widely paired genital markings in bc XVIII, XIX, XX, XXI–XXIV, XXXIII, XXXIV, paired on aa in XII, XIII, XIV, XV, and lacks spermathecae. Differences from *G. yunnanensis* Chen & Xu, 1977 reported from China are that *G. yunnanensis* has longer wings in XXII–XXXII, clitellum in XVIII–XXXVIII, and lacks spermathecae. Differences from *G. mekongensis* Panha & Chanabun, 2012 are that *G. mekongensis* has longer wings in XXIV–½XXXIII, XXXIII, XXXIV, ½XXXV, and lacks spermathecae. It differs from *G. huailuangensis* Chanabun & Panha, 2013 recorded from Najahlauy National Park, Ubon Ratchathani, northeast Thailand by the latter having a larger body size, clitellum in XII, XIII, XVI–XXXII, XXXIII, and lacks spermathecae. It differs from *G. champasakensis* sp. n. from Mekong River at Ban Khonkhen, Champasak, Laos by the latter having longer wings in XXIII, XXIV–½XXXII, XXXII, XXXIII, longer clitellum in XIX, XX–XLIX, L, LI, LII, and spermathecae between 14/15–19/20.

***Glyphidrilus namdonensis* Chanabun & Panha, sp. n.**
http://zoobank.org/64F05048-0871-4BF0-A7F5-B9FB02701348
Figs 1, 10, Table 1

Type material. Holotype: One adult (CUMZ 3424) in the banks of Done River at Ban Namdone, Thakhek, Khammouan, Laos (17°28'39.9"N, 104°45'35.1"E), 161 meters elevation on 6 December 2013. **Paratypes:** 16 adults and 6 juveniles (CUMZ 3425), 2 adults (ZMH 14583), 2 adults (NHMUK), and 2 adults (RMBR), all same collection data as holotype.

Diagnosis. *Glyphidrilus namdonensis* sp. n. is small sized earthworm with distinctly expanded tissues, the clitellar wings on the lateral side of the body in XXIV, XXV–XXVIII, XXX. Clitellum in XIX, XX–XXXVI, XXXVII, XXXVIII. Female pores,

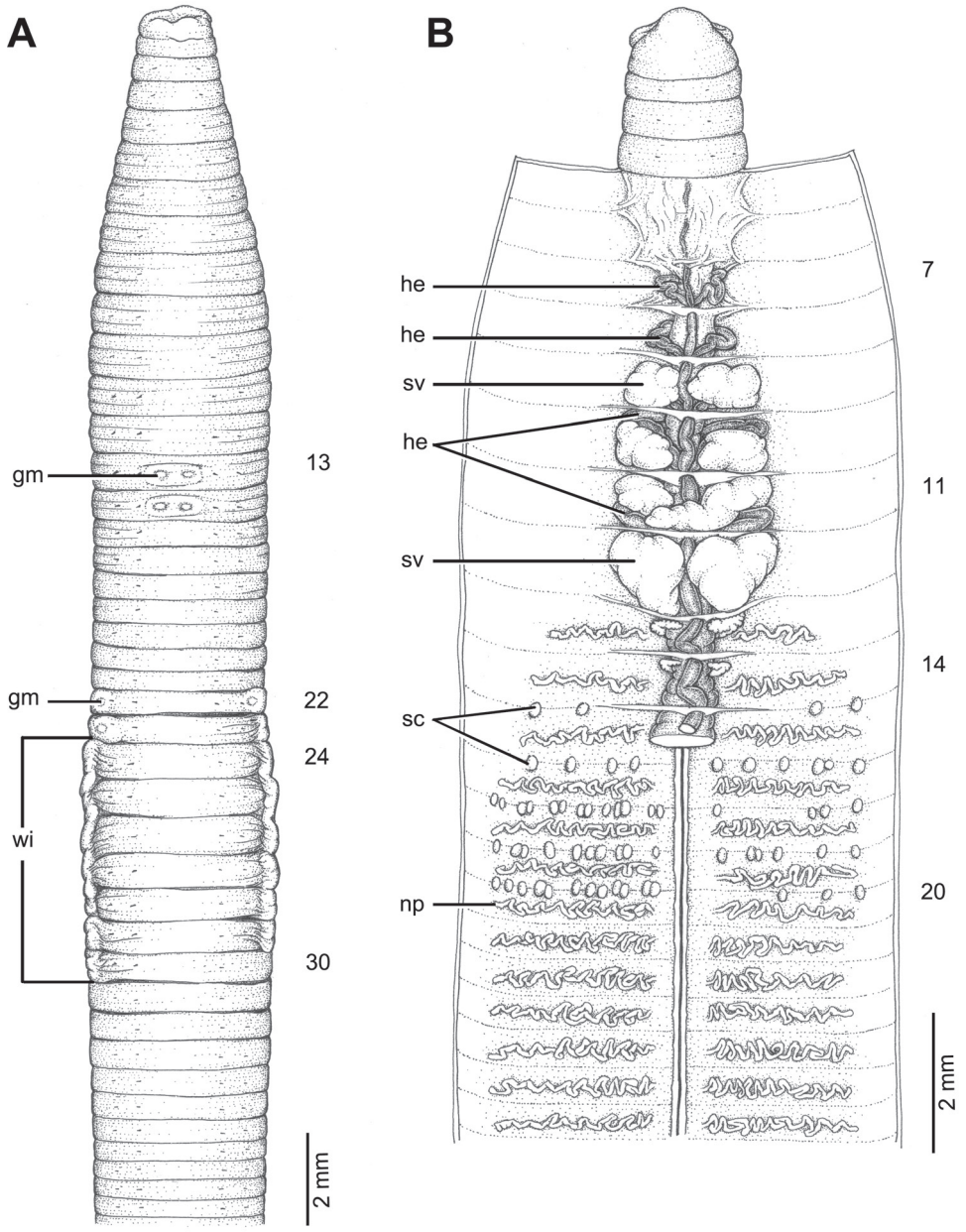


Figure 10. Morphology of holotype (CUMZ 3424) of *Glyphidrilus namdonensis* sp. n. **A** external ventral view, annular clitellum in XX–XXXVII **B** internal dorsal view.

male pores and spermathecal pores not visible. Genital markings: medium paired or asymmetrical on aa in XIII, XIV; paired or asymmetrical on bc in XXII, XXIII, XXIV and XXVII, XXVIII–XXXIII. Four pairs of seminal vesicles in IX–XII. Intestinal origin in XVI. Ovaries in XIII–XIV. Spermathecae between 14/15–18/19.

Description of holotype. Dimensions: body length 92 mm, diameter 3.5 mm in segment VIII, 4.0 mm before the clitellar wing in segment XXIII, 4.0 mm after the wing in segment XXXI in the clitellar region; body cylindrical in the anterior part, quadrangular in transverse section behind clitellum. 216 segments. Body color pale brown with variations from red to pink in adjacent tissues of the wing portions in different individuals of newly collected specimens. Clitellar wing on ventro-lateral part of clitellum in XXIV–XXX, 5.0 mm in height, and 0.2 mm in width on both sides. Prostomium zygolobous. Dorsal pores absent. Clitellum annular in XX–XXXVII. Four pairs of setae per segment from II, setal formula aa:ab:bc:cd:dd = 1.3:0.5:1.0:0.5:2.0 in segment VIII and 1.5:0.5:1.0:0.5:2.3 in postclitellar segments. Female pores, male pores, and spermathecal pores not visible. Genital markings: medium paired on aa in XIII, XIV, laterally paired or asymmetrical on bc in XXII, XXIII.

Septa 5/6–8/9 thicker than between other segments, 9/10–14/15 thick, and 15/16 to the last segment thin. Gizzard small, globular in VIII. Intestine enlarged from XVI. Hearts, five pairs in VII–XI, first in VII and last in XI. A pair of holonephridia in each segment from segment XII onwards. Seminal vesicles, four pairs in IX, X, XI, XII. Ovaries two pairs in XIII and XIV. Testis free in X and XI. Prostate and accessory glands absent. Spermathecae sessile and diverticula absent, small elongated oval or globular between 14/15–18/19, about 0.1–0.2 mm in diameter, two to thirteen on each side per segment.

Variations. Body lengths of adult (n = 23) and juvenile paratypes (n = 6). Adults ranged from 90–139 mm (121.9±25.9), with 183–259 segments. Juveniles ranged from 87–136 mm (103.3±17.4), with 185–223 segments. Wings in XXIV, XXV–XXVIII, XXX, clitellum in XIX, XX–XXXVI, XXXVII, XXXVIII. Genital markings: medium paired or asymmetrical on aa in XIII, XIV; paired or asymmetrical on bc in XXII, XXIII, XXIV and XXVII, XXVIII–XXXIII.

Distribution. The new species was found only from the banks of Done River at Ban Namdone, Thakhek, Khammouan, Laos.

Etymology. The species was named after the Done River.

Remarks. *Glyphidrilus namdonensis* sp. n. is compared with other *Glyphidrilus* having spermathecae and a clitellum starting feom segment XIX. *Glyphidrilus namdonensis* differs from *G. champasakensis* sp. n. by the latter having longer wings in XXIII, XXIV–½XXXII, XXXII, XXXIII, longer clitellum in XIX, XX–XLIX, L, LI, LII, and spermathecae between 14/15–19/20 (see Table 1).

***Glyphidrilus champasakensis* Chanabun & Panha, sp. n.**
http://zoobank.org/32CACB01-68EE-4093-A66E-8EEF939A3B6D
Figs 1, 11, 12, Table 1

Type material. Holotype: One adult (CUMZ 3426) in the banks of Mekong River at Ban Khonkhen, Champasak, Laos (15°02'21.2"N, 105°51'20.4"E), 106 meters elevation on 17 April 2014. **Paratypes:** 17 adults (CUMZ 3427), 2 adults (ZMH 14584), 2 adults (NHMUK), and 2 adults (RMBR), all specimens collected from type locality.

Figure 11. Photographs showing the **A** *Glyphidrilus champasakensis* sp. n. **B** type locality of *G. champasakensis* sp. n. in the banks of Mekong River at Ban Khonkhen, Champasak, Laos and **C** coloration of newly collected paratype (CUMZ 3427) after the first preservation step in 30% (v/v) ethanol.

Diagnosis. *Glyphidrilus champasakensis* sp. n. is a large sized earthworm with distinct expanded tissues of the clitellar wings on the sides of the body in XXIII, XXIV–½XXXII, XXXII, XXXIII. Clitellum in XIX, XX– XLIX, L, LI, LII. Female pores, male pores and spermathecal pores not visible. Genital markings: medium paired or asymmetrical on aa in XII, XIII, XXXIII, XXXIV; paired or asymmetrical on bc in XVII, XVIII, XIX–XXII, XXIII, XXIV and XXXII, XXXIII, XXXIV, XXXV. Four pairs of seminal vesicles in IX–XII. Intestinal origin in XVI. Large ovaries in XIII–XIV. Spermathecae between 14/15–19/20.

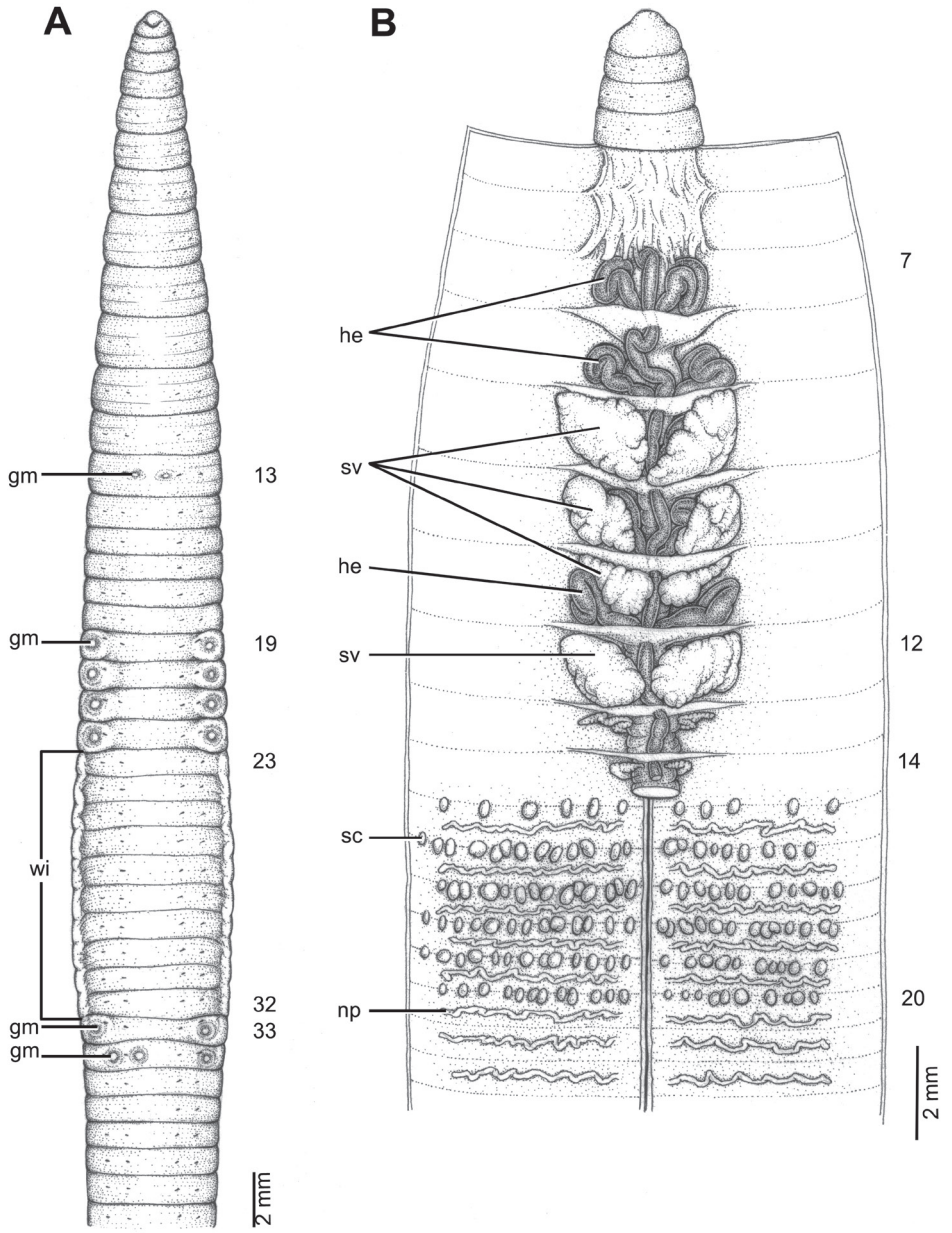


Figure 12. Morphology of holotype (CUMZ 3426) of *Glyphidrilus champasakensis* sp. n. **A** external ventral view, annular clitellum in XIX–XLIX **B** internal dorsal view.

Description of holotype. Dimensions: body length 301 mm, diameter 5.0 mm in segment VIII, 5.0 mm before the clitellar wing in segment XXII, 5.0 mm after wing in segment XXXIII in clitellar region; body cylindrical in anterior part, quadrangular

in transverse section behind clitellum. 424 segments. Body color pale brown with variations from red to pink at adjacent tissues of wing portion in different individuals of newly collected specimens. Clitellar wing on ventro-lateral part of clitellum in XXIII–XXXII, 10.0 mm in height, and 0.2 mm in width on both sides. Prostomium zygolobous. Dorsal pores absent. Clitellum annular in XIX– XLIX. Four pairs of setae per segment from II, setal formula aa:ab:bc:cd:dd = 2.0:1.0:2.0:1.0:3.0 in segment VIII and 2.0:1.0:2.0:1.0:2.5 in postclitellar segments. Female pores, male pores, and spermathecal pores not visible. Genital markings: medium paired or asymmetrical on aa in XIII, XXXIV, laterally paired or asymmetrical on bc in XIX–XXII and XXXIII, XXXIV.

Septa 4/5–8/9 thicker than between other segments others segment, 9/10–11/12 thick and 12/13 to the last segment thin. Gizzard small, globular in VIII. Intestine enlarged from XVI. Hearts, five pairs in VII–XI, first in VII and last in XI. A pair of holonephridia in each segment from segment XIV onwards. Seminal vesicles, four pairs in IX, X, XI, XII. Ovaries, two pairs in XIII and XIV. Testis free in X and XI. Prostate and accessory glands absent. Spermathecae sessile and diverticula absent, small elongated oval or globular between 14/15–19/20, about 0.1–0.3 mm in diameter, five to thirteen on each side per segment.

Variations. Body lengths of adult (n = 24) ranged from 167–301 mm (212.75 ± 42.15), with 248–424 segments. Wings in XXIII, XXIV–½XXXII, XXXII, XXXIII, clitellum in XIX, XX– XLIX, L, LI, LII. Genital markings: paired or asymmetrical on aa in XII, XIII, XXXIII, XXXIV; paired or asymmetrical on bc in XVII, XVIII, XIX–XXII, XXIII, XXIV and XXXII, XXXIII, XXXIV, XXXV.

Distribution. The new species was found only on the banks of the Mekong River at Ban Khonkhen, Champasak, Laos.

Etymology. The new species was named after Champasak, Laos, the type locality.

Remarks. Here we compare *Glyphidrilus champasakensis* sp. n. to the species with spermathecae and a clitellum beginning from segment XIX. It differs from *G. namdonensis* sp. n. by *G. namdonensis* sp. n. having shorter wings in XXIV, XXV–XXVIII, XXX, and spermathecae between 14/15–18/19 (see Table 1).

Discussion

Semi-aquatic earthworms of the genus *Glyphidrilus* are widely recorded on the Asian and African continents. Recently, many species have been described from several ecotone areas in Thailand between freshwater and terrestrial habitats of many river basins, where the soil pH varies from neutral to basic at 7–7.5 (Chanabun et al. 2013).

The seven new species from Thailand and Laos presented in this paper range in size, with respect to the other *Glyphidrilus* members, from large to small, with *G. champasakensis* sp. n. being the longest and *G. nanensis* sp. n. the smallest. The other five species are almost of the same size, as shown in Table 1. However, the locations of clitellum, wings, and spermathecae and other characters of the seven species show clear differences from the closely related species.

Glyphidrilus chiangraiensis sp. n. from Mekong River at Wat Hatkai, Chiangkhong, Chiangrai, north Thailand, was found in many areas of the Mekong River and its tributaries from Thailand and Laos, at elevations from 101–385 meters, and co-existing with *G. mekongensis* Panha & Chanabun, 2012, *Amynthas mekongianus* (Cognetti, 1922), *Amynthas* sp., and *Metaphire* sp. The earthworms and cocoons were found in the soil when digging (Fig. 13) and this is probably the cause of the wide distribution, since cocoons travelled from the high to low elevations during the rainy season. *Glyphidrilus nanensis* sp. n. was found in the rice fields of a tributary of the Nan River, in north Thailand. It was found co-existing with the common terrestrial earthworm *Drawida beddardi* (Rosa, 1890). *Glyphidrilus nanensis* sp. n. is probably an isolated endemic occurring in the Nan River valley. This earthworm was found at soil depths of 5–10 cm in agricultural land. This demonstrates that the species is compatible with at least organic farming and that conservation of the species is not difficult, which is increasingly important in the world. Jouquet et al. (2008b) showed the casts produced by *Glyphidrilus* sp. can be considered as patches of nutrients in paddy fields. Owa et al. (2003) also observed rice development and greater productivity when earthworm casts were associated to rice plants. Choosai et al. (2010) observed rice development in Northeast Thailand and confirmed the positive effect of earthworms on soil properties, rice yield was higher when presence of casts. This strongly suggests that any agricultural system promoting earthworm development, thereby increasing the number of casts per rice field, could be considered as an useful approach for the sustainable management of paddy fields.

Glyphidrilus satunensis sp. n. was found at a pond, part of a wildlife sanctuary at Satun, south Thailand. It has a similar body size with another species described from nearby in the Malay Peninsula (Chanabun et al. 2013); however, the locations of clitellum, wings, and spermathecae show clear differences between the closely related species.

The four new species described from Laos, *G. namphao* sp. n., *G. sekongensis* sp. n., *G. namdonensis* sp. n., and *G. champasakensis* sp. n. live in different habitats. *Glyphidrilus namphao* sp. n. lives in Phao River the river near to the Namphao border between Laos and Vietnam at a higher elevation (525 meters) than the other three new species. *Glyphidrilus sekongensis* sp. n. lives in a stream located within an evergreen and mossy forest at Ban Kiangkong, Lamarm, Sekong; the forest is covers 70% of the area near the stream. The soils are slightly sandy and black with the accumulation of organic matter. *Glyphidrilus namdonensis* sp. n. lives in the sandy banks of the Don River, which is one of the branches of Mekong River at Thathek, Khammouan, Laos. *Glyphidrilus champasakensis* sp. n. is one of the new species along the Mekong River found at a soil depth of 20–40 cm, and has similar characteristics to two other new species (*G. sekongensis* sp. n. and *G. namdonensis* sp. n.).

The following key includes only the species most closely related to those described here. The basis for including the species covered in the key is the same as the basis for including previously described species for comparison in the Remarks sections. The remaining species of *Glyphidrilus* must be identified with other resources.

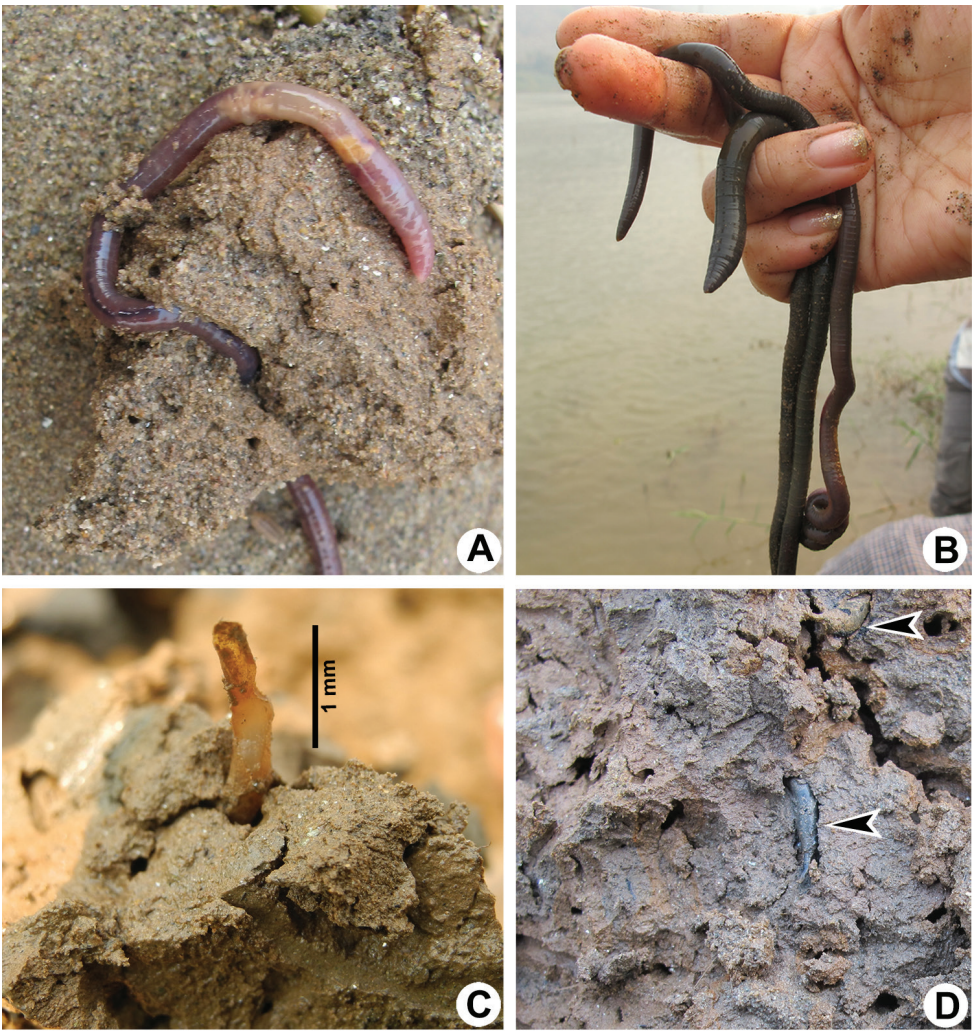


Figure 13. Photographs showing **A** *Metaphire* sp. **B** *Amynthas mekongianus* (Cognetti, 1922) **C** and **D** Cocoons of *Glyphidrilus* in the soil.

Key to species of the newly described species of *Glyphidrilus*, and those most similar to them

1 Spermathecae absent **2**
– Spermathecae present **5**
2 Wings in XXII–XXXII, and clitellum in XVIII–XXXVIII ..... ***G. yunnanensis* Chen & Xu, 1977**
– Wings from XXIV or XXV **3**

3 Clitellum in XII, XIII, XVI–XXXII, XXXIII, wings in XXV, XXVI–XXX, XXXI ..... ***G. huailuangensis* Chanabun & Panha, 2013**

– Clitellum from XIX or XX **4**

4 Wings in XXIV–½XXXIII, XXXIII, XXXIV, ½XXXV, clitellum in XIX–XXXVII, XXXVIII, and intestine beginning from XV ..... ***G. mekongensis* Panha & Chanabun, 2012**

– Wings in XXIV, XXV–XXXI, XXXII, clitellum in XIX, XX–XXXV, XXXVI, XXXVII and intestine beginning from XVI ..... ***G. vangviengensis* Chanabun & Panha, 2011**

5 Heart in VII–XI **6**

– Heart in VIII–XI **15**

6 Wings in XVIII–XXIV, clitellum in XVII–XXVI, XXIX, and spermathecae between 13/14–16/17 ..... ***G. namphao* sp. n.**

– Wings beginning from XX **7**

7 Gizzard beginning from 7/8 ..... **8**

– Gizzard beginning in segment VIII **10**

8 Wings in XXI, XXII–XXVII, XXVIII, XXIX, clitellum in XIV, XVI, XVII–XXXI, XXXII, XXXIII, XXXIV, XXXV, XXXVI and spermathecae between 14/15–18/19 ..... ***G. borealis* Chanabun & Panha, 2013**

– Wings beginning from XXIV or XXV or XXVI **9**

9 Wings in XXIV, XXV, XXVI–XXXI, XXXII, clitellum in XII, XIII, XIV, XV, XVI–XL, XLI, XLII and spermathecae between 12/13–18/19 ..... ***G. vangthongensis* Chanabun & Panha, 2013**

– Wings in XXIV, XXV, XXVI–XXVII, XXVIII, XXIX, clitellum in XVII, XVIII–XXXIII, XXXIV and spermathecae between 13/14–17/18 ..... ***G. nanensis* sp. n.**

10 Intestine beginning from XV **11**

– Intestine beginning from XVI **13**

11 Spermathecae between 12/13 ..... **12**

– Spermathecae between 16/17–22/23, wings in XXIV, XXV–XXXII, XXXIII and clitellum in XX–XLIII, XLIV, XLV ..... ***G. chaophraya* Chanabun & Panha, 2013**

12 Wings in XXIII, XXIV, XXV, XXVI–XXIX, XXX, XXXI, XXXII, clitellum in XVII, XVIII–XXXIII, XXXIV, XXXV, XXXVI, XXXVII, XXXVIII and spermathecae between 12/13–18/19 ..... ***G. chiensis* Chanabun & Panha, 2013**

– Wings in XXIII, XXIV–XXVIII, XXIX, XXX, XXXI, clitellum in XV, XVI, XVII, XVIII–XXXI, XXXII, XXXIII, XXXIV, XXXV, XXXVI and spermathecae between 12/13–17/18 ..... ***G. quadratus* Chanabun & Panha, 2013**

13 Spermathecae begin from 14/15 ..... **14**

– Spermathecae between 15/16–20/21, wings in XXIII, XXIV–XXVI, ½XXVII, XXVII, ½XXVIII, XXVIII and clitellum in XVII, XVIII, XIX, XX, XXI–XXXVI, XXXVII, XXXVIII, XXXIX ..... ***G. chiangraiensis* sp. n.**

14 Wings in XXIV, XXV–XXVIII, XXX, clitellum in XIX, XX–XXXVI, XXXVII, XXXVIII and spermathecae between 14/15–18/19..... ***G. namdonensis* sp. n.**

– Wings in XXIII, XXIV–½XXXII, XXXII, XXXIII, clitellum in XIX, XX– XLIX, L, LI, LII and spermathecae between 14/15–19/20 ..... ***G. champasakensis* sp. n.**

15 Intestine beginning from XIV **16**

– Intestine beginning from XVI **17**

16 Wings in XX, XXI–XXVI, XXVII, clitellum in XI, XII, XIII–XXXIII, XXXIV, XXXV and spermathecae between 13/14–17/18..... ..... ***G. wararamensis* Chanabun & Panha, 2013**

– Wings in XXIII, XXIV–XXVIII, XXIX, XXX, clitellum in XVIII–XXX, XXXI, XXXII and spermathecae between 14/15–17/18..... ..... ***G. kratuensis* Chanabun & Panha, 2013**

17 Spermathecae intrasegmental in XVIII–XXI, wings in XXII, XXIII–XXVII, XXVIII and clitellum in XVII, XVIII–XXX..... ..... ***G. trangensis* Chanabun & Panha, 2013**

– Spermathecae intersegmental in 12/13 or 13/14 ..... **18**

18 Wings in XXIV, XXV, XXVI–XXIX, XXX, XXXI, clitellum in XVII, XVIII–XXXII, XXXIII, XXXIV, XXXV and spermathecae between 13/14–15/16... ..... ***G. satunensis* sp. n.**

– Wings in XXV–XXXI, clitellum in XVI, XVII–XXXVI, XXXVII and spermathecae between 12/13–15/16 ..... ***G. sekongensis* sp. n.**

Acknowledgements

This project was partly funded by Chulalongkorn University Graduate School Postdoctoral Project to RC, while most of the financial support was received from The Thailand Research Fund, The TRF Senior Research Scholar RTA 5880002 (2015–2018) to SP. This research was also partially funded by a grant from WCU-058-016-FW (Food and Water Research Cluster, Chulalongkorn University), Higher Education Research Promotion (HERP; Project Code 2559A15562001). We are grateful to Professor Peter K.L. Ng, Dr. Daren Yeo and Dr. Kelvin Tan (ZRC, Singapore), and to Ms. Emma Sherlock (NHMUK, London) and Dr. Andreas Schmidt-Rhaesa (ZMH, Germany) for kindly permitting us to study the type specimens and relevant reference material. Thanks also to Ms. Thita Krutchuen for excellent drawings, and to all members of the Animal Systematics Research Unit, Chulalongkorn University for assistance in collecting material.

References

Chanabun R, Bantaowong U, Sutcharit C, Tongkerd P, Inkavilay K, James SW, Panha S (2011) A new species of semi-aquatic freshwater earthworm of the genus *Glyphidrilus* Horst, 1889 from Laos (Oligochaeta: Almidae). Tropical Natural History 11: 213–222.

Chanabun R, Bantaowong U, Sutcharit C, Tongkerd P, James SW, Panha S (2012a) A new species of semi-aquatic freshwater earthworms of the genus *Glyphidrilus* Horst, 1889 from the Mekong River (Oligochaeta: Almidae). Raffles Bulletin of Zoology 60(2): 265–277.

Chanabun R, Sutcharit C, Tongkerd P, Shau-Hwai AT, Panha S (2012b) Three new species of semi-aquatic freshwater earthworms of the genus *Glyphidrilus* Horst, 1889 from Malaysia (Clitellata: Oligochaeta: Almidae). Zootaxa 3458: 120–132.

Chanabun R, Sutcharit C, Tongkerd P, Panha S (2013) The semi-aquatic freshwater earthworms of the genus *Glyphidrilus* Horst, 1889 from Thailand (Oligochaeta: Almidae) with re-descriptions of several species. ZooKeys 265: 1–76. https://doi.org/10.3897/zookeys.265.3911

Chanabun R, Panha S (2015) Two New Species of Semi-aquatic Earthworms Genus *Glyphidrilus* Horst, 1889 from Malaysia (Oligochaeta: Almidae). Tropical Natural History 15(2): 179–189.

Choosai C, Jouquet P, Hanboonsong Y, Hartmann C (2010) Effects of earthworms on soil properties and rice production in the rained paddy fields of Northeast Thailand. Applied Soil Ecology 45: 298–303. https://doi.org/10.1016/j.apsoil.2010.05.006

Gates GE (1972) Burmese earthworms. An introduction to the systematics and biology of megadrile oligochaetes with special reference to the Southeast Asia. Transactions of the American Philosophical Society 62: 1–326. https://doi.org/10.2307/1006214

Horst R (1889) Over eene nieuwe soort order de Lumbricinen door Prof. Max Weber uit nedenl. India medegebracht. Tijdschrift der Nederlandsche Dierkundige Vereeniging 2: 77.

Horst R (1893) Earthworms from Malay Archipelago. Zoologische Ergebnisse einer Reise in Niederlandisch Ost Indien 3: 28–83.

Jamieson BGM (1968) A new species of *Glyphidrilus* (Microchaetidae: Oligochaeta) from East Africa. Journal of Natural History 2: 387–395. https://doi.org/10.1080/00222936800770381

Jirapatrasilp P, Prasankok P, Chanabun R, Panha S (2015) Allozyme data reveal genetic diversity and isolation by distance in sympatric *Glyphidrilus* Horst, 1889 (Oligochaeta: Almidae) of the Lower Mekong River Basin. Biochemical Systematics and Ecology 61: 35–43. https://doi.org/10.1016/j.bse.2015.05.003

Jirapatrasilp P, Prasankok P, Sutcharit C, Chanabun R, Panha S (2016) Two new Cambodian semi-aquatic earthworms in the genus *Glyphidrilus* Horst, 1889 (Oligochaeta, Almidae), based on morphological and molecular data. Zootaxa 4189(3): 543–558. https://doi.org/10.11646/zootaxa.4189.3.5

Jouquet P, Hartmann C, Choosai C, Hanboonsong Y, Bruner D, Montoroi JP (2008b) Different of earthworms and ants on soil properties of paddy fields in North-East Thailand. Paddy and Water Environment 6: 381–386. https://doi.org/10.1007/s10333-008-0134-x

Michaelsen W (1896) Oligochaeten. Ergebnisse einer zoologishen Forschungsreise in den Molukken und in Borneo, im Auftrage der Senckenbergischen naturforschenden Gesellschaft ausgeführt von Dr. Willy Kükenthal. Abhandlungen Senckenbergischen Naturforschenden Gesellschaft 23: 193–243.

Michaelsen W (1897) Neue und wenig bekannte afrikanische Terricolen. Mittheilungeb aus dem Naturhistorischen Museum 14: 1–71.

Michaelsen W (1900) Oligochaeta. Das Tierreich 10: 1–575.

Michaelsen W (1902) Neue Oligochaeten und neue Fundorte alt-bekannter. Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten 19: 1–54.

Michaelsen W (1910) Die Oligochätenfauna der vonderindisch-ceylonischen Region. Abhandulngen aus dem Gebiete der Naturwissenschaften 19: 1–108.

Michaelsen W (1918) Die Lumbriciden, mit besonderer Berücksichtigung der bisher als Familie Glossoscolecidae zusammenfassten Unterfamilien. Zoologische Jahrbücher, Abteilung für Systematik 41: 1–398.

Michaelsen W (1922) Oligochäten aus dem Rijks Museum van Natuurlijke Historie zu Leiden. Capita Zoologica 1: 1–68.

Owa SO, Oyenusi AA, Joda AO, Morafa SOA, Yeye JA (2003) Effect of earthworm casting on growth parameters of rice. African Zoology 38: 229–233.

Rao N (1922) Some new species of earthworms belonging to the genus *Glyphidrilus*. Annals and Magazine of Natural History 9: 51–68. https://doi.org/10.1080/00222932208632640

Shen HP, Yeo DCJ (2005) Terrestrial earthworms (Oligochaeta) from Singapore. The Raffles Bulletin of Zoology 53: 13–33.

ZooKeys 672: 35–48 (2017)
doi: 10.3897/zookeys.672.12488
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

First Canadian records of genera *Apimela* Mulsant & Rey and *Gyronycha* Casey from New Brunswick: description of two new species and new provincial distribution records (Coleoptera, Staphylinidae, Aleocharinae)

Jan Klimaszewski[1], Reginald P. Webster[2], Adriano Zanetti[3], Caroline Bourdon[1]

1 *Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 du P.E.P.S., P.O. Box 10380, Stn. Sainte-Foy, Quebec, Quebec, Canada G1V 4C7* **2** *24 Mill Stream Drive, Charters Settlement, NB, Canada E3C 1X1* **3** *Museo Civico di Storia Naturale, Lungadige Porta Vittoria 9, I-37129 Verona, Italy*

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

Academic editor: *A. Brunke* | Received 1 March 2017 | Accepted 14 April 2017 | Published 3 May 2017

http://zoobank.org/CDCE50DB-E70B-4F08-932E-B0001AFB43E5

Citation: Klimaszewski J, Webster RP, Zanetti A, Bourdon C (2017) First Canadian records of genera *Apimela* Mulsant & Rey and *Gyronycha* Casey from New Brunswick: description of two new species and new provincial distribution records (Coleoptera: Staphylinidae: Aleocharinae). ZooKeys 672: 35–48. https://doi.org/10.3897/zookeys.672.12488

Abstract

Two genera, *Apimela* Mulsant & Rey and *Gyronycha* Casey (both Aleocharinae: Oxypodini: Meoticina), are recorded from New Brunswick and Canada for the first time. The following species are newly recorded or described as new in New Brunswick and Canada: *Apimela fusciceps* (Casey); *A. canadensis* Klimaszewski & Webster, **sp. n.**; and *Gyronycha pseudoobscura* Klimaszewski & Webster, **sp. n.** The genera are defined and the key for species identification is provided. Color habitus images and black and white images of the median lobe of the aedeagus, spermatheca, tergite, and sternite VIII are provided for all species occurring in Canada, and *Apimela macella* (Erichson), the type species of genus *Apimela*, and *G. valens* Casey, the type species of *Gyronycha*. New or additional habitat data are provided for the species treated in this contribution. The following new synonym is established: *Gyronycha lepida* Casey, 1911 (NC), is a synonym of *G. fusciceps* Casey, 1894 (NC).

Keywords

Coleoptera, Staphylinidae, Aleocharinae, *Apimela*, *Gyronycha*, taxonomy, new species, Canada

Copyright *Her Majesty the Queen in Right of Canada.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

The genus *Gyronycha* was described by Casey (1894) to accommodate his seven new species distributed in the USA (CA, NC, NJ, NY, NV, TX). Later, Casey (1911) added two *Gyronycha* species from North Carolina and New York. Casey (1885) described *Calodera attenuata* from California, which was transferred by Seevers (1978) to *Apimela* Mulsant & Rey. The species of *Gyronycha* described by Casey represent a mixed group and most of them belong to the genus *Apimela*, for details see the checklist of *Apimela* and *Gyronycha* further in the text. Moore and Legner (1975) reported three valid Nearctic species of *Apimela*: *A. attenuata* (Casey, 1885) [with *G. lineata* Casey, 1894, as its synonym], *A. fenyesi* (Bernhauer, 1906), and *A. longipennis* (Casey, 1911), all from California. Seevers (1978) redefined the two genera and distinguished *Apimela* from *Gyronycha* by smaller and more slender body, transverse pronotum [both genera have distinctly or slightly transverse pronotum], transverse antennomeres IV-X, presence of a tubercle arising from the margin of first and fifth visible male tergites, and the distinctive form of the spermatheca (Fig. 25E, spermatheca of *A. attenuata* (Casey) in Seevers 1978). Seevers (1978) designated *G. valens* Casey as a type species of *Gyronycha*, because Casey (1894) did not designate specifically one species as the types of the genus. Seevers (1978) synonymized genus *Gyronychina* Casey, 1911, described from one California species (*G. longipennis* Casey), with the genus *Apimela*. The generic type of *Apimela* is the Palaearctic species *A. macella* (Erichson, 1839) [Figs 27–29; male median lobe of aedeagus in lateral view, illustrated by Seevers 1978, Fig. 5f, g]. We have modified Seevers's (1978) diagnosis in separating *Apimela* from *Gyronycha* when making generic assignment of the new species discovered in New Brunswick (NB). Our modified diagnoses of the two genera are included in the key to species. For all Nearctic species of *Apimela* and *Gyronycha* see below the checklist in this paper.

Materials and methods

All specimens in this study were dissected to examine the genital structures. Extracted genital structures were dehydrated in absolute alcohol, mounted in Canada balsam on celluloid micro-slides, and pinned with the specimen from which they originated. Images of the entire body and the genital structures were taken using an image processing system (Nikon SMZ 1500 stereoscopic microscope; Nikon Digital Camera DXM 1200F, and Adobe Photoshop software).

Morphological terminology mainly follows that used by Seevers (1978). The ventral side of the median lobe of the aedeagus is considered to be the side of the bulbus containing the foramen mediale, the entrance of the ductus ejaculatorius, and the adjacent ventral side of the tubus of the median lobe with the internal sac and its structures (this part is referred to as the parameral side in some recent publications); the opposite side is referred to as the dorsal part. In the species descriptions, microsculpture refers to the surface of the upper forebody (head, pronotum and elytra).

Species within genera are arranged alphabetically in the text and in the table.

Depository/institutional abbreviations

CNC Canadian National Collection of Insects, Arachnids, and Nematodes, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada.
FMNH Field Museum of Natural History, Chicago, USA.
LFC Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, R. Martineau Insectarium, Quebec City, Quebec, Canada.
RWC R. Webster collection, Fredercton, New Brunswick, Canada.
USNM United States National Museum, Washington, D.C, USA.

USA state abbreviations follow those of the US Postal Service.

Discussion. We have discovered that the spermathecal capsule in *Apimela* and *Gyronycha* has an apical or apico-lateral, narrow, tubular projection, which may be indicative of close phylogenetic relationship between both genera. Known males of *Gyronycha* species, have carniform tubercules on the first and fifth visible tergites (Figs 16, 21, 22), and females are lacking these structures. These tubercles are absent in *Apimela*. Seevers (1978) pointed out that *Apimela* and *Meotica* Mulsant & Rey are similar in having small, slender and compressed body but considered *Apimela* to be closely related to *Gyronycha* due to elongate elytra and mesoventrite, and the distinctive form of spermatheca. Externally, species of *Apimela* are very similar to those of *Alisalia* Casey, which live in very similar habitats, but the latter have shorter elytra and mesoventrite and have different type of genitalia (Klimaszewski et al. 2009). Casey (1894) considered *Gyronycha* as allied to Central American *Bamona* Sharp but his hypothesis needs further studies to be confirmed.

Key to Canadian species of *Apimela* and *Gyronycha*

1 Antennomeres VII-X slightly to strongly transverse (Figs 1, 9, 27); tarsal claws small (Figs 1, 9); males without tubercles on first and fifth visible tergites; spermatheca with sinuate stem, coils partial and not overlapping (Figs 8, 15, 29) (*Apimela*) **2**

– Antennomeres VII-X moderately to strongly elongate (Figs 16, 23); tarsal claws large (Fig. 16); males with strong tubercles on first and fifth visible tergites (Figs 21, 22); spermatheca with broadly and irregularly coiled stem, coils overlapping (Fig. 26) (*Gyronycha*) ..... ***Gyronycha pseudoobscura* Klimaszewski & Webster, sp. n.** [male unknown]

2 Antennomeres VI-X strongly transverse (Fig. 9); elytra slightly broader than maximum width of pronotum (Fig. 9); eyes moderately large (Fig. 9); spermatheca with spherical apical part of capsule bearing elongate and multiply micro-coiled projection (Fig. 15)..... ***Apimela canadensis* Klimaszewski & Webster, sp. n.**

– Antennomeres VI-X slightly transverse (Fig. 1); elytra distinctly broader than maximum width of pronotum (Fig. 1); eyes large (Fig. 1); spermatheca with tubular apical part of capsule and with short and few microcoiled projection *Gyronycha* (Fig. 8) ..... ***Apimela fusciceps* (Casey)**

Taxonomy

Apimela Mulsant & Rey, 1874

Figs 1–15, 27–29

Type species. *Homalota macella* Erichson, 1839

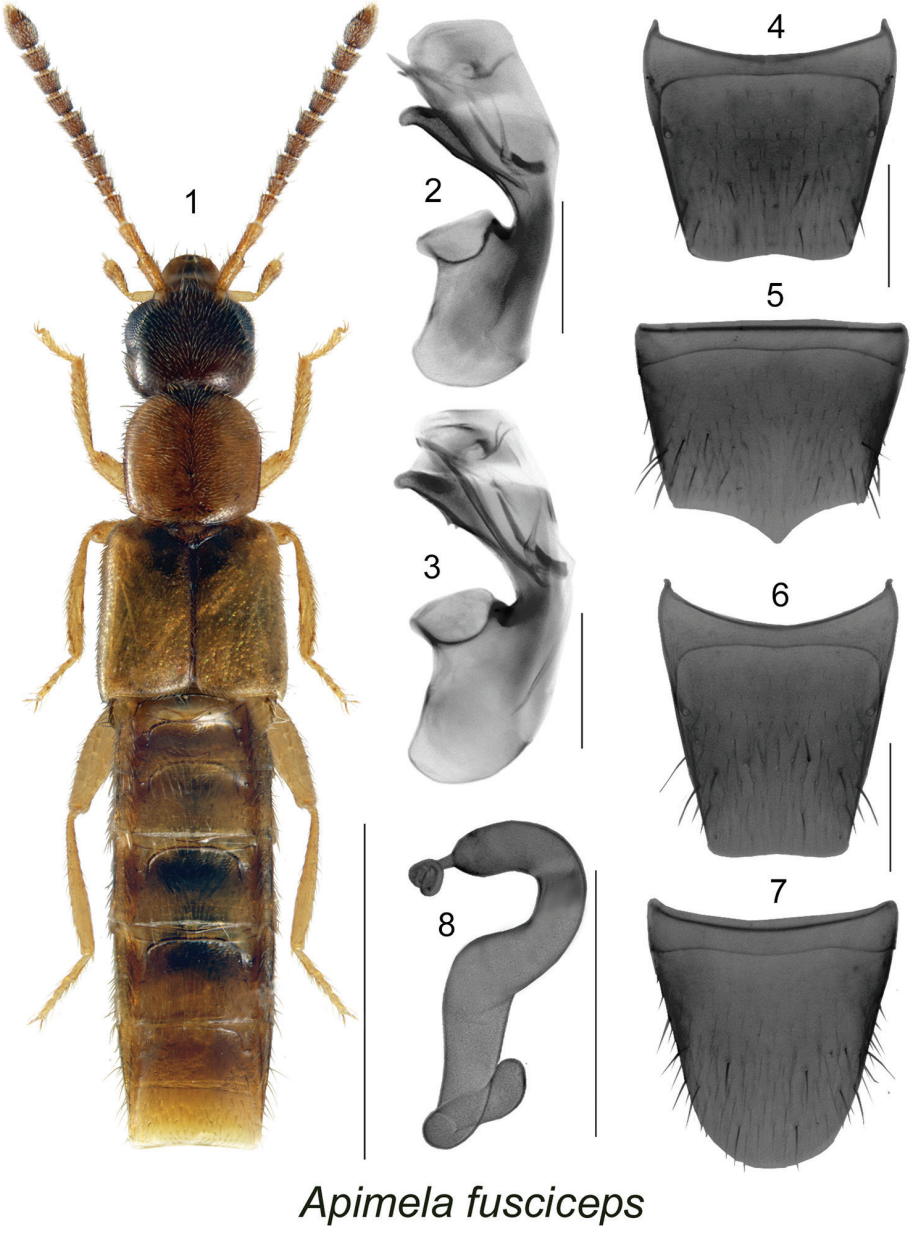
Diagnosis. Body yellowish brown, narrow and linear, length 2.0-3.0 mm; forebody densely and finely pubescent; head subquadrate as large as or slightly larger than pronotum, eyes moderately large, usually shorter than postocular area of head and visible from above, posterior angles of head angular, basal carina vestigial and visible only basally; antennomeres V-X slightly to strongly transverse; last palpomere needle-shaped; pronotum slightly transverse, widest in apical third, as long as head, densely pubescent, pubescence on midline of disc directed anteriad except posteriad basally, on sides anteriad and laterad, forming arcuate lines; elytra strongly elongate, one six/seventh broader than pronotum, at suture longer than pronotum, pubescence directed obliquely postriad; mesoventrite long, mesocoxae close; abdomen parallel sided, first four visible tergites with deep arcuate impressions, males without tubercles on first and fifth visible tergites; basal metatarsus as long as two following combined, tarsi small; median lobe of aedeagus with sinuate venter of tubus in lateral view, crista apicalis of bulbus from moderately-sized to large, internal sac with complex sclerites; spermatheca with sinuate stem, coils partial and not overlapping. Species of this genus occur in riparian habitats.

Apimela fusciceps (Casey), comb. n.

Figs 1–8

Gyronycha fusciceps Casey, 1894: 376. **Lectotype** (female). USA: N.Y. [New York]; *fusciceps* Casey; TYPE USNM 38789; Casey bequest 1925; our lectotype designation label (USNM). There is an unpublished Gusarov's lectotype designation label. The genitalia were probably treated by KOH as they are barely recognizable and the spermatheca is missing. Lectotype - present designation. PARALECTOTYPE: USA: N.Y. [New York]; *fusciceps* Casey; TYPE USNM 38789, *fusciceps*-2; Casey bequest 1925; our paralectotype designation label (USNM) 1 male. There is an unpublished Gusarov's paralectotype label. The genitalia were probably treated by KOH as they are barely recognizable.

Gyronycha lepida Casey, 1911: 217, syn. n. **Lectotype** (male). USA: N.C. [North Carolina]; *lepida* Casey; TYPE USNM 38790; Casey bequest 1925; our lectotype designation label (USNM). There is an unpublished Gusarov's lectotype designation label. Lectotype - present designation. PARALECTOTYPES: N.C. [North Carolina]; *lepida* Casey; TYPE USNM 38790, *lepida*-2; Casey bequest 1925; our paralectotype designation label (USNM) 1 female [there is an unpublished Gusarov's paralectotype designation label]; N.C. [North Carolina]; TYPE USNM 38790, *lepida*-3 Casey; Casey bequest 1925; our paralectotype designation label (USNM)1 female [there is an unpublished Gusarov's paralectotype designation label].



Figures 1–8. *Apimela fusciceps* (Casey): **1** habitus in dorsal view **2–3** median lobe of aedeagus in lateral view **4** male tergite VIII **5** male sternite VIII **8** spermatheca **6** female tergite VIII **7** female sternite VIII. Scale bars: 1 mm for habitus; 0.2 mm for remaining structures.

Material examined. Canada, New Brunswick, Carleton Co., Belleville, Meduxnekeag Valley Nature Preserve, 46.1944°N, 67.6832°W, 2.VI.2008, R.P. Webster, coll.// River margin, under cobblestones in sand/gravel, among scattered grasses (RWC, LFC) 2 females; Belleville, Meduxnekeag Valley Nature Preserve, 46.1942°N, 67.6832°W, 9.VI.2008,

R.P. Webster, coll. // River margin, under cobblestones among grasses away from water's edge (RWC) 2 females; Belleville, Meduxnekeag Valley Nature Preserve, 46.1921°N, 67.6815°W, 11.VI.2010, R.P. Webster, coll. // River margin, under cobblestones set in sand (RWC) 1 female; Belleville, Meduxnekeag Valley Nature Preserve, 46.1941°N, 67.6830°W, 31.V.2013, R.P. Webster, coll. // River margin, under small rock (RWC) 1 male.

Diagnosis. Body length 3.0–3.4 mm, subparallel, yellowish brown, head and scutellar region of elytra dark brown, strongly glossy, forebody with fine and moderately dense pubescence, punctation fine; head subquadrate, eyes large and about as long as postocular region of head, posterior angles rounded, pubescence directed straight and obliquely anteriad; antennomeres V-X slightly to strongly transverse, head broader than pronotum; pronotum slightly transverse, posterior angles angular; elytra elongate, at suture longer than pronotum, and about one fourth wider than pronotum, abdomen subparallel, with first visible four tergites deeply impressed basally, males lacking tubercles on first and fifth visible tergites. MALE. Median lobe of aedeagus with tubus strongly produced ventrally, in lateral view its venter sinuate with two more or less visible minute teeth in apical third, internal sac with complex structures as illustrated (Figs 2 [NB], 3 [holotype]); tergite VIII truncate apically (Fig. 4); sternite VIII produced apically and sharply pointed (Fig. 5). FEMALE. Spermatheca S-shaped, capsule tubular, slightly arched and with apical narrow, tubular projection coiled apically, stem sinuate and twisted (Fig. 8); tergite VIII truncate apically (Fig. 6); sternite VIII rounded apically (Fig. 7).

Distribution. Formerly known from New York and North Carolina in the United States (Casey 1894, 1911). Here, reported in New Brunswick, Canada, for the first time.

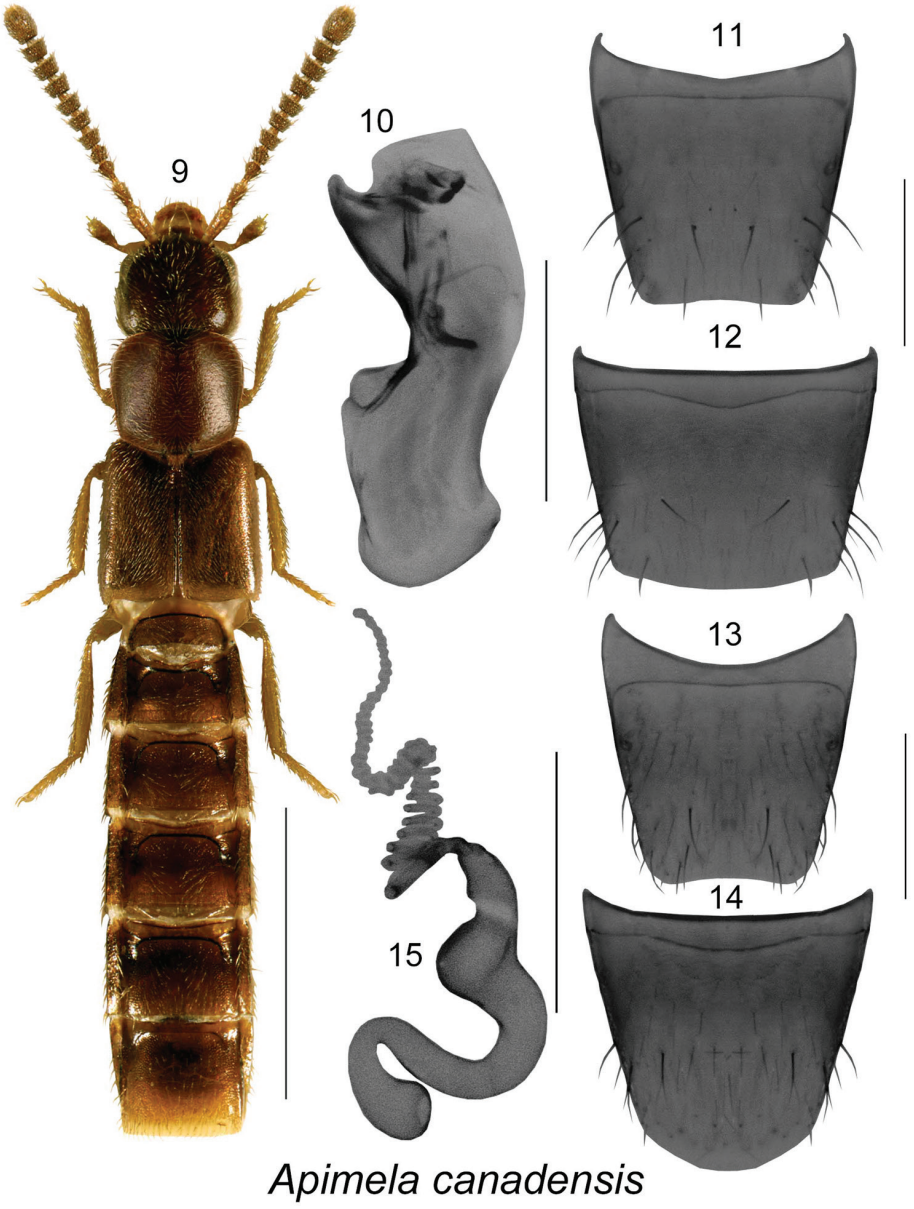
Collection and habitat data. In New Brunswick, this species was found along a river margin under cobblestones set in sand/gravel, often in areas with scattered grasses, sometimes away from water's edge. Adults were collected in late May and June.

Comments. This species belongs to a distinct species group and has spermatheca type similar to that of *A. attenuata* (Casey).

Apimela canadensis Klimaszewski & Webster, sp. n.

http://zoobank.org/2CAD6FD2-6B89-45A8-93E1-4DE94B359B60
Figs 9–15

Holotype (male). CANADA, New Brunswick, Restigouche Co., Jacquet River Gorge PNA, 47.8257°N, 66.0779°W, 14.V.2010, R.P. Webster, coll. // Partially shaded cobblestone bar near outflow of brook at Jacquet River, under cobblestones and gravel on sand (LFC). PARATYPES: New Brunswick, Restigouche Co., Jacquet River Gorge PNA, 47.8257°N, 66.0779°W, 14.V.2010, R.P. Webster coll. // Partially shaded cobblestone bar near outflow of brook at Jacquet River, under cobblestones & gravel on



Figures 9–15. *Apimela canadensis* Klimaszewski & Webster, sp. n.: **9** habitus in dorsal view **10** median lobe of aedeagus in lateral view; 11, male tergite VIII **12** male sternite VIII **13** female tergite VIII **14** female sternite VIII **15** spermatheca. Scale bars: 1 mm for habitus; 0.2 mm for remaining structures.

sand (RWC) 2 males, 1 female; Carleton Co., Belleville, Meduxnekeag Valley Nature Preserve, 46.1942°N, 67.6832°W, 9.VI.2008, R.P. Webster coll. // River margin, under cobblestones among grasses away from water's edge (RWC) 1 female.

Etymology. Named after Canada, the country of origin, and to commemorate the 150th anniversary of Canada.

Description. Body length 2.0–3.0 mm, subparallel, yellowish brown with slightly darker head, moderately glossy, forebody with fine and dense pubescence, punctation fine; head subquadrate, eyes moderately large and shorter than postocular region of head, posterior angles rounded and slightly angular, pubescence directed straight and obliquely anteriad; antennomeres V-X strongly transverse, head slightly broader than pronotum; pronotum slightly transverse, posterior angles angular; elytra elongate, at suture longer than pronotum, and about one sixth wider than pronotum, abdomen subparallel, with first four visible tergites deeply impressed basally, males lacking tubercles on first and fifth visible tergites. MALE. Median lobe of aedeagus with tubus strongly produced ventrally, its venter sinuate with apex turned slightly upward, internal sac with complex structures as illustrated (Fig. 10); tergite VIII truncate apically (Fig. 11); sternite VIII truncate and broadly arcuate apically (Fig. 12). FEMALE. Spermatheca S-shaped, capsule spherical, slightly arched with a narrow apical tubular multiple micro-coiled projection, stem sinuate, S-shaped (Fig. 15); tergite VIII truncate apically (Fig. 13); sternite VIII rounded apically (Fig. 14).

Distribution. Known only from New Brunswick, Canada.

Collection and habitat data. The holotype and three paratypes were captured on a partially shaded cobblestone bar near the outflow of brook along the Jacquet River. The adults were found under cobblestones and gravel in sand. One paratype was found along a river margin under a cobblestone among grasses away from the water's edge. Adults were collected in May and June.

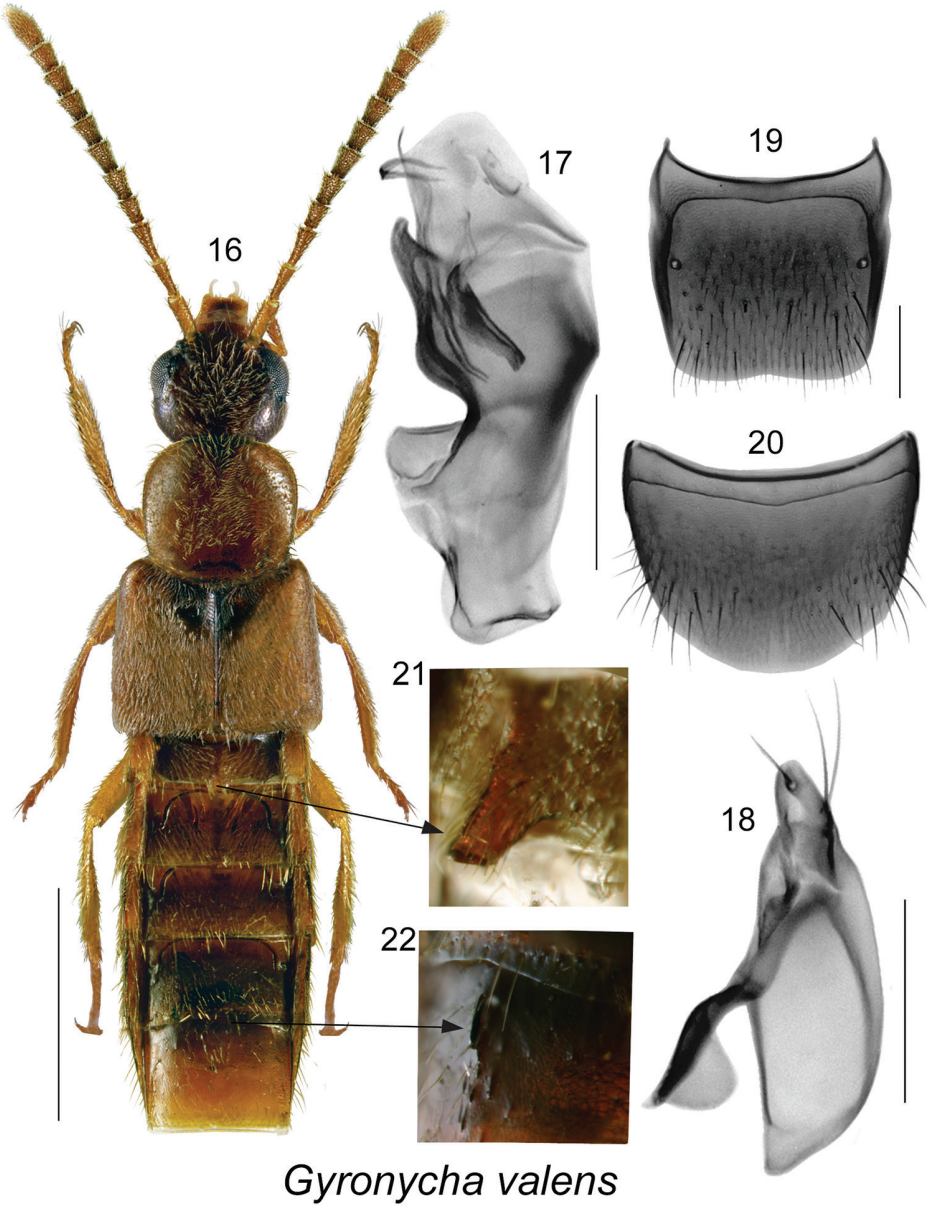
Comments. This species clearly belongs to a different species group than *A. fusciceps*, which has capsule of spermatheca entirely tubular.

Gyronycha Casey, 1894

Figs 16–26

Type species. *Gyronycha valens* Casey, 1894.

Diagnosis. Body yellowish brown, narrow and linear, length 2.5–4.2 mm; forebody densely and finely pubescent; head round, as large as pronotum, eyes large, about as long as postocular area of head visible from above, posterior angles of head rounded, basal carina vestigial and visible only basally; antennomeres V-X slightly to strongly elongate; last palpomere needle-shaped; pronotum slightly transverse, widest in apical third, as long as head, densely pubescent, pubescence on midline of disc directed anteriad except posteriad basally, on sides anteriad and laterad, forming arcuate lines; elytra strongly elongate, one fifth broader than pronotum, at suture longer than pronotum, pubescence directed obliquely postriad; mesoventrite long, mesocoxae close; abdomen parallelsided, first four visible tergites with deep arcuate impressions, males with tubercles on first and fifth visible tergites; basal metatarsus as long as the following two combined, tarsi large; median lobe of aedeagus with strongly sinuate venter of tubus in lateral view, crista apicalis of bulbus moderately



Figures 16–22. *Gyronycha valens* Casey (type species of the genus): **16** habitus in dorsal view **17** median lobe of aedeagus in lateral view **18** Paramere **19** male tergite VIII **20** male sternite VIII **21** dorsal projection on first visible male tergite; and **22** on fourth visible male tergite. Scale bars: 1 mm for habitus; 0.2 mm for remaining structures.

large, internal sac with complex sclerites; spermatheca with broadly and irregularly coiled stem, coils overlapping. New Brunswick specimens of this genus were found in gravel in a riparian habitat.

***Gyronycha pseudoobscura* Klimaszewski & Webster, sp. n.**
http://zoobank.org/484B63FF-BE0A-4BBF-A0E1-8E71F0D71C49
Figs 23–26

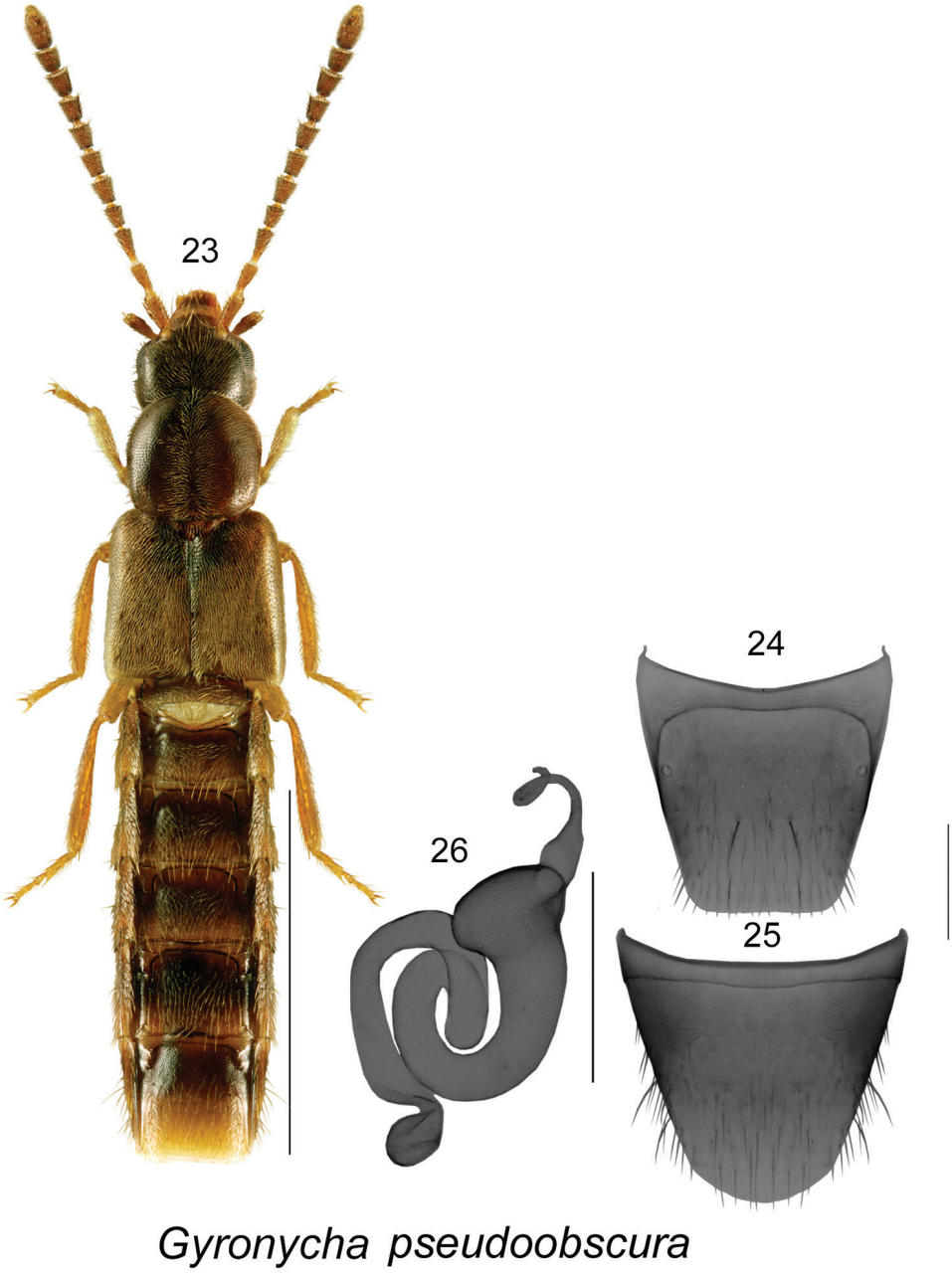
Holotype (female). CANADA, New Brunswick, Restigouche Co., Jacquet River Gorge PNA, 47.8257°N, 66.0779°W, 24.V.2010, R.P. Webster coll. // partially shaded cobblestone bar near outflow of brook at Jacquet River, under cobblestones and gravel on sand (LFC). PARATYPE: New Brunswick, Queens Co., Bayard at Nerepis River, 45.4426°N, 66.3280°W, 24.V.2008, R.P. Webster coll. // River margin, in gravel (RWC) 1 female.

Etymology. The name of this new species, *pseudoobscura*, derives from a similar species of *Gyronycha obscura* Casey described from California, USA.

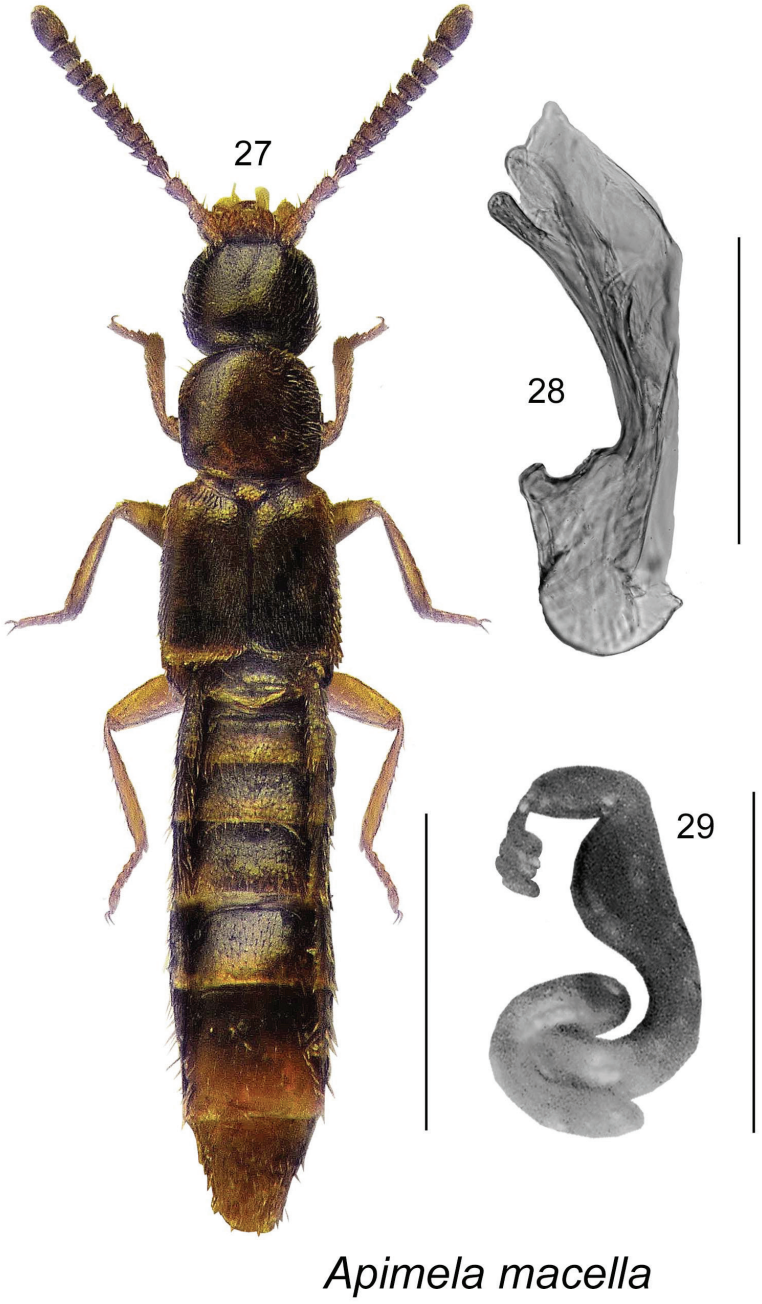
Description. Body length 3.9–4.0 mm, subparallel, yellowish brown with head and pronotum and antennae dark brown, moderately glossy, forebody with fine and dense pubescence, punctation fine; head round, eyes moderately large and shorter than postocular region of head, posterior angles rounded, pubescence directed straight and obliquely anteriad; antennomeres V-X slightly elongate, head about as broad as pronotum; pronotum slightly transverse, posterior angles slightly angular; elytra elongate, at suture longer than pronotum, and about one fifth wider than pronotum, abdomen subparallel, with first visible four tergites deeply impressed basally. MALE. Unknown. FEMALE. Spermatheca with subspherical capsule, and with apical narrow, tubular and coiled apically projection, stem sinuate, with large, overlapping coils (Fig. 26); tergite VIII truncate apically (Fig. 24); sternite VIII rounded apically (Fig. 25).

Collection and habitat data. The holotype was captured under cobblestones and gravel on sand on a partially shaded cobblestone bar near the outflow of a brook flowing into the Jacquet River. The paratype was captured in gravel along a river margin.

Comments. This species is similar externally and has similar shape of spermatheca and female tergite and sternite VIII to those of *G. obscura* Casey. *Gyronycha pseudoobscura* may be distinguished from *G. obscura* by narrower body, dark brown color of head and pronotum (light brown in *G. obscura*), and the differently shaped pronotum with anterior angles rounded and strongly converging apically in apical part of the disc, while the pronotal angles are rectangular and moderately converging apically in *G. obscura*. The two species have allopatric distribution, and are known from remote and disjunctive localities in New Brunswick, Canada, and California, United States of America.



Figures 23–26. *Gyronycha pseudoobscura* Klimaszewski & Webster (female): **23** habitus in dorsal view **24** tergite VIII **25** sternite VIII **26** spermatheca. Scale bars: 1 mm for habitus; 0.2 mm for remaining structures.



Figures 27–29. *Apimela macella* (Erichson) (type species of the genus from Europe): **27** habitus in dorsal view **28** median lobe of aedeagus in lateral view **29** spermatheca. Scale bars: 1 mm for habitus; 0.2 mm for remaining structures.

Checklist of *Apimela* and *Gyronycha* species in Canada and USA, valid species and new records are in bold

Taxon	Author	Original generic assignment	Distribution	Present taxonomic status
Apimela attenuata	Casey, 1885: 306	*Calodera*	CA, NV	Valid species [spermatheca illustrated by Seevers 1978: 207]
Apimela lineata	Casey, 1894: 376	*Gyronycha*	NV	Synonymized with *A. attenuata* by Moore & Legner 1975: 343. As valid in Seevers (1978: 252).
Apimela fenyesi	Bernhauer, 1906: 337	*Aleuonota*	CA	Valid species: Moore & Legner 1975: 343; confirmed here. As valid in Seevers (1978: 252).
Apimela fusciceps	Casey,1894: 376	*Gyronycha*	**NB**, NC, NY	Valid species. **New combination**
Apimela lepida	Casey, 1911: 217	*Gyronycha*	NC	**New synonymy**
Apimela longipennis	Casey, 1911: 219	*Gyronychina*	CA	As valid species Moore & Legner 1975: 343, Seevers (1978: 252).
Apimela longicornis	Casey, 1911: 217	*Gyronycha*	NC, NY	Tentatively affiliated with *Apimela.* **New combination**
Apimela pertenuis	Casey, 1894: 377	*Gyronycha*	NJ	Status uncertain, not examined. **New combination**
Apimela canadensis	Klimaszewski & Webster, sp. n.	*Apimela*	**NB**	Valid species
Gyronycha obscura	Casey 1894: 375	*Gyronycha*	CA	Male unknown, species tentatively affiliated with *Gyronycha*
Gyronycha pseudoobscura	Klimaszewski & Webster, sp. n.	*Gyronycha*	**NB**	Male unknown, species tentatively affiliated with *Gyronycha*
Gyronycha texana	Casey, 1894: 374	*Gyronycha*	TX	Valid species
Gyronycha valens	Casey, 1894: 373	*Gyronycha*	AZ, CA, IN, NC, NM, NY, SO, TX	Valid species

Acknowledgements

We thank Floyd Shockley for loan of types form USNM, and Crystal A. Maier for loan of type of *A. fenyesi* from FMNH. I. Lamarre (LFC) is thanked for editing and D. Paquet (LFC) for formatting this manuscript. We are obliged to R. Pace (Verona, Italy) for some useful comments on the genus *Apimela*. The New Brunswick Department of Natural Resources (Fish & Wildlife Branch) is thanked for issuing a permit for sampling in the Jacquet River Gorge Protected Natural Area and the Meduxnekeag River Association is thanked for permission to sample beetles at the Meduxnekeag Valley Nature Preserve. This research was supported by Natural Resources Canada.

References

Bernhauer M (1906) Neue Aleocharinen aus Nord-Amerika. Deutsche Entomologische Zeitschrift [1907]: 337–348.

Casey TL (1885) New genera and species of Californian Coleoptera. California Academy of Sciences 1: 283–336. https://doi.org/10.5962/bhl.title.8839

Casey TL (1894) Coleopterological notices. V. Annals of the New York Academy of Sciences 7(6/12): 281–606, pl. 1. [the actual year of publication was 1894 but it is cited often as 1893]

Casey TL (1911) New American species of Aleocharinae and Myllaeninae. Memoirs on the Coleoptera 2. The New Era Printing Co., Lancaster, Pennsylvania, 245 pp.

Erichson WF (1839) Erster Band. In: Morrin FH (Ed.) Genera et species Staphylinorum insectorum coleopterorum familiae. Berlin, 400 pp.

Klimaszewski J, Webster RP, Savard K, Couture J (2009) First record of the genus *Alisalia* Casey from Canada, description of two new species, and a key to all Nearctic species of the genus (Coleoptera, Staphylinidae, Aleocharinae). Zookeys 25: 1–18. https://doi.org/10.3897/zookeys.25.280

Moore I, Legner EF (1975) A catalogue of the Staphylinidae of America north of Mexico (Coleoptera). University of California, Division of Agricultural Science, Special Publication No. 3015: 1–514.

Mulsant E, Rey C (1874). Tribu des brévipennes: Famille des aléochariéns: Septième branche: Myrmédoniares. Annales de la Société d'Agriculture Histoire Naturelle et Arts Utiles de Lyon (4) 6[1873]: 33–727.

Seevers CH (1978) A generic and tribal revision of the North American Aleocharinae (Coleoptera: Staphylinidae) [with additions and annotations by Lee H. Herman]. Fieldiana: Zoology 71: 1–289.

ZooKeys 672: 49–120 (2017)
doi: 10.3897/zookeys.672.10624
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Molecular, morphological and acoustic assessment of the genus *Ophryophryne* (Anura, Megophryidae) from Langbian Plateau, southern Vietnam, with description of a new species

Nikolay A. Poyarkov Jr.[1,2], Tang Van Duong[1,3], Nikolai L. Orlov[4], Svetlana S. Gogoleva[2,5,6], Anna B. Vassilieva[1,2,5], Luan Thanh Nguyen[7], Vu Dang Hoang Nguyen[7], Sang Ngoc Nguyen[7], Jing Che[8,9], Stephen Mahony[10,11]

1 *Department of Vertebrate Zoology, Biological faculty, Lomonosov Moscow State University, Leninskiye Gory, Moscow, GSP-1, 119991, Russia* **2** *Joint Russian-Vietnamese Tropical Research and Technological Center, 63 Nguyen Van Huyen Road, Nghia Do, Cau Giay, Hanoi, Vietnam* **3** *Vietnam National Museum of Nature, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet Road, Hanoi, Vietnam* **4** *Zoological Institute, Russian Academy of Sciences, Universitetskaya nab., 1, St. Petersburg, 199034, Russia* **5** *A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii pr., 33, Moscow 119071, Russia* **6** *Zoological Museum of the Lomonosov Moscow State University, Bolshaya Nikitskaya st. 6, Moscow 125009, Russia* **7** *Institute of Tropical Biology, Vietnam Academy of Science and Technology (VAST), 85 Tran Quoc Toan St., District 3, Ho Chi Minh City, Vietnam* **8** *State Key Laboratory of Genetic Resources and Evolution State, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, Yunnan, China* **9** *Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences, Yezin, Nay Pyi Taw 05282, Myanmar* **10** *UCD School of Biology and Environmental Science, UCD Science Centre (West), University College Dublin, Belfield, Dublin 4, Ireland* **11** *Department of Life Sciences, The Natural History Museum, South Kensington, London, United Kingdom*

Corresponding authors: *Nikolay A. Poyarkov* (n.poyarkov@gmail.com); *Stephen Mahony* (stephenmahony2@gmail.com)

Academic editor: *A. Crottini* | Received 23 September 2016 | Accepted 20 March 2017 | Published 3 May 2017

http://zoobank.org/DFAF2079-2883-4BA9-931E-07F00D1F7D76

Citation: Poyarkov Jr NA, Duong TV, Orlov NL, Gogoleva SS, Vassilieva AB, Nguyen LT, Nguyen VDH, Nguyen SN, Che J, Mahony S (2017) Molecular, morphological and acoustic assessment of the genus *Ophryophryne* (Anura, Megophryidae) from Langbian Plateau, southern Vietnam, with description of a new species. ZooKeys 672: 49–120. https://doi.org/10.3897/zookeys.672.10624

Abstract

Asian Mountain Toads (*Ophryophryne*) are a poorly known genus of mostly small-sized anurans from southeastern China and Indochina. To shed light on the systematics within this group, the most com-

Copyright *Nikolay Poyarkov Jr. et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

prehensive mitochondrial DNA phylogeny for the genus to date is presented, and the taxonomy and biogeography of this group is discussed. Complimented with extensive morphological data (including associated statistical analyses), molecular data indicates that the Langbian Plateau, in the southern Annamite Mountains, Vietnam, is one of the diversity centres of this genus where three often sympatric species of *Ophryophryne* are found, *O. gerti*, *O. synoria* and an undescribed species. To help resolve outstanding taxonomic confusion evident in literature (reviewed herein), an expanded redescription of *O. gerti* is provided based on the examination of type material, and the distributions of both *O. gerti* and *O. synoria* are considerably revised based on new locality records. We provide the first descriptions of male mating calls for all three species, permitting a detailed bioacoustics comparison of the species. We describe the new species from highlands of the northern and eastern Langbian Plateau, and distinguish it from its congeners by a combination of morphological, molecular and acoustic characters. The new species represents one of the smallest known members of the genus *Ophryophryne*. At present, the new species is known from montane evergreen forest between 700–2200 m a.s.l. We suggest the species should be considered Data Deficient following IUCN's Red List categories.

Keywords
12S rRNA, 16S rRNA, advertisement call, amphibian, biodiversity, Da Lat Plateau, frog, Indochina, southeast Asia, taxonomy, Truong Son

Introduction

Asian Mountain toads (*Ophryophryne* Boulenger, 1903) are a small group of frogs from southeast Asia with a rather limited distribution mostly in mountains of eastern Indochina and adjacent parts of southern China (Yunnan and Guangxi) and northern Thailand (Inger et al. 1999, Ohler 2003, Orlov and Ananjeva 2007, Yang 2008). The genus *Ophryophryne* is still poorly studied, to date five (Ohler 2003, Stuart et al. 2006) or six (Stuart et al. 2010) species are recognized, with little consensus on the taxonomic status of several forms. All of the known *Ophryophryne* species have been reported from the Truong Son or Annamite mountains in Vietnam, which may be considered as an area of highest diversity for this group (Orlov and Ananjeva 2007).

The systematic status of *Ophryophryne* has long been a source of confusion. Boulenger (1903) described the genus and species *O. microstoma* Boulenger, 1903, and though he clearly stated that *Ophryophryne* is closely allied to *Megophrys* Kuhl & Van Hasselt, 1822, he noted characters also shared by members of the family Bufonidae Gray, 1825 (lacking vomerine and maxillary teeth and presence of horizontal pupil). Subsequently, Noble (1926), mostly based on morphology of the pectoral girdle, clearly demonstrated that the genus *Ophryophryne* is a member of Pelobatidae (at the time including the subfamily Megophryinae Bonaparte, 1850), and assumed its close affinities to *Megophrys*. However, due to widespread misinterpretation of Boulengers' original statement, the genus *Ophryophryne* was nevertheless incorrectly listed as a member of Bufonidae in several classical works on batrachians (Bourret 1937, 1942, Gorham 1974, Guibé 1950, Nguyen and Ho 1996, Taylor 1962).

The systematic status of the genus *Ophryophryne* among the Megophryidae has been discussed in several works. Liu and Hu (1962) provided the first description of the *Ophryophryne* tadpole which was remarkably similar to those of *Megophrys*, which led Dubois (1980) to rank *Ophryophryne* at the level of subgenus within *Megophrys*. Soon afterwards, Dubois re-evaluated his proposition and elevated *Ophryophryne* back to the genus-level status (Dubois 1987). Summarizing available cytological, morphological and ecological evidence, Rao and Yang (1997) proposed to split *Megophrys s. lato*, regarding *Ophryophryne* as a separate genus, as well as the former *Megophrys* subgenera, *Megophrys s. stricto*, *Atympanophrys* Tian & Hu, 1983, *Brachytarsophrys* Tian & Hu, 1983 and *Xenophrys* Günther, 1864. Several studies indicated close affinities of *Ophryophryne* to the genus *Xenophrys* (Tian and Hu 1983, 1985, Frost 1985, Ye et al. 1993, Rao and Yang 1997, Manthey and Grossmann 1997). Delorme et al. (2006) recognized the tribe Xenophryini, containing two genera *Ophryophryne* and *Xenophrys*, and most recent faunal reviews have treated *Ophryophryne* as a valid genus within Megophryidae (Fei et al. 1999, 2005, 2009, 2010, 2012, Orlov and Ananjeva 2007, Nguyen et al. 2009). Recently, Mahony (2011a) suggested that insufficient evidence was available for the morphological distinction of *Xenophrys* from *Megophrys* and suggested to retain the historical usage of *Megophrys s. lato* for species of both genera pending a taxonomic review of the group, however, he did not discussed the status of *Ophryophryne*.

Though a comprehensive phylogeny of the genus *Ophryophryne* is still pending, preliminary molecular data were contradictory, suggesting both as sister-clade relationships of *Ophryophryne* with respect to a monophyletic group composed of *Xenophrys*, *Megophrys*, and *Brachytarsophrys* (Pyron and Wiens 2011), or providing evidence of the paraphyly of *Xenophrys* with respect to *Ophryophryne* (e.g. Wang et al. 2012). A recent phylogenetic study on Megophryinae by Chen et al. (2017) provides new insights on evolutionary relationships within this group, indicating contrasting (though poorly supported) phylogenetic positions of *Ophryophryne* in their multilocus nuclear-gene based phylogeny and matrilineal mtDNA genealogy. Chen et al. (2017) preliminarily recognized *Ophryophryne* as one of the five monophyletic genera within Megophryinae (*Ophryophryne*, *Brachytarsophrys*, *Xenophrys*, *Atympanophrys* and *Megophrys*). However, Mahony et al. (2017) provides an alternative hypothesis based on extensive morphological studies and a larger nuclear gene dataset. They provided compelling evidence (recent diversification, insufficient morphological or biological distinction of major clades) for the consideration of Megophryinae to be treated as a single genus, *Megophrys*, with seven sub-clades (including *Ophryophryne*) being treated as subgenus level taxa. Their phylogenetic analyses provided strong support for the sister taxa relationship of *Ophryophryne* and a clade corresponding to *Panophrys* (previously considered a synonym or subclade of *Xenophrys*, e.g., Delorme et al. 2006, Chen et al. 2017).

For a long time after its' description, the genus *Ophryophryne* was thought to include a single species, *O. microstoma*, described from "Mau Son" in Tonkin (northern Vietnam). Later, Bourret (1937) described a second species, *O. poilani* Bourret, 1937, based upon a single, badly preserved specimen from "Dong Tam Ve" in Quang Tri Prov. of Annam (central Vietnam). Almost half a century later a third species, *O. pachy-*

proctus Kou, 1985, was described by Kou (1985) from Mengla County in Yunnan Prov. (southern China). Ohler (2003) revised the available material on the genus and, mostly based on samples collected by M. Smith (in southern Vietnam), and I.S. Darevsky and N.L. Orlov (in central Vietnam), described two more species: *O. gerti* Ohler, 2003 and *O. hansi* Ohler, 2003, respectively (the type series of *O. gerti* included specimens from the Langbian Plateau in southern Vietnam, and Laos). Ohler (2003) revised diagnostic characters and provided a key for the genus; she also examined the type specimen of *O. poilani* and considered it to be a junior synonym of *O. microstoma* (opinion not shared by Stuart et al. 2010). The last major progress on the taxonomy of *Ophryophryne* was made by Stuart et al. (2006), who reported the genus for Cambodia and described one more species, *O. synoria* Stuart, Sok & Neang, 2006 from Mondolkiri Prov. in eastern hilly Cambodia, near the Vietnamese border. A recent review of southern Vietnamese herpetofauna by Vassilieva et al. (2016) based on morphological evidence recorded *O. synoria* for lowland areas of Dong Nai and Binh Phuoc provinces.

The Langbian (or Da Lat) Plateau forms the southernmost edge of the Annamite Mountains, or Truong Son Range, a mountain chain spanning the breadth of Indochina, including parts of Vietnam, Laos and Cambodia. To date, following the review by Ohler (2003), only *Ophryophryne gerti* has been recorded from the high elevations (above 1000 m a.s.l.) of the Langbian Plateau (Stuart et al. 2010, Nguyen et al. 2009, 2014). However, our recent fieldwork in this area from 2007 until 2016 revealed the presence of at least three morphologically distinct species, often recorded in syntopy (Orlov et al. 2008, Poyarkov and Vassilieva 2011). Further investigation of partial 12S rRNA–16S rRNA mtDNA gene sequences, as well as the study of advertisement calls from the Langbian *Ophryophryne* populations, herein confirm their specific status and reveals that one of the lineages represents a previously undescribed species. We also provide the first preliminary mtDNA phylogeny for the genus *Ophryophryne* and discuss the biogeography of the genus in Indochina in light of our new data.

Materials and methods

Sample collection. All specimens were collected during fieldwork in southern Vietnam in 2007–2016. Frogs were collected mostly during night excursions by opportunistic visual searching, or by sound when calling. Geographic coordinates were obtained using a Garmin GPSMAP 60CSx GPS receiver and recorded in datum WGS 84. The geographic position of the surveyed localities and the distribution of *Ophryophryne* species in the southern Annamite Mountains (Truong Son) and adjacent regions of southern Indochina (eastern Cambodia) are shown in Fig. 1. The newly collected specimens were deposited in the herpetological collection of the Zoological Museum of Moscow State University in Moscow, Russia (ZMMU) and the Institute of Tropical Biology Zoological Collection, Ho Chi Minh City, Vietnam (ITBCZ). Examined specimens of compared species are stored in herpetological collections of ZMMU, Zoological Institute R.A.S., St. Petersburg, Russia (ZISP), Natural History Museum,

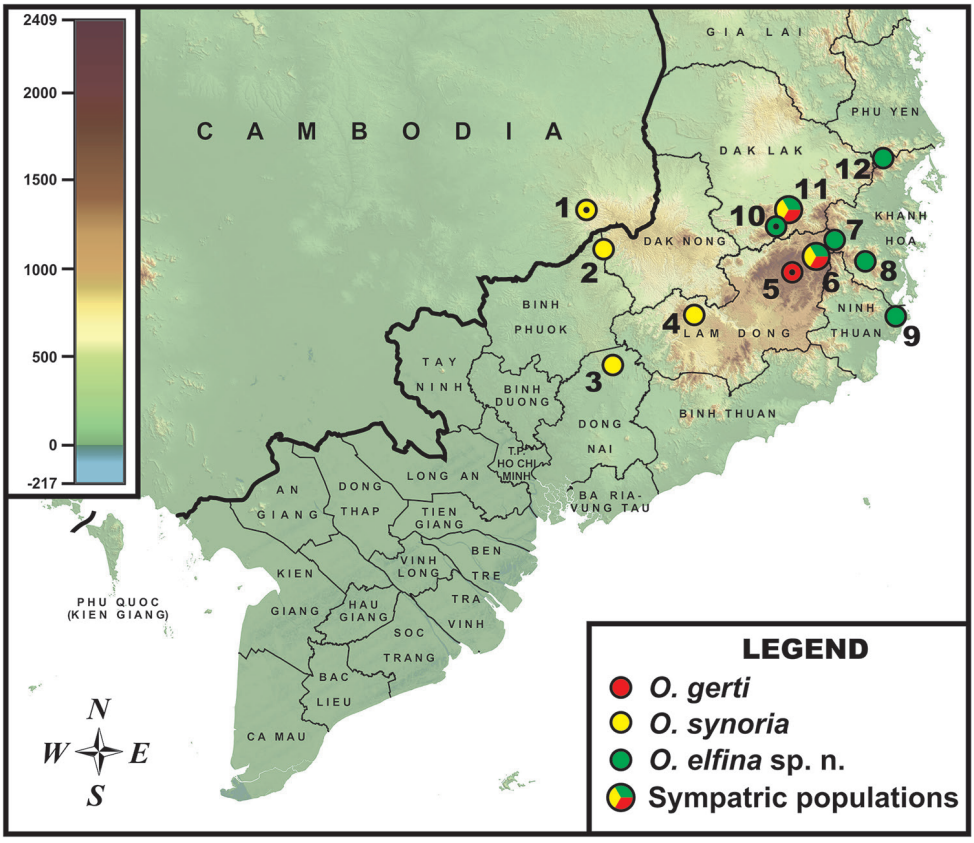


Figure 1. Distribution of *Ophryophryne* species in southern Indochina (Langbian Plateau in Vietnam, and adjacent regions of Camodia), indicating localities sampled in this study. Black dot in the center of an icon indicates the type locality of the new species. Locality information. **1** O Chung Chry stream, Samling Logging Concession, O'Rang Dist., Mondolkiri Prov., Cambodia (500 m a.s.l.) (Stuart et al. 2006; type locality of *O. synoria*; Mahony et al. 2017) **2** Bu Gia Map N.P., Binh Phuoc Prov., Vietnam (400 m a.s.l.) (Vassilieva et al. 2016; this study) **3** Nam Cat Tien sector of Dong Nai Biosphere Reserve, Dong Nai Prov., Vietnam (200 m a.s.l.) (Vassilieva et al. 2016; this study) **4** Loc Bac forest, Bao Loc Dist., Lam Dong Prov., Vietnam (830 m a.s.l.) (this study) **5** Cam Ly River (Ohler 2003: type locality of *O. gerti*) and Nui Ba Mt. in environs of Dalat city, Lam Dong Prov., Vietnam (ca. 1000–1800 m a.s.l.) (this study) **6** Environs of Bidoup Mt. (2000 m a.s.l.), and Giang Ly Ranger Station (1500 m a.s.l.), Bidoup–Nui Ba N.P., Lam Dong Prov., Vietnam (Poyarkov [Paiarkov] and Vassilieva 2011; this study) **7** Hon Giao Mt., Bidoup–Nui Ba N.P., Lam Dong and Khanh Hoa provincial border, Vietnam (1900–2000 m a.s.l.) (this study) **8** Hon Ba Mt., Hon Ba N.R., Dien Khanh Dist., Khanh Hoa Prov., Vietnam (950–1510 m a.s.l.) (Nguyen et al. 2014; this study) **9** Nui Chua Mt., Nui Chua N.P., Ninh Hai Dist., Ninh Thuan Prov., Vietnam (780 m a.s.l.) (this study) **10** Chu Pan Fan Mt., Chu Yang Sin N.P., Lak Dist., Dak Lak Prov., Vietnam (1900 m a.s.l.) (type locality of *Ophryophryne elfina* sp. n.; this study) **11** Chu Yang Sin Mt. environs, Krong Kmar Commune, Krong Bong Dist., Dak Lak Prov., Vietnam (700–2000 m a.s.l.) (Orlov et al. 2008; this study) **12** Tay Hoa Dist., Phu Yen Prov., Vietnam (700 m a.s.l.) (this study).

London, United Kingdom (NHMUK, formerly BMNH, though the latter acronym is retained for specimen numbers for comparability with older literature), Field Museum of Natural History, Chicago, USA (FMNH), and Yunnan University, Faculty of Biology, Kunming, China (YU).

Morphology. Specimens were photographed in life, and tissue samples for genetic analyses were taken prior to preservation, and stored in 96% ethanol. We recorded morphological data from specimens fixed and stored in 75% ethanol.

Measurements to the nearest 0.1 mm were taken using either a digital caliper, or a dissecting microscope; morphometrics of adult frogs and character terminology follows Mahony (2011a) and Mahony et al. (2013). Morphometric abbreviations are as follows:

SVL snout to vent length;
HW head width;
HL head length;
ED eye diameter;
TYD tympanum diameter;
TYE eye to tympanum distance;
SL snout length;
EN eye to narial distance;
SN narial to snout distance;
IUE interorbital distance, between upper eyelids;
IN internarial distance;
UEW upper eyelid width;
FAL forearm length;
HAL hand length;
FIL first finger length;
FIIL second finger length;
FIIIL third finger length;
FIVL fourth finger length;
SHL shank length;
TL thigh length;
FOL foot length;
TFOL tibiotarsal articulation to tip of fourth toe distance;
IMT inner metatarsal tubercle length.

Additionally, for the description of the type series we measured the distance between anterior orbital borders (**IFE**); distance between posterior orbital borders (**IBE**); first toe length (**TIL**); second toe length (**TIIL**); third toe length (**TIIIL**); fourth toe length (**TIVL**); fifth toe length (**TVL**). All measurements were taken on the right side of the specimen, except when a character was damaged, in which case the measurement was taken on the left side. Entire skin surface of all specimens were examined by microscope for the presence of dermal microstructures. Sex was determined by direct observation of calling in life and/or gonadal inspection by dissection.

Morphological description of larval stages included the following 15 measurements: total length (**TOL**); body length (**BL**); tail length (**TAL**); maximum body width (**BW**); maximum body height (**BH**); maximum tail height (**TH**); snout to vent length (**SVL**); snout to spiracle distance (**SSp**); maximum upper tail fin height (**UF**); maximum lower tail fin height (**LF**); internarial distance (**IN**); interpupilar distance (**IP**); rostro-narial distance (**RN**); naro-pupilar distance (**NP**); eye diameter (**ED**). The oral disk width and the labial tooth row formula were not recorded since in *Ophryophryne* the oral disk is modified to an extensive funnel which is closed when fixed in preservative, and oral disk structures typical for most other anurans are absent. Tadpoles were staged after Gosner (1960); morphometrics followed Grosjean (2001, 2003) and Poyarkov et al. (2015b).

All statistical analyses were performed with Statistica 6.0 (StatSoft, Inc. 2001). Morphometric characters were used for univariate analyses, corrected by body size. Sexes were separated for subsequent comparisons among the samples. One-way ANOVA and Duncan's post hoc test were used for morphometric comparisons. Multivariate statistical analyses were conducted for examination of overall morphological variation among studied populations. If some characters showed high correlation between each other, all but one of them were omitted in order to exclude the overweighting effect of these characters on the analyses. After metric values were log e-transformed, a principal component analysis (PCA) was conducted. The additional specimens of the undescribed *Ophryophryne* species, measured by LTN, were not included in the PCA to avoid potential error due to inter-observer variation of measurement techniques. A significance level of 95% was used in all statistical tests.

Comparative morphological data were obtained from museum specimens of *Ophryophryne* and (when available) photographs of these specimens in life (see Appendix 1). Data on morphology and taxonomy of *Ophryophryne* are also available from the following literature: *O. microstoma* (Bourret 1942, Liu and Hu 1962, Yang 1991, Ye et al. 1993, Fei et al. 1999, 2009, 2010, 2012, Zhang and Wen 2000, Ohler 2003, Bain et al. 2007, Yang 2008), *O. pachyproctus* (Kou 1985, Yang 1991, Ye et al. 1993, Fei et al. 1999, 2009, 2010, 2012), *O. poilani* (Bourret 1937, 1942, Ohler 2003). However, due to the considerable undiagnosed diversity within Megophryidae (Chen et al. 2017, Mahony et al. 2017), where available, we relied on the examination of type specimens, topotypic material and/or original species descriptions. Only characters verified on all specimens in the type series and referred specimens are used to represent the new species in the comparison and diagnosis sections. Specimens of *O.* cf. *poilani* listed in Appendix 1 were not used in the comparison of the undescribed species with *O. poilani*.

DNA isolation and sequencing. For molecular analysis, total genomic DNA was extracted from ethanol-preserved muscle or liver tissues using either standard phenol-chloroform extraction procedures (Hillis et al. 1996) followed with isopropanol precipitation (at Moscow State University: hereafter MSU), or a Qiagen DNeasy® Blood & Tissue Kit primarily following manufacturers' instructions, with the exception of an extended (10 minute) soaking step prior to the elution of extracted DNA from the column, and additional final elution step using 40 μl H_2O (at University College Dub-

lin: hereafter UCD). The isolated total genomic DNA was visualized using agarose gel electrophoresis in the presence of ethidium bromide (MSU), or SafeView™ (Applied Biological Materials Inc. — at UCD). The concentration of total DNA was measured using NanoDrop 2000 (MSU) or NanoDrop 1000 (Thermo Scientific) (UCD), and consequently either adjusted to ca. 100 ng DNA/µl (MSU), or 10 ng DNA/µl (UCD).

We amplified sequences of a continuous fragment including partial sequences of 12S rRNA and 16S rRNA genes and complete t-val gene sequence, to obtain a fragment of up to 2077 bp (base pairs) of mtDNA. For some adult specimens and larvae a partial ca. 460–500 bp fragment of the 16S rRNA gene was sequenced for molecular identification purposes. 16S rRNA is a molecular marker widely applied for biodiversity surveys in amphibians (Vences et al. 2005a, 2005b, Vieites et al. 2009), and has proven to be particularly useful in studies of megophryid diversity (Matsui et al. 2010, 2014, Ohler et al. 2011, Stuart et al. 2011, 2012, Hamidy et al. 2012, Rowley et al. 2010a, 2011a, 2012, Jiang et al. 2013, Poyarkov et al. 2015a and references therein). Amplification was performed in 25 µl reactions using either ca. 50 ng genomic DNA, 10 nmol of each primer, 15 nmol of each dNTP, 50 nmol additional $MgCl_2$, Taq PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.1 mM $MgCl_2$ and 0.01% gelatine) and 1 U of Taq DNA polymerase (MSU), or 2.0 µl of genomic DNA (10 ng/µl), 2.5 µl Sigma 10x PCR buffer (excluding $MgCL_2$), 0.5 µl $MgCL_2$, 0.5 µl dNTP mix, 0.5 µl forward and reverse primer (10 ng/µl), 0.2 µl Platinum® *Taq* DNA Polymerase (Invitrogen) and 18.3 µl PCR grade H_2O (UCD). Primers used in PCR and sequencing were as follows: forward primers: 12SAL (AAACTGGGATTAGATACCCCACTAT; Zhang et al. 2008), LX12SN1 (TACACACCGCCCGTCA; Zhang et al. 2008), 12SA (CTGGGATTAGATACCCCACTA; Palumbi 1996), L1879 (CGTACCTTTTGCATCATGGTC; Matsui et al. 2010), L2188 (AAAGTGGGCCTAAAAGCAGCCA; Matsui et al. 2006), 16L-1 (CTGACCGTGCAAAGGTAGCGTAATCACT; Hedges 1994); reverse primers: 16S2000H (GTGATTAYGCTACCTTTGCACGGT; Zhang et al. 2008), LX16S1R (GACCTGGATTACTCCGGTCTGAACTC; Zhang et al. 2008), 16SBr (CCGGTCTGAACT-CAGATCACGT; Palumbi 1996), H1923 (AAGTAGCTCGCTTAGTTTCGG; Matsui et al. 2010), H2317 (TTCTTGTTACTAGTTCTAGCAT; Shimada et al. 2011), Will6 (CCCTCGTGATGCCGTTGATAC; Wilkinson et al. 2002). Tadpoles were assigned to species based on short 16S rRNA sequences obtained using the primer pair 16L-1 (see above) and 16H-1 (CTCCGGTCTGAACTCAGATCACGTAGG; Hedges 1994). Two Touch-Down (TD) PCR reaction protocols (Murphy and O'Brien 2007) were used: TD 63–57 for 12SA and 16SBr primers and TD 55 for all other primer pairs. Slight differences in reaction protocol were used between MSU, and UCD reactions (in parentheses). TD 55 included an initial denaturation step of 5 (2) min. at 94°C and followed with 10 cycles of denaturation for 30 (45) sec. at 96°C, primer annealing for 30 (40) sec. with annealing temperature decreasing by 1°C per cycle from 65°C to 55°C and extension step for 1 min. at 72°C, followed with 35 cycles of 30 (45) sec. at 96°C, 30 (40) sec. at 55°C and 4 (1) min. at 60°C (72°C), with the final extension step for 10 min. at 72°C. TD 63–57 consisted of 2 min. at 95°C, 6 cycles of 45 sec. at 95°C, 40 sec. at 63°C with a

reduction of 1°C each cycle, 1 min. at 72°C, followed by 35 cycles of 45 sec. at 95°C, 40 sec. at 57°C and 1 min at 72°C, and a final step of 10 min. at 72°C. PCR products were loaded onto 1% agarose gels, stained with either GelStar gel stain (Cambrex: at MSU) or SafeView™ (at UCD), and visualized in a Dark reader transilluminator (Clare Chemical). If distinct bands were produced, PCR products were purified either using 2 µl, from a 1:4 dilution of ExoSapIt (Amersham), per 5 µl of PCR product prior to cycle sequencing (MSU), or using TSAP (Promega) following manufacturers' instructions (UCD). At MSU, a 10 µl sequencing reaction included 2 µL of template, 2.5 µl of sequencing buffer, 0.8 µl of 10 pmol primer, 0.4 µl of BigDye Terminator version 3.1 Sequencing Standard (Applied Biosystems) and 4.2 µl of water. The cycle-sequencing reaction was 35 cycles of 10 sec. at 96°C, 10 sec. at 50°C and 4 min. at 60°C. Cycle sequencing products were purified by ethanol precipitation. Sequence data collection and visualization were performed on an ABI 3730xl automated sequencer (Applied Biosystems). At UCD, purified PCR products were Sanger sequenced in both directions by Macrogen (Europe). The forward and reverse sequences were checked visually either in Chromas Pro software (Technelysium Pty Ltd., Tewntin, Australia: at MSU) and a consensus sequence was compiled with BioEdit 5.0.9 (Hall 1999: at MSU), or using CodonCodeAligner 3.7.1 (CodonCode Corporation, Dedham, Massachusetts: at UCD). Sequences were submitted to a BLAST search in GenBank to confirm that the intended sequences had been amplified. The obtained sequences are deposited in GenBank under the accession numbers KY425352–KY425411 and KY515232–KY515233 (see Table 1).

Phylogenetic analyses. Sequences coding for the 12S rRNA–16S rRNA mtDNA genes of 66 megophryid specimens: 53 *Ophryophryne*, representing all currently recognized species, and outgroup sequences of two *Brachytarsophrys* species, eight *Megophrys s. lato* species (including seven *Xenophrys* and one *Megophrys s. stricto* species), two *Leptobrachium* Tschudi, 1838, and one *Leptolalax* Dubois, 1980 species (Table 1), were included in the final alignment and subjected to phylogenetic analyses. Nucleotide sequences were initially aligned using ClustalX 1.81 (Thompson et al. 1997) with default parameters, and then optimized manually in BioEdit 7.0.5.2 (Hall 1999) and MEGA 6.0 (Tamura et al. 2013). Mean uncorrected genetic distances (*p*-distances) between sequences were determined with MEGA 6.0 (Tamura et al. 2013); the existence of "barcode gap" was estimated using the online version of ABGD (Puillandre et al. 2012). MODELTEST v.3.06 (Posada and Crandall 1998) was used to estimate the optimal evolutionary models to be used for the data set analysis. The best-fitting model as suggested by the Akaike Information Criterion (AIC) was the general time-reversible (GTR) model of DNA evolution with a gamma shape parameter (G).

Maximum Likelihood (ML) analysis was conducted using Treefinder (Jobb et al. 2004). Transitions and transversions were equally weighted, and gaps were treated as missing data. Confidence in tree topology was tested by non-parametric bootstrap analysis (Felsenstein 1985) with 1000 replicates. Bayesian inference (BI) was conducted using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003); Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses were

Table 1. Specimens and GenBank sequences of *Ophryophryne* and outgroup Megophryidae representatives used in molecular analyses. AN – Accession number. Numbers of localities (No. 1–12) correspond to those in Figures 1 and 2. For detailed specimen information see Appendix 1. Asterisk marks holotype specimen, double asterisk marks topotype specimens (continues on next two pages). Museum abbreviations for the specimens from which sequences were generated in this study are explained in the Materials and methods section.

GenBank AN	Voucher ID	Species	Locality	Elevation (m a.s.l.)	Reference
KY022198	FMNH 262778*	*O. synoria*	**1** – Cambodia, Mondolkiri Prov., O'Reang	500	Mahony et al. 2017
KY425353	ZMMU ABV-00379	*O. synoria*	**2** – Vietnam, Binh Phuoc Prov., Bu Gia Map N.P., Dac Ca River	400	this paper
KY425354	ZMMU NAP-00731	*O. synoria*	**2** – Vietnam, Binh Phuoc Prov., Bu Gia Map N.P., Dac Ca River	400	this paper
KY425355	ZMMU ABV-00380	*O. synoria*	**2** – Vietnam, Binh Phuoc Prov., Bu Gia Map N.P., Dac Ca River	400	this paper
KY425356	ZMMU ABV-00376	*O. synoria*	**2** – Vietnam, Binh Phuoc Prov., Bu Gia Map N.P., Dac Ca River	400	this paper
KY425357	ZMMU NAP-00834	*O. synoria*	**3** – Vietnam, Dong Nai Prov., Nam Cat Tien N.P., Da Ta Po River	200	this paper
KY425358	ZMMU ABV-00209	*O. synoria*	**4** – Vietnam, Lam Dong Prov., Bao Loc, Loc Bac forestry	830	this paper
KY425359	ZMMU ABV-00159	*O. synoria*	**4** – Vietnam, Lam Dong Prov., Bao Loc, Loc Bac forestry	830	this paper
KY425360	ZMMU NAP-01756	*O. synoria*	**6** – Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Giang Ly	1500	this paper
KY425361	ZMMU NAP-01787	*O. synoria*	**6** – Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Giang Ly	1500	this paper
KY425362	ZMMU NAP-01835	*O. synoria*	**6** – Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Giang Ly	1500	this paper
KY425363	ZISP NLO-36349	*O. synoria*	**11** – Vietnam, Dak Lak Prov., Chu Yang Sin N.P., Chu Yang Sin Mt.	1000	this paper
KY425364	ZISP NLO-36554	*O. synoria*	**11** – Vietnam, Dak Lak Prov., Chu Yang Sin N.P., Chu Yang Sin Mt.	1000	this paper
KY425365	KIZ-013663**	*O. gerti*	**5** – Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Langbian Mt.	1800	this paper
KY425366	KIZ-013664**	*O. gerti*	**5** – Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Langbian Mt.	1800	this paper
KY425367	KIZ-013662**	*O. gerti*	**5** – Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Langbian Mt.	1800	this paper
KY425368	ZMMU NAP-01878	*O. gerti*	**6** – Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Giang Ly	1500	this paper
KY425369	ZMMU NAP-01789	*O. gerti*	**6** – Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Giang Ly	1500	this paper
KY425370	ZMMU NAP-02471	*O. gerti*	**6** – Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Giang Ly	1500	this paper
KY425371	ZMMU NAP-01790	*O. gerti*	**6** – Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Giang Ly	1500	this paper
KY425372	ZMMU NAP-01788	*O. gerti*	**6** – Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Giang Ly	1500	this paper
KY425373	ZMMU NAP-02758	*O. gerti*	**11** – Vietnam, Dak Lak Prov., Chu Yang Sin N.P.	1000	this paper
KY425374	ZMMU ABV-00530	*O. gerti*	**11** – Vietnam, Dak Lak Prov., Chu Yang Sin N.P.	1000	this paper
KY425375	ZMMU NAP-02759	*O. gerti*	**11** – Vietnam, Dak Lak Prov., Chu Yang Sin N.P.	1000	this paper

GenBank AN	Voucher ID	Species	Locality	Elevation (m a.s.l.)	Reference
KY425376	ZMMU ABV-00577	*O. gerti*	**11 –** Vietnam, Dak Lak Prov., Chu Yang Sin N.P.	1000	this paper
KY425377	ZMMU NAP-02760	*O. gerti*	**11 –** Vietnam, Dak Lak Prov., Chu Yang Sin N.P.	1000	this paper
KY425378	ZISP NLO-36510	*O. gerti*	**11 –** Vietnam, Dak Lak Prov., Chu Yang Sin N.P.	1000	this paper
KY425379	ZMMU ABV-00454	*O. elfina* sp. n.	**6 –** Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Bidoup Mt.	2000	this paper
KY425380	ZMMU ABV-00455	*O. elfina* sp. n.	**6 –** Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Bidoup Mt.	2000	this paper
KY515233	ZMMU NAP-01169	*O. elfina* sp. n. (larva)	**6 –** Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Bidoup Mt.	2000	this paper
KY425381	ZMMU NAP-01782	*O. elfina* sp. n.	**7 –** Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Hon Giao Mt.	2000	this paper
KY425382	ZMMU NAP-01783	*O. elfina* sp. n.	**7 –** Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Hon Giao Mt.	2000	this paper
KY425383	ZMMU NAP-01757	*O. elfina* sp. n.	**7 –** Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Hon Giao Mt.	2000	this paper
KY425384	ZMMU NAP-01758	*O. elfina* sp. n.	**7 –** Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Hon Giao Mt.	2000	this paper
KY425385	ZMMU ABV-00316	*O. elfina* sp. n.	**8 –** Vietnam, Khanh Hoa Prov., Hon Ba N.R., Hon Ba Mt.	1500	this paper
KY425386	KIZ YPX-05429	*O. elfina* sp. n.	**9 –** Vietnam, Ninh Thuan Prov., Nui Chua N.P.	780	this paper
KY425387	KIZ YPX-05457	*O. elfina* sp. n.	**9 –** Vietnam, Ninh Thuan Prov., Nui Chua N.P.	780	this paper
KY425388	KIZ YPX-05428	*O. elfina* sp. n.	**9 –** Vietnam, Ninh Thuan Prov., Nui Chua N.P.	780	this paper
KY425389	ZMMU NAP-02658*	*O. elfina* sp. n.	**10 –** Vietnam, Dak Lak Prov., Chu Yang Sin N.P., Chu Pan Fan Mt.	1900	this paper
KY515232	ZMMU NAP-02673**	*O. elfina* sp. n. (larva)	**10 –** Vietnam, Dak Lak Prov., Chu Yang Sin N.P., Chu Pan Fan Mt.	1900	this paper
KY425390	ZISP NLO-36522	*O. elfina* sp. n.	**11 –** Vietnam, Dak Lak Prov., Chu Yang Sin N.P., Chu Yang Sin Mt.	2000	this paper
KY425391	ZMMU ABV-00581	*O. elfina* sp. n.	**11 –** Vietnam, Dak Lak Prov., Chu Yang Sin N.P., Chu Yang Sin Mt.	1800	this paper
KY425392	ZMMU ABV-00580	*O. elfina* sp. n.	**11 –** Vietnam, Dak Lak Prov., Chu Yang Sin N.P., Chu Yang Sin Mt.	1975	this paper
KY425393	DVT-00393	*O. elfina* sp. n.	**12 –** Vietnam, Phu Yen Prov., Tay Hoa	700	this paper
KY022203	AMNH A163680	*O. hansi*	Vietnam, Quang Nam Prov., Tra My, Tra Don	930	Mahony et al. 2017
DQ283377	AMNH A163669	*O. hansi*	Vietnam, Quang Nam Prov., Tra My, Tra Don	970	Frost et al. 2006
KY425395	ZMMU NAP-06485	*O. hansi*	Vietnam, Kon Tum Prov., Thac Nham forest	1100	this paper
KY425396	ZMMU NAP-06501	*O. hansi*	Vietnam, Gia Lai Prov., Kon Chu Rang N.R.	1000	this paper
KY425397	ZMMU NAP-06524	*O. hansi*	Vietnam, Gia Lai Prov., Kon Chu Rang N.R.	1000	this paper
KY425398	ZMMU NAP-06502	*O. hansi*	Vietnam, Gia Lai Prov., Kon Chu Rang N.R.	1000	this paper
KY425399	ZMMU NAP-06525	*O. hansi*	Vietnam, Gia Lai Prov., Kon Chu Rang N.R.	1000	this paper
KY022200	KUH 311601	*O. microstoma*	China, Guangxi Prov., Shiwan Dashang N.R., Fulong	500	Mahony et al. 2017
KY022199	AMNH A168682	*O. microstoma*	Vietnam, Lao Cai Prov., Van Ban Dist., Nam Tha	330	Mahony et al. 2017

GenBank AN	Voucher ID	Species	Locality	Elevation (m a.s.l.)	Reference
KY022201	AMNH A163668	*O.* cf. *poilani*	Vietnam, Quang Nam Prov., Tra My, Tra Don	980	Mahony et al. 2017
KY022202	AMNH A169287	*O.* cf. *poilani*	Vietnam, Thua Thien–Hue Prov., A Luoi Dist., A Roang	680	Mahony et al. 2017
JX564854	ZP-AM 44	*Brachytarsophrys carinense*	–	–	Zhang et al. 2013
KY425404	ZMMU NAP-06324	*Brachytarsophrys feae*	Vietnam, Vinh Phuc Prov., Tam Dao	–	this paper
KY425405	ZMMU NAP-03994	*Xenophrys* cf. *aceras*	Thailand, Satun Prov.	–	this paper
KY425406	ZMMU NAP-05005	*Xenophrys* cf. *daweimontis*	Vietnam, Dien Bien Prov., Muong Nhe, Muong Nhe N.R.	–	this paper
KY425407	ZMMU NAP-04137	*Xenophrys* cf. *parva*	Thailand, Suratthani Prov.	–	this paper
KY425408	ZMMU NAP-04423	*Xenophrys* cf. *maosonensis*	Vietnam, Dien Bien Prov., Muong Nhe, Muong Nhe N.R.	–	this paper
AY561308	ZYC1500	*Xenophrys minor*	China	–	Zheng et al. 2004
AY561307	ZYC1513	*Xenophrys omeimontis*	China, Sichuan	–	Zheng et al. 2004
KY425409	DVT-04135	*Xenophrys* sp.	Vietnam, Lao Cai Prov.	–	this paper
KY425410	ZMMU NAP-05095	*Megophrys nasuta*	Malaysia, Sarawak	–	this paper
KJ630505	SCUM120630	*Leptobrachium boringii*	China, Sichuan Prov., Emei Shan Mt.	–	Xu et al. 2014
KY425411	DVT-00298	*Leptobrachium banae*	Vietnam, Phu Yen Prov., Tay Hoa	–	this paper
JX564874	MVZ-Herp-223642	*Leptolalax* cf. *pelodytoides*	–	–	Zhang et al. 2013

run with one cold chain and three heated chains for four million generations and sampled every 1,000 generations. Five independent MCMCMC runs were performed and 1,000 trees were discarded as burn-in. Confidence in tree topology was assessed by posterior probability (Huelsenbeck and Ronquist 2001).

We *a priori* regarded tree nodes with bootstrap (BS) values 75% or greater and posterior probabilities (BPP) values over 0.95 as sufficiently resolved, BS values between 75% and 50% (BPP between 0.95 and 0.90) were regarded as tendencies, and BS values below 50% (BPP below 0.90) were considered to be unresolved (Huelsenbeck and Hillis 1993).

Acoustic analyses. Calls were recorded using a portable digital audio recorder Zoom h4n (ZOOM Corporation, Tokyo, Japan) in stereo mode with 96 kHz sampling frequency and 16-bit precision, or using a Marantz 660 digital tape recorder (D&M Professional, Kanagawa, Japan) in mono mode with sampling rate at 48 kHz and 16-bit precision with a high-sensitivity Sennheiser K6 ME66 cardioid electret condenser microphone (Sennheiser electronic, Wedemark, Germany), or using a Nikon D 600 digital SLR camera (Nikon Corporation, Japan) in video mode with audio tracks removed from video recordings using Avisoft SASLab Pro software v. 5.2.05 (Avisoft Bioacoustics, Germany) with a 48 kHz sampling frequency and 16-bit precision. Temperature was measured at the calling sites immediately after audio recording using a digital thermometer, KTJ TA218A Digital LCD Thermometer-Hydrometer. All recordings were made in situ in the natural habitats of respective specimens. Advertisement calls of the undescribed *Ophryophryne* species were recorded on the mountain summit of Bidoup in the Bidoup-Nui Ba National Park (hereafter N.P.), Lam Dong Prov., eastern edge of the Langbian Plateau, Vietnam (12°06'42.4"N; 108°39'33.6"E, 1930–1940 m a.s.l.), on 10 and 15 April 2014, and 10 February 2015, between 16:05–18:35 h and at temperatures from 11.3°C in February to 17.5°C in April. In total, we made five recordings from three vocalizing males. Advertisement calls of *O. gerti* were recorded in Chu Yang Sin N.P., Dak Lak Prov., northern edge of the Langbian Plateau, Vietnam (12°24'01.6"N; 108°21'11.0"E, 1020 m a.s.l. and 12°25'25.7"N; 108°21'52.5"E, 1040–1045 m a.s.l.), on 22–27 May 2014, between 20:40–23:10 h at 22–22.5°C. In total, we made three recordings from three males. Advertisement calls of *O. synoria* were recorded in Chu Yang Sin N.P., Dak Lak Prov., Tay Nguyen region, Vietnam (12°28'0.94"N; 108°20'45.4"E, 700–800 m a.s.l.) on 25 May 2008, between 21:56–22:30 h at 21°C. In total, three recordings from three males were made.

All recordings were standardized by Avisoft SASLab Pro software v. 5.2.05 in mono format with sampling rate at 48 kHz and 16-bit precision, and low-frequency noise was reduced using the low-pass filter (up to 1000 Hz). Calls were analyzed using Avisoft SASLab Pro software v. 5.2.05; all parameters were measured using the reticule and standard cursors in the spectrogram window of Avisoft. Spectrograms for analyses were created using the Hamming window, FFT-length 1024 points, frame 100%, and overlap 87.5%. Figure spectrograms were created using the Hamming window, FFT-length 512 points, frame 100%, and overlap 75%. In total, we measured 1797 calls of the new *Ophryophryne* sp., 533 calls of *O. gerti* and 200 calls of *O. synoria*.

Four temporal parameters were measured: the duration of each call, the interval between successive calls within each series, the duration of series, the interval between successive series, and five frequency parameters: the initial and final fundamental frequency, the minimum and maximum of fundamental frequency and the frequency of maximum amplitude (also "F peak"). Then we calculated the frequency range as the difference between the maximum and minimum of fundamental frequencies and the call repetition rate per recording/series (calls/s) for each recording/series as a ratio of number of all calls within the recording/series (excluding series consisting of just one call) to recording/series duration. All numerical parameters are given as mean ± SE, the minimum and maximum values are given in parentheses (min–max).

To compare acoustic characteristics between three species of *Ophryophryne* we applied one-way ANOVA with Tukey post hoc for the values of the parameters for which distributions did not differ from normality ($p > 0.05$, Kolmogorov–Smirnov test). Otherwise we used nonparametric Kruskal-Wallis ANOVA with Mann-Whitney U post hoc test.

The records of advertisement calls were deposited at the Fonoteca Zoologica and are available at the website http://www.fonozoo.com (under the accession numbers 9954–9964).

Results

Sequence data

The final alignment of the studied 12S rRNA–16S rRNA mtDNA gene fragment consisted of 2077 sites: 1439 sites were conserved and 567 sites were variable, of which 465 were found to be parsimony-informative. The transition–transversion bias (R) was estimated as 2.06. Nucleotide frequencies were A = 32.8%, T = 27.6%, C = 21.6%, and G = 17.9% (all data given for ingroup only).

Phylogenetic relationships and geographic distribution of mtDNA haplotypes

We achieved high resolution of phylogenetic relationships among taxa within *Ophryophryne*, with all major nodes fully resolved (BPP = 1.0; BS = 100%: Fig. 2). Monophyly of species-level groups and species complexes in *Ophryophryne* were also significantly supported (BS > 90%; BPP ≥ 0.95). However, phylogenetic relationships between the taxa of outgroup Megophryinae are poorly resolved with major nodes in the tree having low or insignificant levels of support (BPP < 0.95; BS < 75%). Bayesian and Maximum Likelihood analyses resulted in essentially similar topologies (see Fig. 2) slightly differing from each other only in associations for several poorly supported outgroup nodes.

Our analyses (Fig. 2) inferred the following set of phylogenetic relationships among studied megophryid taxa:

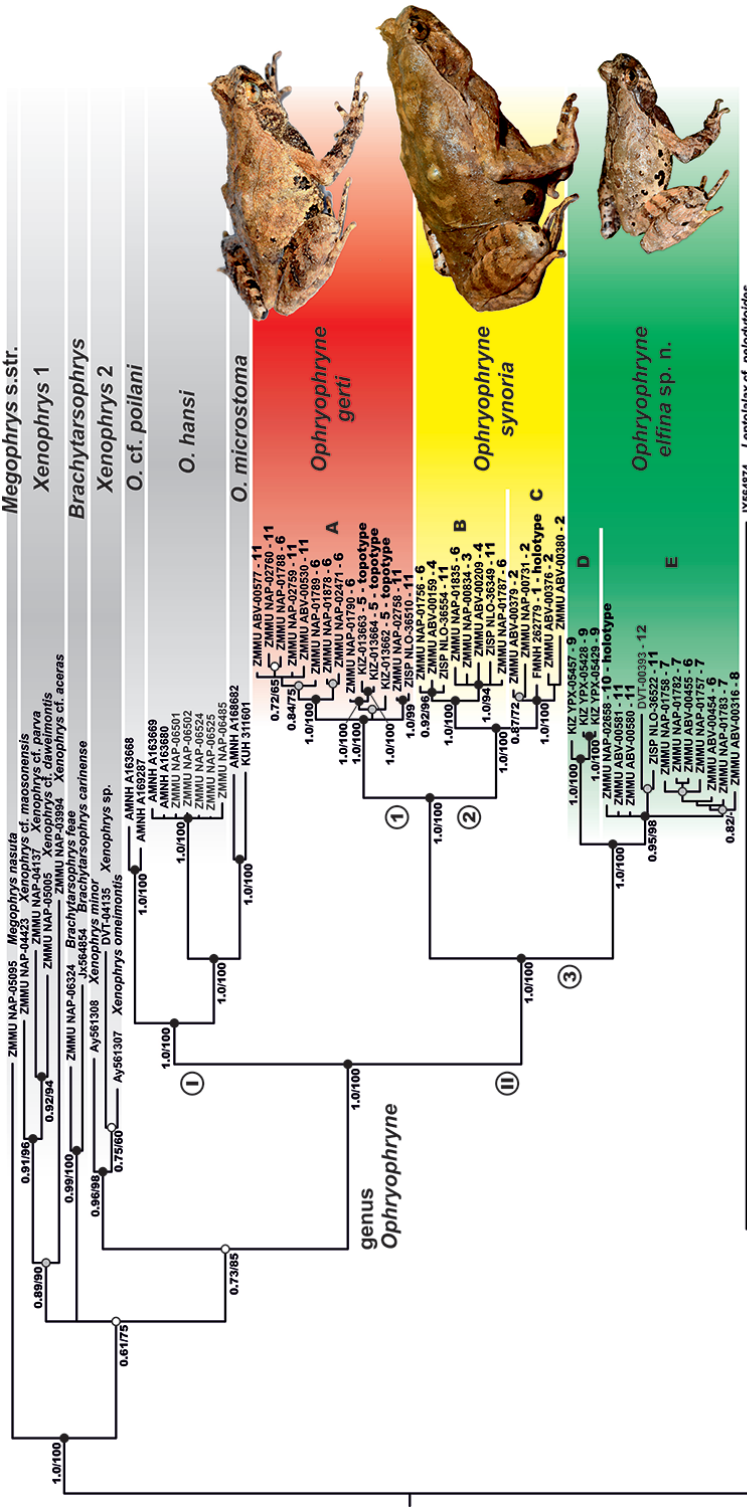


Figure 2. Bayesian inference dendrogram of *Ophryophryne* (including other megophryid outgroups) derived from the analysis of a 2077 bp DNA sequences of the 12S rRNA–16S rRNA mtDNA gene. Voucher specimen IDs and GenBank accession numbers are given in Table 1. Numbers near branches represent BPP/BS support values for BI/ML inferences respectively. Color of clade labels for *Ophryophryne* corresponds to icon colors on Fig. 1. **I** and **II** represent Group I and Group II defined in the results section; numbers **1**, **2** and **3** next to nodes in Group II represent species level clades; **A–E** represent subclades within species level clades.

Our data confirm the monopyly of Megophryinae with respect to outgroup taxa (*Leptobrachium* and *Leptolalax*) (1.0/100; hereafter node support values are given for BPP/BS respectively). Within Megophryinae, the sample of *Megophrys nasuta* (Schlegel, 1858), representing the genus *Megophrys s. stricto*, forms the most basal split; this lineage is recovered as a sister group with respect to all other Megophryinae. Phylogenetic relationships among other genera of Megophryinae remain essentially unresolved; while monophyly of *Brachytarsophrys* received high support (0.99/100), species assigned to *Xenophrys* form two weakly supported groups, paraphyletic with respect to *Ophryophryne* and *Brachytarsophrys*.

Monophyly of the genus *Ophryophryne* is strongly supported by all analyses (1.0/100). General topology of the Bayesian tree suggests that the genus *Ophryophryne* is divided into two major groups: the first group joins taxa from southern China, northern and central Indochina (Group I, see Fig. 2), while the second group comprises lineages of *Ophryophryne* confined to the Langbian Plateau in southern Vietnam and adjacent Cambodia (Group II, see Fig. 2). Both clades are reciprocally monophyletic with high support values (1.0/100).

Within Group I, the clade consisting of two specimens from central Vietnam (AMNH A-169287, Thua Thien-Hue Prov., and AMNH A-163668, Quang Nam Prov.: identified as *O. gerti* by Bain et al. 2007) forms a sister clade to all other species in this group (1.0/100). This lineage is determined to be distantly related to the topotype specimens of *O. gerti* from the Langbian Plateau, and based on examination of these specimens we tentatively regard these specimens as *O.* cf. *poilani* (see discussion below).

Within Group I, specimens of *O. microstoma* from southern China (KUH 311601) and northern Vietnam (AMCC 141231) are clustered together (1.0/100) forming a sister clade to a group comprising specimens identified as *O. hansi* Ohler, 2003, from Kon Tum Plateau in central Vietnam; monophyly of the latter group is also strongly supported (1.0/100) (Fig. 2).

Within Group II, Subclade A joins the medium-sized specimens from environs of the type locality of *O. gerti* (Langbian Mt., environs of Da Lat city, Lam Dong Prov., Vietnam, up to 1800 m a.s.l.; see Fig. 1: Loc. 5) with two other populations found at medium elevation (1000–1500 m a.s.l.) on the eastern (Bidoup–Nui Ba N.P., Lam Dong Prov., Vietnam, see Fig. 1: Loc. 6) and northern (Chu Yang Sin N.P., Dak Lak Prov., Vietnam; see Fig. 1: Loc. 11) edges of the Langbian Plateau (Fig. 2). In the present paper we treat this lineage as the species level clade representing *O. gerti s. stricto* (see discussion below).

The second species level clade comprises large-sized *Ophryophryne* from comparatively lowland populations in the western foothills of the Langbian Plateau (Fig. 1, Locs 1–4, < 1000 m a.s.l.) and large-sized *Ophryophryne* from medium elevations in the northern parts of the plateau (Fig. 1, Locs 6 and 11; 1000–1500 m a.s.l.). Two reciprocally monophyletic (1.0/100) subclades are revealed in this clade (Fig. 2). Subclade B joins montane populations from 1000–1500 m a.s.l. (Fig. 1, Locs 4, 6 and 11) and a recently discovered lowland (200 m a.s.l.) population from Cat Tien N.P. in Dong Nai Prov. Subclade C joins two populations from the westernmost edge of the Langbian Plateau, which include the holotype of *O. synoria* from Mondolkiri Prov. in eastern

Cambodia (Fig. 1, Loc. 1; 500 m a.s.l.) and a population from Bu Gia Map N.P. in the adjacent Vietnamese province of Binh Phuoc (Fig. 1, Loc. 2; 400 m a.s.l.). These localities are close to each other and geographically belong to one hilly region on the western edge of the Langbian Plateau. Based on phylogenetic and morphological data we herein regard both subclades B and C as the species level clade representing *O. synoria*.

The third species level clade forms a sister clade with respect to a clade comprised of *O. gerti s. stricto* and *O. synoria* (Fig. 2). It joins small-sized *Ophryophryne* specimens, all collected from both high elevations (> 1750 m a.s.l.) in the northern and eastern parts of the Langbian Plateau (see Fig. 1: Bidoup and Hon Giao Mts., Lam Dong Prov., Locs 6–7; and Chu Pan Fan and Chu Yang Sin Mts., Dak Lak Prov., Locs 10–11), and from lower elevations (700–1510 m a.s.l.) on the summits of three mountains representing the easternmost outcrops of the Langbian Plateau (see Fig. 1: Hon Ba Mt., Khanh Hoa Prov., Loc. 8; Nui Chua Mt., Ninh Thuan Prov., Loc. 9; Tay Hoa, Phu Yen Prov., Loc. 12). Among these populations, samples from the summit of Nui Chua Mt. (Fig. 1, Loc. 9) form Subclade D (Fig. 2), forming a sister clade with respect to all other populations (Fig. 2, Subclade E; monophyly support 0.95/98). This clade of small-sized *Ophryophryne* from the northern and eastern parts of the Langbian Plateau currently cannot be assigned to any of the recognized species and represents a new species described herein.

Intra- and interspecific differentiation of mtDNA haplotypes

The observed interspecific sequence divergence within the genus *Ophryophryne* varied from p = 4.1% to p = 13.0% (Table 2). The values of uncorrected genetic p-distances in ingroup and outgroup comparisons slightly overlapped: sequence divergence between *Ophryophryne* and outgroup taxa varied from p = 8.8% to p = 24.7%. The minimal interspecific p-distance between recognized nominal species in our analysis was found between the sister species *O. gerti* and *O. synoria* (p = 4.1%–5.0%). The maximum p-distance for *Ophryophryne* was observed between *O. synoria* and *O. hansi* (p = 12.6%–13.0%) (see Table 2). The ABGD analysis revealed the existence of a "barcoding gap" at genetic distance value p = 4.0% in the 16S rRNA gene.

Intraspecific distances within *Ophryophryne* species in our analysis varied from p = 0.5% (in *O. gerti*), to p = 3.3% among two samples of *O.* cf. *poilani* from Quang Nam and Thua Thien-Hue provinces of Vietnam, and to p = 3.7% among two samples of *O. microstoma* from China and Vietnam respectively. The latter two values are higher than usual intraspecific distances in the 16S rRNA gene in Anura (Vences et al. 2005a, 2005b, Vieites et al. 2009); we recognize that identification of these lineages as conspecific is preliminary, based on morphology and topology of the mtDNA tree. Further studies are needed to clarify their taxonomic status. We also found significant genetic differentiation between intraspecific lineages in two of the three *Ophryophryne* species inhabiting the Langbian Plateau. Sequence divergence between Subclades B and C of *O. synoria* is p = 2.6%, while the differentiation between the Nui Chua population (Subclade D) and all other populations of the new species (Subclade E) was even greater at p = 3.1%.

The newly discovered lineage of *Ophryophryne* from highlands of the northern and eastern parts of the Langbian Plateau was found to have the lowest genetic distance with respect to *O. gerti* (p = 8.2%–9.1%). This value is much higher than the minimum genetic distances observed in intraspecific comparisons between species of *Ophryophryne* included in this study (Table 2).

Morphological differentiation

Among the three species examined, mean SVL varied significantly, ranging from 26.9 to 53.7 mm in males and from 35.1 to 70.7 mm in females (Table 3). For SVL, *post hoc* analyses of one-way ANOVA revealed that males were significantly smaller than females in all three species of Langbian *Ophryophryne* (one-way ANOVA, $p < 0.05$; Duncan test, $p < 0.05$). Body size variation among adult males and females of Langbian *Ophryophryne* is shown in Figure 3. All three species are clearly different in body size, with *O. synoria* being the largest, and the undescribed *Ophryophryne* species being the smallest species known for the genus (male SVL values overlap with values for *O. pachyproctus*, N.A. Poyarkov, pers. observ.). *Ophryophryne gerti* occupies an intermediate position between these two species, with SVL values of males (31.7–42.2 mm) slightly overlapping both with those for *O. synoria* (38.2–53.7 mm) and the undescribed *Ophryophryne* species (26.9–33.9 mm).

The results of the multivariate PCA-analysis of the morphometric data are shown in Fig. 4 (data given for males only). The discriminative power of PCA factors derived from analysis of morphometric characters is shown in Appendix 2. For males, F1 explained 77.80% of the variability and F2 explained 5.99%. The two-dimensional plots of both the first two principal components (Factor 1 and Factor 2; Fig. 4A) and the first vs. the third principal components (Factor 1 and Factor 3; Fig. 4B) for males completely discriminated the following three morpho-groups: (I) *O. gerti*, (II) *O. synoria*, and (III) the undescribed *Ophryophryne* species. Our multivariate analysis included meristic data for the holotypes of two *Ophryophryne* species: *O. synoria* (FMNH 262779, male) and *O. gerti* (BMNH 1921.4.1.324, male). Both holotypes were assigned correctly to the respective groups, representing *O. synoria* and *O. gerti*, in full concordance with the results of molecular analyses (Fig. 4).

In summary, both in body size and other meristic characters, all three examined lineages of Langbian *Ophryophryne* form clearly separated morphological groups, also supported by multivariate statistical analysis. The small-sized population of the undescribed *Ophryophryne* species appears to be morphologically distinct from sympatric *O. gerti* and *O. synoria*, as well as from other congeners (see Comparisons for details).

Acoustic differentiation

Measurements of advertisement call parameters for three *Ophryophryne* species found on the Langbian Plateau are given in Table 4, oscillograms and sonograms are given

Table 2. Uncorrected *p*-distance (percentage) between 16S rRNA sequences of *Ophryophryne* species and outgroup species included in phylogenetic analyses (below the diagonal), and standard error estimates (above the diagonal). The ingroup mean uncorrected *p*-distances are shown on the diagonal and shaded with grey.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	*O. gerti* (Subclade A)	**0.5**	1.1	1.1	1.7	1.6	1.9	1.8	1.7	2.0	1.9	1.9	2.1	2.2	2.1	2.2	2.2	2.1	1.9	2.2	2.4
2	*O. synoria* (Subclade B)	4.1	**0.4**	0.9	1.6	1.6	1.9	1.8	1.8	1.8	1.8	1.7	2.0	2.1	2.1	2.2	2.2	2.0	1.9	2.2	2.3
3	*O. synoria* (Subclade C)	5.0	2.6	**0.9**	1.7	1.6	1.8	1.9	1.7	1.8	1.8	1.6	2.0	2.1	2.1	2.1	2.1	2.0	1.9	2.2	2.2
4	*O. elfina* sp. n. (Subclade D)	9.1	9.3	10.0	**0.0**	0.9	1.8	1.6	1.7	2.1	1.8	1.9	2.2	2.0	2.2	2.2	2.3	2.1	2.1	2.3	2.2
5	*O. elfina* sp. n. (Subclade E)	8.2	8.9	8.7	3.1	**0.8**	1.8	1.6	1.6	2.1	2.0	2.1	2.2	1.8	2.3	2.2	2.1	2.1	2.0	2.3	2.3
6	*O. microstoma*	10.9	11.8	11.6	9.7	9.5	**3.7**	1.3	1.5	1.6	1.9	1.7	1.7	1.8	1.9	2.1	2.2	2.0	1.9	2.4	2.4
7	*O. hansi*	10.9	12.6	13.0	9.7	10.0	7.6	**0.0**	1.3	1.7	1.8	1.7	1.9	1.8	2.1	2.2	2.3	1.9	1.8	2.4	2.5
8	*O.* cf. *poilani*	9.1	10.5	10.6	8.6	9.4	7.8	7.3	**3.3**	1.8	1.7	1.7	1.8	1.7	2.0	2.1	2.5	1.9	1.7	2.2	2.2
9	*Xenophrys minor*	12.8	13.0	12.7	10.3	10.9	8.0	10.8	8.8	–	1.8	1.4	1.9	2.0	2.0	2.2	2.1	1.5	1.7	2.3	2.4
10	*Xenophrys* sp.	11.6	12.2	11.9	11.1	11.7	10.3	9.5	8.8	7.8	–	1.3	1.8	1.8	1.8	2.2	2.4	1.7	1.7	2.2	2.2
11	*Xenophrys omeimontis*	10.8	11.4	11.0	9.9	10.2	8.8	9.6	8.4	5.3	3.7	–	1.7	1.8	1.8	2.1	2.1	1.2	1.6	2.2	2.2
12	*Xenophrys* cf. *maosonensis*	13.6	14.7	15.1	14.4	15.2	10.5	12.8	9.7	11.1	10.3	9.9	–	1.4	1.4	1.9	2.2	1.8	1.7	2.4	2.4
13	*Xenophrys* cf. *parva*	13.6	14.2	14.3	11.1	10.7	10.1	11.4	9.7	9.5	9.1	9.1	7.4	–	1.6	1.9	2.3	1.7	1.8	2.5	2.4
14	*Xenophrys* cf. *daweimontis*	12.8	13.4	14.3	14.8	14.5	11.3	14.0	11.5	11.1	9.5	9.1	6.6	6.2	–	2.0	2.3	2.0	2.1	2.6	2.3
15	*Xenophrys* cf. *aceras*	16.5	16.3	16.0	18.1	18.3	16.9	18.9	14.8	14.8	14.0	12.8	13.6	14.4	13.6	–	2.5	2.0	1.9	2.3	2.4
16	*Megophrys nasuta*	14.5	16.0	16.4	16.0	14.5	15.8	17.8	15.8	14.0	14.4	13.6	13.6	13.2	14.8	16.5	–	2.0	2.2	2.6	2.4
17	*Brachytarsophrys feae*	12.8	13.0	13.7	10.7	11.5	11.5	11.7	10.9	7.0	7.4	5.3	11.1	9.1	11.1	12.3	14.4	–	1.4	2.4	2.4
18	*Brachytarsophrys carinense*	11.5	11.4	12.0	11.5	11.5	11.5	11.3	10.1	8.6	7.4	7.4	10.3	9.5	11.9	12.3	12.8	4.9	–	2.2	2.3
19	*Leptolalax*	17.5	18.8	19.4	18.5	19.9	21.0	21.1	18.9	21.8	19.8	21.0	20.2	19.3	21.4	22.6	23.9	20.2	18.9	–	2.2
20	*Leptobrachium*	23.1	23.9	24.0	22.2	21.8	23.5	24.7	23.1	22.8	22.0	21.8	23.3	22.4	23.7	23.7	22.2	20.4	20.8	21.8	–

Table 3. Measurements of the three species of *Ophryophryne* found on the Langbian Plateau (southern Vietnam) and in adjacent Cambodia. For abbreviations see Material and methods. Values are given as means ± SE (min–max), *N* — number of specimens. All measurements are given in mm (continues on next page).

Species	SVL	HW	HL	ED	TYD	TYE	SL	EN	NS	IUE	IN	UEW
Ophryophryne gerti												
Males	35.9 ± 3.4	10.3 ± 0.8	10.2 ± 0.9	4.4 ± 0.4	2.4 ± 0.4	2.0 ± 0.3	3.2 ± 0.5	1.4 ± 0.3	1.6 ± 0.3	3.3 ± 0.5	2.7 ± 0.4	3.1 ± 0.4
N = 15	(31.7–42.2)	(9.2–12.0)	(9.0–12.0)	(3.8–5.3)	(1.8–3.1)	(1.7–2.5)	(2.6–3.8)	(0.7–1.8)	(1.1–2.2)	(2.2–4.1)	(1.9–3.4)	(2.2–3.8)
Females	45.1 ± 2.2	11.3 ± 0.4	11.4 ± 0.6	4.8 ± 0.4	2.7 ± 0.2	2.5 ± 0.2	3.4 ± 0.4	1.6 ± 0.3	1.7 ± 0.4	3.8 ± 0.2	2.8 ± 0.1	3.9 ± 0.3
N = 3	(43.1–47.4)	(10.9–11.7)	(10.9–12.1)	(4.4–5.1)	(2.5–3.0)	(2.3–2.6)	(2.9–3.6)	(1.2–1.9)	(1.4–2.2)	(3.6–4.0)	(2.7–2.9)	(3.5–4.2)
Ophryophryne elfina sp. n.												
Males	29.7 ± 1.8	8.6 ± 0.7	8.9 ± 0.6	3.7 ± 0.3	2.2 ± 0.2	1.5 ± 0.2	2.7 ± 0.4	1.2 ± 0.2	1.4 ± 0.3	2.7 ± 0.3	2.3 ± 0.5	2.9 ± 1.0
N = 29	(26.9–33.9)	(7.2–10.1)	(7.4–9.8)	(3.1–4.3)	(1.6–2.7)	(1.1–2.1)	(2.0–3.3)	(0.8–1.6)	(1.0–2.2)	(2.1–3.4)	(1.5–3.3)	(2.4–7.9)
Females	35.6 ± 0.5	9.7 ± 0.5	9.8 ± 1.1	4.0 ± 0.2	2.3 ± 0.4	1.7 ± 0.1	3.1 ± 0.5	1.4 ± 0.3	1.7 ± 0.3	2.9 ± 0.5	2.5 ± 0.4	2.9 ± 0.2
N = 6	(35.1–36.5)	(9.0–10.2)	(8.6–11.0)	(3.6–4.2)	(1.9–3.0)	(1.6–1.9)	(2.4–3.6)	(1.0–1.7)	(1.2–2.1)	(2.3–3.6)	(1.7–2.9)	(2.6–3.1)
Ophryophryne synoria												
Males	45.7 ± 4.3	13.8 ± 0.9	13.5 ± 1.0	5.1 ± 0.3	3.5 ± 0.5	2.8 ± 0.4	3.8 ± 0.5	1.9 ± 0.4	1.6 ± 0.3	4.5 ± 0.6	3.1 ± 0.3	3.7 ± 0.4
N = 14	(38.2–53.7)	(12.5–15.6)	(12.2–15.4)	(4.7–5.6)	(3.0–4.7)	(1.9–3.6)	(3.0–4.6)	(1.3–2.5)	(1.2–2.2)	(3.5–5.5)	(2.5–3.5)	(3.0–4.1)
Females	60.8 ± 9.7	16.6 ± 1.8	16.1 ± 1.8	5.3 ± 0.4	3.7 ± 0.2	3.3 ± 0.3	4.0 ± 0.3	2.0 ± 0.4	1.8 ± 0.4	4.7 ± 0.2	3.5 ± 0.6	4.1 ± 0.2
N = 3	(51.4–70.7)	(14.6–18.0)	(14.0–17.4)	(5.0–5.7)	(3.4–3.9)	(3.0–3.6)	(3.7–4.3)	(1.6–2.4)	(1.3–2.2)	(4.4–4.9)	(3.0–4.1)	(3.8–4.2)

Table 3 Continued. Measurements of the three species of *Ophryophryne* found on the Langbian Plateau (southern Vietnam) and in adjacent Cambodia. For abbreviations see Material and methods. Values are given as means ± SE (min–max), *N* — number of specimens. All measurements are given in mm.

Species	FAL	HAL	FIL	FIIL	FIIIL	FIVL	SHL	TL	FOL	TFOL	IMT
Ophryophryne gerti											
Males	8.7 ± 1.0	8.7 ± 0.9	3.7 ± 0.6	3.7 ± 0.4	5.9 ± 0.6	3.5 ± 0.6	17.2 ± 2.8	16.8 ± 2.5	14.7 ± 2.4	23.7 ± 2.6	2.0 ± 0.5
N = 15	(7.2–11.0)	(7.2–10.1)	(2.7–5.0)	(3.1–4.4)	(4.2–7.2)	(2.3–4.6)	(14.4–24.6)	(13.9–23.3)	(11.2–18.8)	(19.2–27.6)	(0.9–2.7)
Females	10.4 ± 0.6	10.5 ± 1.4	4.7 ± 0.7	4.3 ± 0.6	7.2 ± 0.5	4.6 ± 0.7	19.7 ± 1.0	19.6 ± 1.6	16.7 ± 0.5	27.0 ± 0.9	2.9 ± 0.2
N = 3	(9.8–10.9)	(9.0–11.9)	(3.9–5.3)	(3.8–4.9)	(6.7–7.7)	(4.1–5.4)	(18.7–20.7)	(17.8–20.6)	(16.3–17.2)	(26.0–27.9)	(2.6–3.0)
Ophryophryne elfina sp. n.											
Males	7.3 ± 0.8	7.0 ± 0.9	2.6 ± 0.5	2.9 ± 0.4	4.8 ± 0.5	3.0 ± 0.6	14.0 ± 1.7	13.7 ± 1.3	11.9 ± 1.0	19.8 ± 2.2	1.6 ± 0.4
N = 29	(6.1–8.9)	(4.8–9.4)	(1.1–3.5)	(2.0–4.1)	(3.7–6.0)	(1.4–4.1)	(11.7–19.6)	(11.3–16.1)	(10.4–14.7)	(17.1–28.0)	(1.2–2.5)
Females	9.0 ± 1.1	8.8 ± 0.7	3.7 ± 0.7	3.9 ± 0.5	5.8 ± 0.8	4.1 ± 0.9	17.1 ± 2.0	16.3 ± 1.0	14.9 ± 0.7	24.3 ± 2.1	2.0 ± 0.3
N = 6	(7.7–10.1)	(7.7–9.7)	(2.9–4.5)	(3.3–4.7)	(4.9–7.0)	(3.1–5.2)	(14.9–19.8)	(15.5–17.8)	(13.9–15.7)	(21.8–27.2)	(1.5–2.4)
Ophryophryne synoria											
Males	12.6 ± 1.3	11.0 ± 1.1	4.6 ± 0.6	4.7 ± 0.6	7.5 ± 0.6	4.5 ± 0.6	20.6 ± 1.6	20.7 ± 2.2	18.4 ± 2.0	29.3 ± 2.8	2.5 ± 0.4
N = 14	(10.4–14.7)	(9.2–11.1)	(3.7–5.8)	(3.7–5.6)	(6.2–8.4)	(3.6–5.5)	(17.7–23.3)	(18.1–25.1)	(15.0–22.4)	(24.0–33.5)	(1.9–3.1)
Females	14.3 ± 1.0	13.7 ± 3.3	5.1 ± 0.5	5.9 ± 1.0	8.4 ± 0.9	5.1 ± 1.4	23.4 ± 2.0	24.3 ± 1.5	20.7 ± 4.6	32.3 ± 5.0	3.2 ± 0.4
N = 3	(13.6–15.5)	(11.0–17.4)	(4.7–5.7)	(5.1–7.0)	(7.6–9.3)	(4.3–6.7)	(21.3–25.3)	(22.7–25.7)	(15.7–24.8)	(26.8–36.4)	(2.8–3.6)

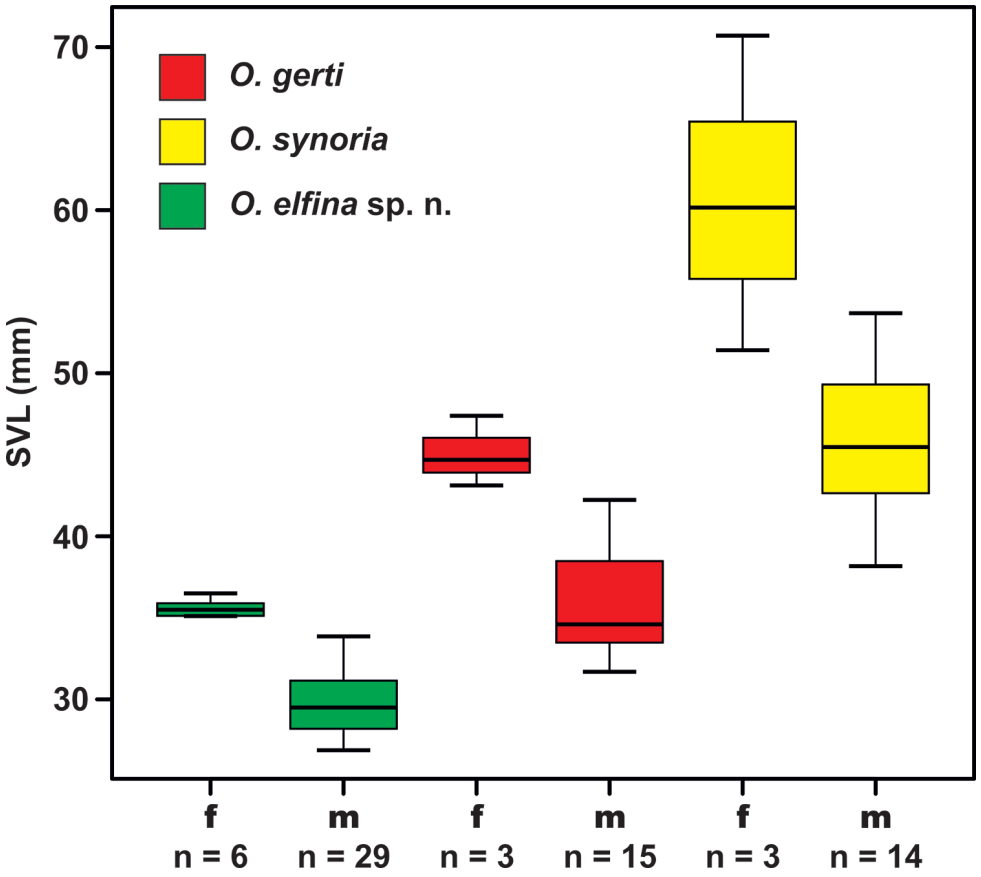


Figure 3. Boxplots of SVL showing body size variation among adult *Ophryophryne* males and females from the Langbian Plateau. Horizontal lines within each box represent the median, and boxes encompass the 75th and 25th quartiles. Color of boxes/*Ophryophryne* species corresponds to icon colors on Figs 1, 2.

in Fig. 5. Advertisement calls of all three *Ophryophryne* species found on the Langbian Plateau represent a series of high whistling sounds, resembling vocalizations of passerine birds. All males vocalized in the evening from dusk until late night. The males of the undescribed *Ophryophryne* species called from stone walls above small streams or from fern leaves, the males of *Ophryophryne gerti* usually called while sitting on bush branches and fern leaves above small streams, and the males of *Ophryophryne synoria* called from stones on the rocky stream banks.

Advertisement calls of all studied species were similarly uttered in series (Fig. 5). The call repetition rate/recording/series were one of the most significant differentiating parameters between the three species (Table 4). Parameters values varied both between and within recordings for each of the three species. Though some parameters values overlapped between species, the means of most of the parameters differed significantly.

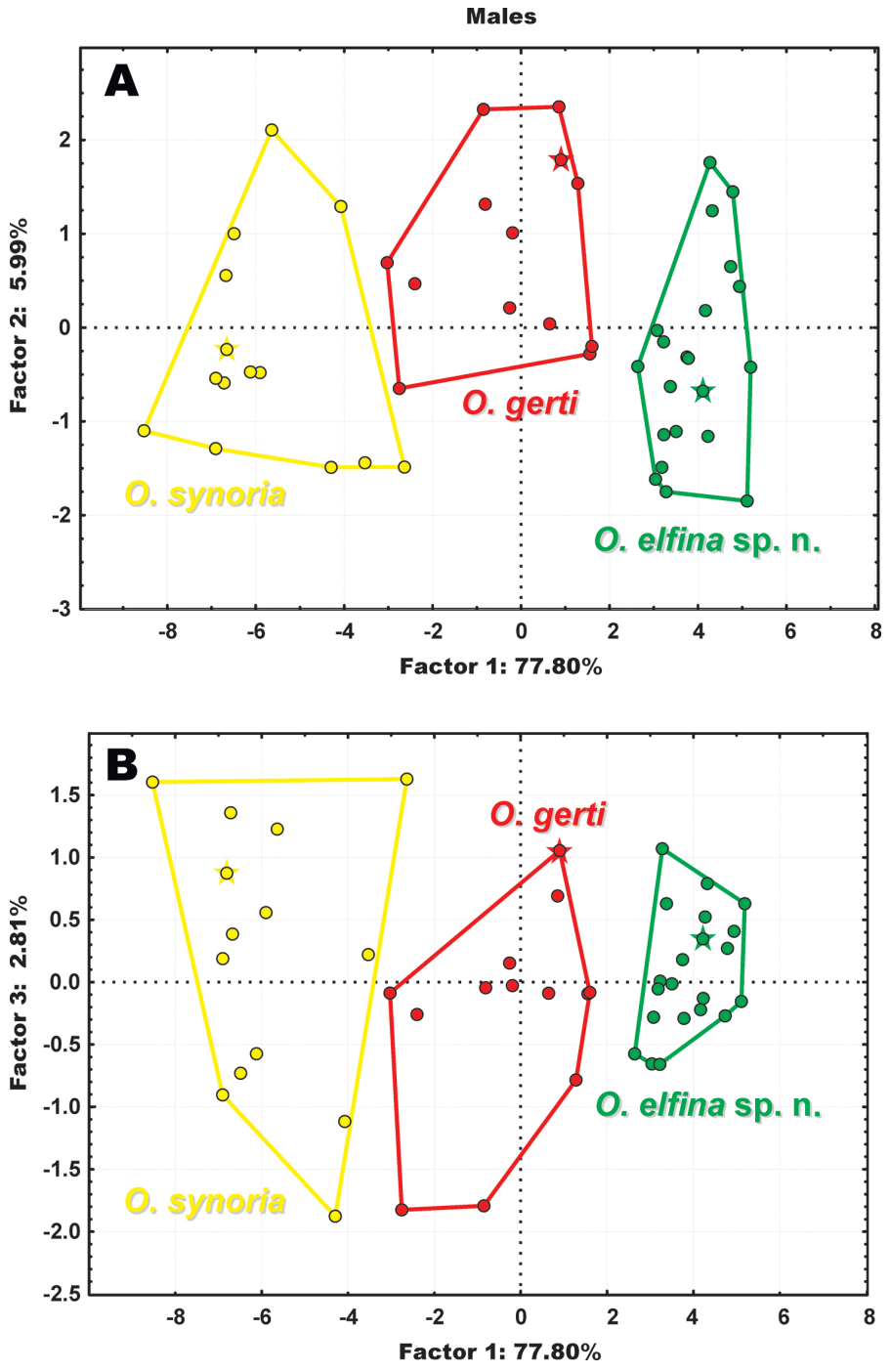


Figure 4. Two-dimensional plots of **A** the first two factors of PCA, and **B** the first and third factors of morphological characters for the *Ophryophryne* specimens examined. Data are given for males only. Star indicates the holotype specimen for each species. Circle color corresponds to those given in Fig. 1.

The frequency of maximum amplitude always coincided with the fundamental frequency and greatly varied within recordings: from 4030 to 4920 Hz for the undescribed *Ophryophryne* species, from 4450 to 5100 Hz for *O. gerti*, and from 3600 to 3890 Hz for *O. synoria*. The values of the maximum amplitude frequency in *O. synoria* were the lowest and least variable among the three species. The minimum fundamental frequency always coincided with the initial fundamental frequency whereas the maximum fundamental frequency either could coincide with the final fundamental frequency, or was close to it. Thus, the frequency modulation was expressed either in lift of fundamental frequency during the whole call, or in an unsymmetrical arch with the peak shifted to the end of the frequency band. The form of frequency modulation varied between these two forms for each species but arch-formed calls appeared most of time in *O. synoria*. The frequency range expressing depth of frequency modulation also varied within each species' calls: from 40 to 840 Hz for the undescribed *Ophryophryne* species, from 370 to 890 Hz for *O. gerti* and from 230 to 650 Hz for *O. synoria* (Table 4).

Number of harmonics varied between/within recordings but this characteristic mostly depended on recording quality (e.g., sensitivity of recording equipment, distance from vocalizing animal, signal volume and background noise). Calls from the highest quality recordings (of the undescribed *Ophryophryne* species) contained two harmonics but a major portion of other calls contained only one harmonic.

We had two sets of the undescribed *Ophryophryne* species recordings which were made at different temperatures (11.3–11.4°C in February and 17.0–17.5°C in April). Values of frequency call parameters didn't significantly differ between the two sets. However, statistically significant differences were found in several temporal parameters of the calls (data summarized in Appendix 3). For instance, the number of calls per series was 10.53 ± 0.72 (3–21, $N = 47$) in February vs. 14 ± 0.45 (2–22, $N = 93$) in April ($F_{1.138} = 18.2$, $p < 0.001$, one-way ANOVA), the inter-call duration comprised 271 ± 4.92 ms (102–942 ms, $N = 448$) in February vs. 184 ms ± 1.72 (96–621 ms, N = 1209) in April ($H_{1.1657} = 349.2$, $p < 0.001$, Kruskal-Wallis ANOVA), the call repetition rate per series was 3.18 ± 0.1 calls/s (1.33–4.91 calls/s, $N = 47$) in February vs. 4.22 ± 0.08 calls/s (2.92–5.49 calls/s, $N = 93$) in April ($F_{1.138} = 63.9$, $p < 0.001$, one-way ANOVA), call duration was also significantly longer in February 79 ± 0.32 ms (48–102 ms, $N = 496$) than in April 70 ± 0.27 ms (25–112 ms, $N = 1301$) ($H_{1.1798} = 380.2$, $p < 0.001$, Kruskal-Wallis ANOVA). April and February recordings showed no significant differences in series duration, inter-series interval duration, and call repetition rate per recording.

Taxonomic implications

Our study, based on three lines of evidence — phylogenetic analysis and distribution of mtDNA haplotypes (Figs 1, 2), multivariate statistical analysis of 23 standard morphometric traits (Fig. 4), and acoustic analysis of advertisement calls (Fig. 5), strongly indicate the presence of three independent and distinct evolutionary lineages of *Ophryophryne* on

Table 4. Measurements of advertisement call parameters for three species of *Ophryophryne* from the Langbian Plateau, and one-way ANOVA/Kruskal-Wallis results for comparison (*p < 0.001) between *Ophryophryne elfina* sp. n., *O. gerti* and *O. synoria*. Parameter values are given as means ± SE (min–max). Abbreviations: *N* — number of recordings/series/calls, F — frequency, s — seconds, ms — milliseconds, Hz — hertz (continues on next page).

Parameters	*O. elfina* sp. n.	*O. gerti*	Tukey/ Mann-Whitney U post hoc tests	*O. synoria*	Tukey/ Mann-Whitney U post hoc tests	ANOVA/ Kruskal-Wallis results
Number of males	3	3	–	3	–	–
Number of recordings	5	3	–	3	–	–
Number of series	140	115	–	15	–	–
Number of calls	1797	533	–	200	–	–
Call repetition rate per recording (calls/s)	1.18 ± 0.2 (0.77–1.95) *N* = 5	0.35 ± 0.14 (0.11–0.59) *N* = 3	p < 0.05	3.07 ± 0.13 (2.82–3.24) *N* = 3	p < 0.001	$F_{2.8}$ = 46.7*
Number of calls per series	12.84 ± 0.41 (2–22) *N* = 140	4.64 ± 0.16 (1–8) *N* = 115	p < 0.001	13.33 ± 1.4 (3–24) *N* = 15	p < 0.05	$F_{2.267}$ = 151.4*
Series duration (s)	3.42 ± 0.11 (0.43–9.00) *N* = 140	2.18 ± 0.09 (0.57–8.31) *N* = 108	p < 0.001	2.59 ± 0.33 (0.62–5.21) *N* = 15	p = 0.88	$F_{2.267}$ = 40.4*
Call repetition rate per series (calls/s)	3.87 ± 0.07 (1.33–5.49) *N* = 140	2.33 ± 0.03 (0.96–3.53) *N* = 108	p < 0.001	5.34 ± 0.15 (4.4–6.59) *N* = 15	p < 0.001	$F_{2.260}$ = 220.7*
Call duration (ms)	73 ± 0.23 (25–112) *N* = 1797	104 ± 0.56 (75–152) *N* = 533	p < 0.001 (U = 12535.5)	62 ± 0.46 (37–85) *N* = 200	p < 0.001 (U = 64227.5)	$H_{2.2530}$ = 1345.1*
Inter-calls interval (ms)	207 ± 2.06 (96–942) *N* = 1657	421.54 ± 4.17 (275–813) *N* = 418	p < 0.001 (U = 24725.5)	143 ± 3.32 (56–528) *N* = 185	p < 0.001 (U = 64860)	$H_{2.2260}$ = 1008.5*
Inter-series interval (s)	6.51 ± 0.41 (1.26–31.65) *N* = 135	7.98 ± 0.55 (0.65–39.76) *N* = 112	p < 0.001 (U = 5593.5)	1.64 ± 0.19 (0.88–2.83) *N* = 12	p < 0.001 (U = 51)	$H_{2.259}$ = 42.7*

Parameters	*O. elfina* sp. n.	*O. gerti*	Tukey/ Mann-Whitney U post hoc tests	*O. synoria*	Tukey/ Mann-Whitney U post hoc tests	ANOVA/ Kruskal-Wallis results
F initial (Hz)	4348.02 ± 2.96 (3980–4680) *N* = 1797	4414.17 ± 5.12 (4070–4640) *N* = 533	p < 0.001 (U = 332858)	3449.55 ± 6.41 (3230–3700) *N* = 200	p < 0.001 (U = 0)	$H_{2.2530}$ = 655.7*
F final (Hz)	4715.3 ± 3.29 (4260–5010) *N* = 1797	4888.76 ± 3.82 (4640–5150) *N* = 533	p < 0.001 (U = 142738)	3708.9 ± 9.28 (3420–3980) *N* = 200	p < 0.001 (U = 0)	$H_{2.2530}$ = 1075.9*
F maximum (Hz)	4807.74 ± 3.46 (4260–5060) *N* = 1797	4998.74 ± 4.27 (4780–5250) *N* = 533	p < 0.001 (U = 138045)	3907.05 ± 4.22 (3750–4070) *N* = 200	p < 0.001 (U = 0)	$H_{2.2530}$ = 1094.2*
F minimum (Hz)	4348.02 ± 2.96 (3980–4680) *N* = 1797	4414.17 ± 5.12 (4070–4640) *N* = 533	p < 0.001 (U = 309752.5)	3449.55 ± 6.41 (3230–3700) *N* = 200	p < 0.001 (U = 0)	$H_{2.2530}$ = 689.5*
F range (Hz)	459.71 ± 3.27 (40–840) *N* = 1797	584.58 ± 3.87 (370–890) *N* = 533	p < 0.001 (U = 229934)	457.5 ± 5.86 (230–650) *N* = 200	p = 0.61 (U = 175721)	$H_{2.2530}$ = 367.1*
F peak (Hz)	4645.94 ± 4.39 (4030–4920) *N* = 1797	4845.99 ± 4.22 (4450–5100) *N* = 533	p < 0.001 (U = 157981.5)	3798.9 ± 4.87 (3600–3890) *N* = 200	p < 0.001 (U = 0)	$H_{2.2530}$ = 1030.2*

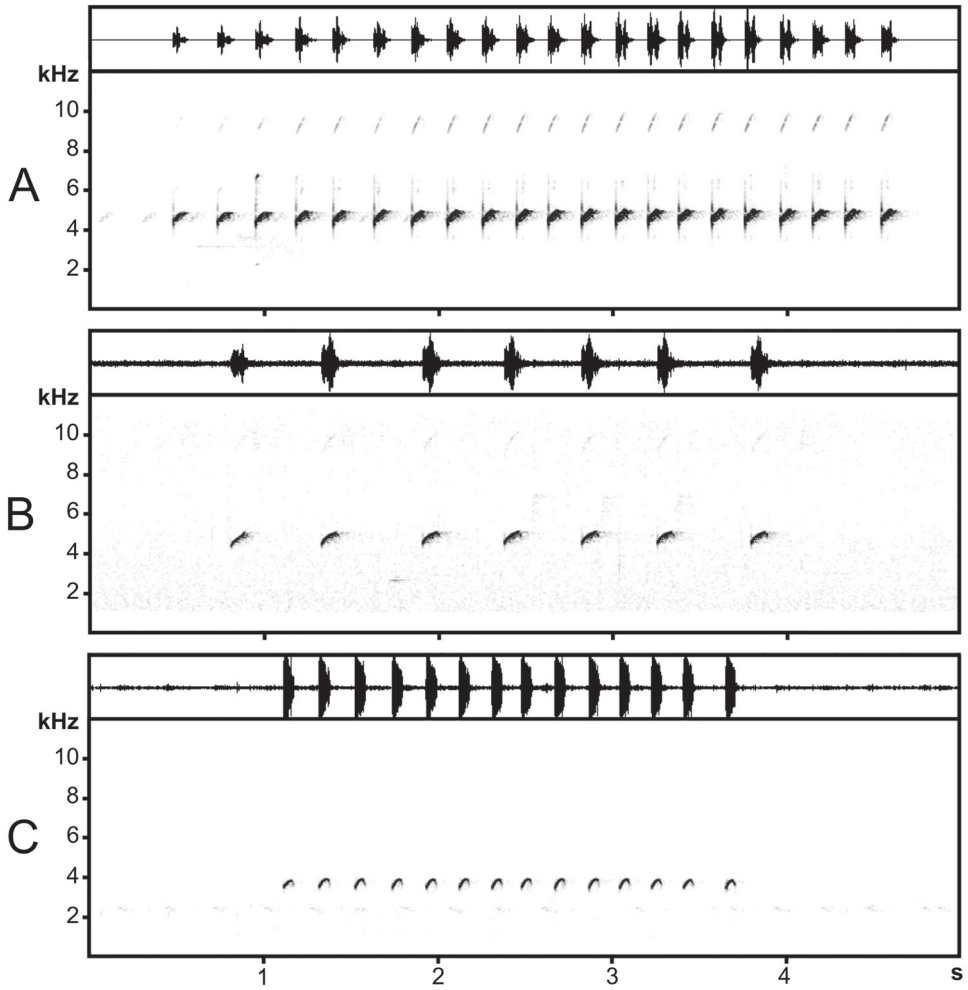


Figure 5. Oscillograms (top) and sonograms (bottom) of male advertisement calls of **A** *Ophryophryne elfina* sp. n. (Lam Dong Prov., Bidoup-Nui Ba N.P., 1935 m a.s.l., 17°C) **B** *O. gerti* (Dak Lak Prov., Chu Yang Sin N.P., 1020 m a.s.l., 22°C) **C** *O. synoria* (Dak Lak Prov., Chu Yang Sin N.P., 750 m a.s.l., 21°C). The sampling rate lowered to 22.05 kHz.

the Langbian Plateau and in surrounding areas of southern Truong Son Mountains in southern Vietnam and eastern Cambodia. Our examination of type material allowed us to identify the two larger lineages as *O. synoria* and *O. gerti*, whereas the smaller species of *Ophryophryne* represents an undescribed taxon (Fig. 6). In two of the 12 surveyed localities (see Fig. 1) all three species were recorded in sympatry, with *O. synoria* and *O. gerti* recorded syntopically in the same streams in environs of Giang Ly Ranger Station, Bidoup–Nui Ba N.P., Lam Dong Prov. (Loc. 6, Fig. 1) and in Chu Yang Sin N.P. in Dak Lak Prov. (Loc. 11, Fig. 1); the small-sized undescribed *Ophryophryne* species was usually found in higher elevations, but was nevertheless recorded synbiotopically with *O. gerti*

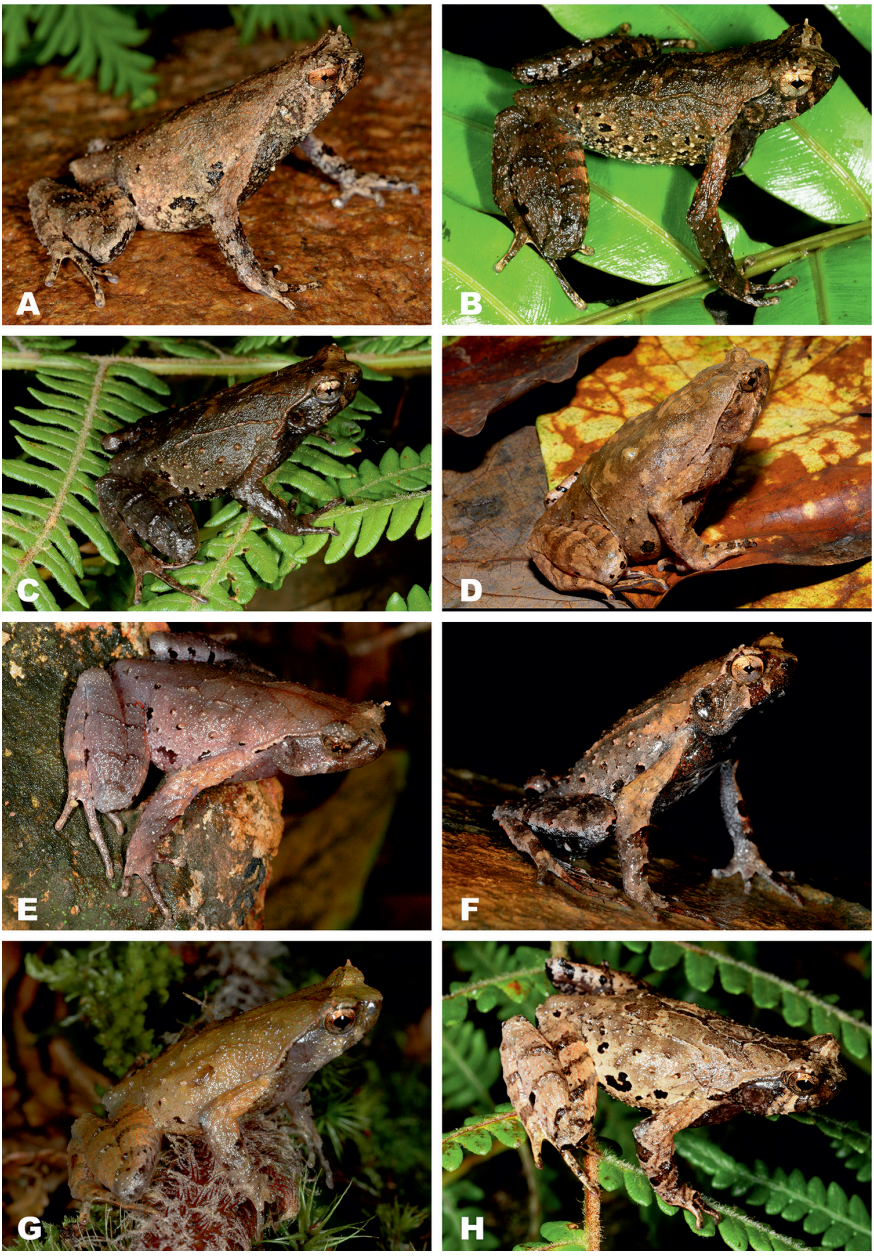


Figure 6. *Ophryophryne* species of the Langbian Plateau (Vietnam): **A** *O. gerti*, female, Chu Yang Sin N.P., Dak Lak Prov., 1000 m a.s.l. **B** *O. gerti*, male, Chu Yang Sin N.P., Dak Lak Prov., 1000 m a.s.l. **C** *O. gerti*, male, Bidoup–Nui Ba N.P., Lam Dong Prov., 1550 m a.s.l. **D** *O. synoria*, female, Bu Gia Map N.P., Binh Phuoc Prov., 400 m a.s.l. **E** *O. synoria*, male, Chu Yang Sin Mt., Chu Yang Sin N.P., Dak Lak Prov., 1000 m a.s.l. **F** *O. synoria*, male, Bidoup–Nui Ba N.P., Lam Dong Prov., 1550 m a.s.l. **G** *Ophryophryne elfina* sp. n., male, Chu Yang Sin Mt., Chu Yang Sin N.P., Dak Lak Prov., 2000 m a.s.l. **H** *Ophryophryne elfina* sp. n., male, Hon Giao Mt., Bidoup–Nui Ba N.P., Lam Dong and Khanh Hoa provincial border, 2000 m a.s.l. Photos by N.A. Poyarkov and N.L. Orlov.

in Chu Yang Sin N.P. in Dak Lak Prov. (Loc. 11, Fig. 1). Below we provide taxonomic remarks on *Ophryophryne* species of the Langbian Plateau along with the description of the new species.

Taxonomic remarks on *Ophryophryne gerti* Ohler, 2003

Chresonymy:

[?] *Ophryophryne microstoma* — Orlov et al. 2002:84 (partim—Dac Lac [Dak Lak] and Lam Dong provinces, Vietnam; no reffered specimens indicated, inclusion *fide* N.L. Orlov).

Ophryophryne gerti Ohler 2003:25, fig. 1 (partim—BMNH 1921.4.1.324, BMNH 1921.4.1.323).

[?] *Ophryophryne gerti* — Ohler 2003:25; Stuart et al., 2006:135 (FMNH 252899, FMNH 252901).

[?] *Ophryophryne microstoma* — Nguyen et al. 2005:15 (partim—"Dak Lak and Lam Dong" provinces, Vietnam; following indication by Orlov et al. 2002).

"*Ophryophryne* sp. 2" — Orlov et al. 2008:82 (Chu Yang Sin N.P., Dak Lak Prov., Vietnam; ZISP 12836–12879).

Ophryophryne gerti — Nguyen et al. 2009:84–85 (partim—"Lam Dong (Cam Li, Dran)", Vietnam).

[?] *Ophryophryne microstoma* — Nguyen et al. 2009:86 (partim—"Dak Lak, Lam Dong", Vietnam; based on the record by Orlov et al. 2002).

[?] *Ophryophryne gerti* — Stuart et al. 2010:40 (eight uncataloged "topotype" females from Bidoup–Nui Ba N.P., Langbian Plateau, Vietnam).

Ophryophryne gerti — Poyarkov [Paiarkov] and Vassilieva 2011:202 (Bidoup–Nui Ba N.P., Lam Dong Prov., Vietnam; ZMMU A-4715, ZMMU A-4718).

Removed from chresonymy:

Ophryophryne gerti Ohler 2003:25 (partim—BMNH "1972.15.2.4" [sic. BMNH 1972.1524])

Ophryophryne gerti — Stuart 2005:475 (FMNH 258564)

Ophryophryne gerti — Bain et al. 2007:108 (AMNH A-169287, AMNH A-163668)

[?] *Ophryophryne gerti* — Orlov et al. 2015:2010, fig. 9 (Gia Lai Prov., Vietnam; based on identification of the specimen illustrated in fig. 9 as *Ophryophryne* cf. *poilani sensu auctorum*).

Holotype. BMNH 1921.4.1.324, adult male from "Cam Ly (river), south-east of Da Lat (11°56'N; 108°25'E), Lang Bian Plateau, sLam [sic. = Lam] Dong Province, Vietnam", collected by M.A. Smith, presented to BMNH in 1921 (Ohler 2003; NHMUK specimen catalogue). Re-examined by SM.

Paratypes. BMNH 1921.4.1.323, immature female from "Dran (11°50'N; 108°34'E), Lang Bian Plateau, Lam Dong Province, Vietnam", collected by M.A.

Smith, presented to BMNH in 1921; BMNH 1972.1524 (see 'Remarks') adult male, "Huey Sapan, Pak Maat (precise location not found), Mekong, Laos", collector M.A. Smith, accessioned in the BMNH from Smith's private collection in 1972, collection date unknown (Ohler 2003; NHMUK specimen catalogue).

Remarks. Ohler (2003) provides the paratype number BMNH 1972.15.2.4 for the Laos paratype specimen, however, the number on the specimen tag and NHMUK specimen catalogue reads BMNH 1972.1524 (Mahony 2011b). Our present data suggests that *O. gerti* is not found beyond the limits of the Langbian Plateau, thus we are confident that the paratype BMNH 1972.1524 does not represent this biological taxon. Ohler (2003: and by implication Stuart et al. 2006) preliminarily identified two specimens from Ankhe Dist. in northern Gia Lai Prov. as *O. gerti*. This locality is disconnected from the Langbian highlands by a wide lowland area, indicating that these specimens are biogeographically isolated from *O. gerti s. stricto*. Further work is necessary to ascertain the taxanomic status of these specimens. Orlov et al. (2002), without providing data on examined specimens, considered that the distribution of *O. microstoma* extended south in Vietnam as far as the Lam Dong and Dak Lak provinces. Our results suggest that these southern Vietnamese populations most likely represented the superficially similar *O. gerti*, *O. synoria*, or possibly the new taxon described below. Stuart (2005) reports *O. gerti* from Champasak Prov. in southern Laos, based on a single specimen (FMNH 258564: not examined here). This locality is biogeographically not connected to the known range of this species, as redefined here, thus the taxonomic status of this specimen requires further confirmation. Bain et al. (2007) identifies two specimens AMNH A-169287 (Thua Thien-Hue Prov., Vietnam) and AMNH A-163668 (Quang Nam Prov., Vietnam) as *O. gerti*. We re-examined these specimens and regard them to be morphologically more similar to *O. poilani*. Furthemore, both specimens were included in our molecular analysis and found to be distantly related to *O. gerti s. stricto* (Fig. 2). Stuart et al. (2010) provides the SVL range (SVL 37.5–42.5 mm, mean ± SD 40.4 ± 1.6, N = 8) for 'topotype' female specimens of *O.* "*gerti*", however these specimens are smaller than females of *O. gerti* provided herein (SVL 43.1–47.4 mm, mean ± SD 45.07 ± 2.16, N = 3), and larger than the new taxon described below (SVL 35.1–36.5 mm, mean ± SD 35.6 ± 0.5, N = 6) (see Table 3 for details). The taxonomic status of these specimens remains unknown.

Vernacular name. English: "*Gerti's Mountain Toad*"; Vietnamese: "*Cóc Núi Got*" (Nguyen et al. 2009), "*Cóc Núi Goti*" (Nguyen et al. 2014).

Redescription of the holotype. Mature male (SVL 35.7 mm), habitus slender (Fig. 7A, B). Specimen in good state of preservation; two incisions are present on trunk, one longitudinally orientated on mid-abdomen, another longitudinally oriented on upper flank on right side; liver and testes observable through incisions, testes enlarged; jaw is dislocated on right allowing visual access to buccal cavity.

Head moderately small (HL/SVL 25.5%; HW/SVL 29.7%), wider than long (HW/HL 116.5%), relatively deep; top of head flat; snout comparatively short (SL/HL 33.0%), truncated in dorsal view (Fig. 7A), projecting significantly beyond margin of lower jaw in profile (Fig. 7C); loreal region vertical; canthus rostralis distinct, moderately sharp; dorsal region of snout flat; eyes large (ED/HL 38.5%), slightly protuberant in dorsal view and in profile, horizontal eye diameter less than twice as long

as maximum tympanum diameter (TYD/ED 62.9%) and longer than snout (ED/SL 116.7%); eye to tympanum distance approximately equal to maximum tympanum diameter (TYE/TYD 95.5%); tympanum distinct, circular, moderately large (TYD/HL 24.2%); pupil in preservation oval (Fig. 7C), vertically orientated; nostril opening oval, vertical, laterally orientated, medially located between eye and snout (EN/NS 100.0%); internarial distance subequal to upper eyelid width (IN/UEW 103.2%), and significantly less than narrowest point between upper eyelids (IN/IUE 145.5%); pineal ocellus not visible externally; vomerine ridges absent; maxillary and vomerine teeth absent; vocal sac gular, its' openings not discernable; tongue moderately large, posterior end free, with weak notch posteriorly, lacking medial lingual process.

Forelimbs slender, forearm moderately long (FAL/SVL 22.4%) slightly enlarged relative to upper forelimb, and shorter than hand (FAL/HAL 95.2%); fingers long and narrow, dorsoventrally flattened; lateral fringes and webbing absent (Fig. 7D); finger length formula F1 = F2 < F4 < F3; subarticular tubercles absent, replaced by low callous dermal ridges; supernumerary tubercles absent; outer palmar (metacarpal) tubercle weak, longitudinally oval, elevated but with indistinct borders (Fig. 7D); thenar tubercles weak; finger tips rounded, weakly expanded relative to digit widths (wider than distal-most finger articulation), with circular pads (Fig. 7D); terminal grooves absent.

Hindlimbs slender, relatively long, shanks overlap when thighs are held at right angle to body; shank length less than half of snout to vent length (SHL/SVL 44.5%); thighs shorter than shanks (SHL/TL 106.0%), and feet (FOL/TL 106.4%); toes long and slightly dorsoventrally flattened (Fig. 7E), relative toe lengths T1 < T2 < T5 < T3 < T4; lateral fringes on toes, outer metatarsal tubercle, subarticular and supernumerary tubercles absent; inner metatarsal tubercle well developed with distinct borders, oval-shaped (IMT/FOL 11.3%) (Fig. 7E); weak ridge of callous tissue present on ventral surface of all toes, not continuing onto metatarsus; webbing between digits rudimentary; tarsal fold absent; toe tips not expanded relative to digit widths, with circular pads; terminal grooves absent.

Skin texture and skin glands in preservation. Skin of dorsal and lateral surfaces of head, body and limbs smooth with numerous small tubercles finely and relatively evenly scattered on dorsal surfaces of trunk, head and limbs (Fig. 7A); small tubercles present on temporal region, tympanum smooth with borders weakly raised; tubercles arranged in distinct transverse ridges on dorsal surfaces of forearms, shanks and thighs; numerous large tubercles on flanks irregularly scattered from axilla to groin, intermixed with smaller tubercles; central portion of outer edge of upper eyelids slightly thickened, with a single short tubercular spine (Fig. 7C), transverse fold on posterior edges of upper eyelids absent; well-developed glandular supratympanic folds, narrow anteriorly, considerably widening posteriorly, from posterior corner of orbits, extending along upper margin of tympanum, terminating above forelimb insertions (Fig. 7C); dorsolateral glandular ridge well-developed, extending from posterior to supratympanic ridges to ca. 75% of trunk length, on each side; a moderately well-developed " >–< " shaped glandular parietoscapular-sacral ridge present on dorsum (see Fig. 7A); two small tubercles present above vent; gular

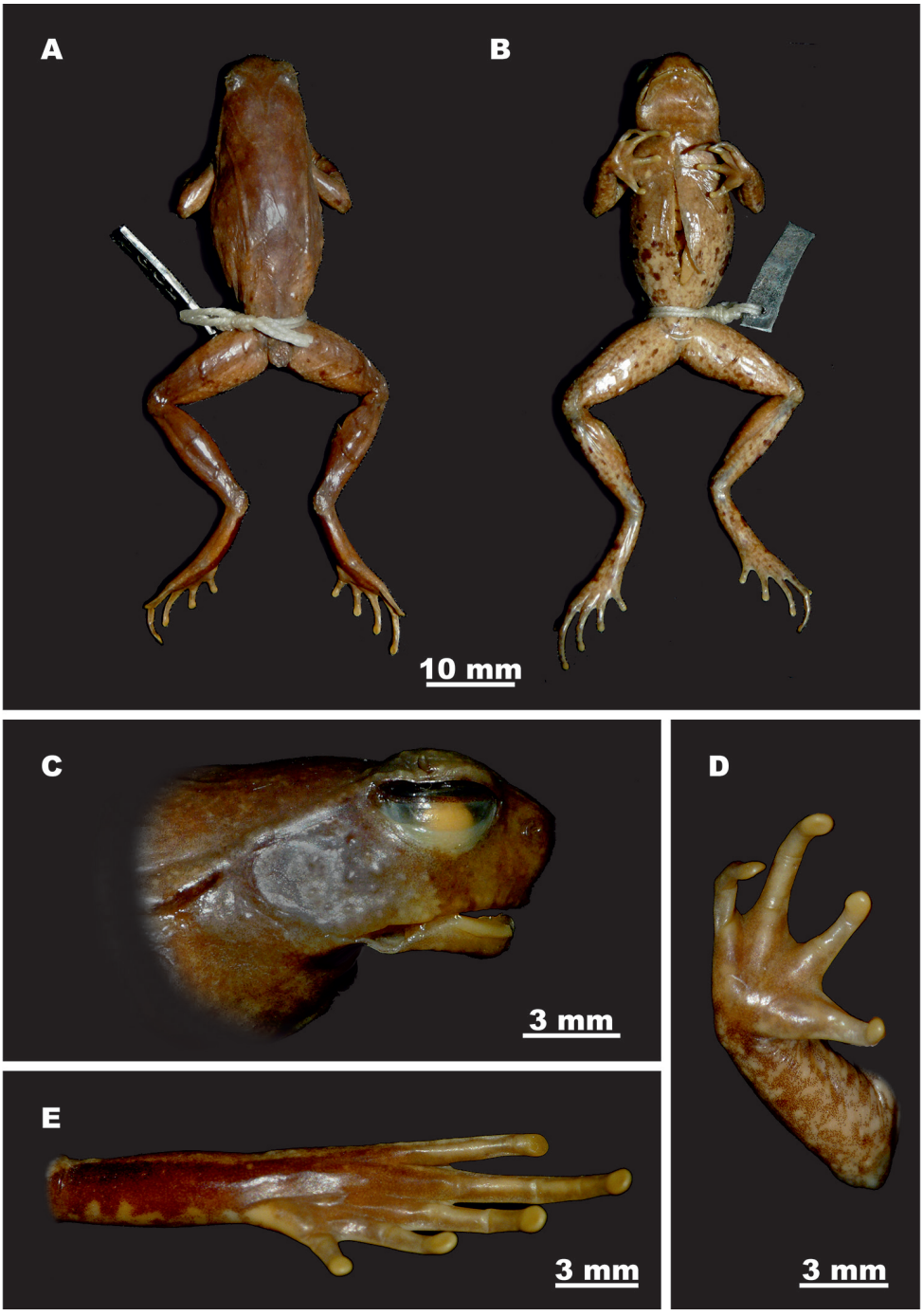


Figure 7. Holotype *Ophryophryne gerti* (BMNH 1921.4.1.324, male) in preservative: **A** dorsal view **B** ventral view **C** head, lateral view **D** volar view of the left hand **E** plantar view of the left foot. Photos by S. Mahony.

region, chest, and ventral surfaces of limbs smooth to weakly shagreened, abdomen weakly granular; two nuptial pads per limb, one large on dorsal surface of F1 from base of metacarpal to near distal joint, another small pad on inner dorsal surface of F2 on metacarpal; pectoral glands round, weakly raised, positioned level with axilla; femoral glands slightly raised, average size, on posterior surface of thighs, situated slightly closer to knee than to cloaca; numerous small white asperities present on posterior half of dorsum, sparse anteriorly, increasing in density posteriorly to above cloaca, absent from all remaining surfaces.

Color of holotype in preservative (Figure 7). Dorsal surfaces of head, body, forearms and hindlimbs mid to light brown, slightly lighter on flanks and dorsal surface of upper arms; a distinct darker brown "V"-shaped marking on dorsal surface of head; no distinct "X"-shaped or hourglass marking on mid dorsum; most flank tubercles are bordered below by a small dark brown spot anteriorly, increasing in size posteriorly towards groin; a broad brown stripe extends around lateral surfaces of snout, from anterior borders of orbits between canthus rostralis and the level of lower border of nostrils; two broad darker brown vertical stripes below orbits, one at level of anterior orbital border, and a second extends from central lower border of orbits to edge of jaw, a faint darker brown stripe extends from posterior border of orbits to cover tympanum; color of supratympanic folds same as surrounding sufaces, but lower border dark brown; edge of lower eyelid dark brown; dorsal surfaces of forearms each with two dark brown transverse blotches, and thighs and shanks with faint darker brown transverse stripes; ventral surfaces of throat, chest and anterior half of abdomen, and ventral surface of hands primarily plain light brown, fading to a mottled brownish beige with small dark brown blotches on posterior half of abdomen, and ventral surfaces of forelimbs, thighs and shanks, and dorsal surfaces of tarsi and feet; ventral surface of tarsi and feet dark brown fading distally on toes to a mid-brown; area surrounding cloaca dark brown, fading distally on lateral surfaces of thighs.

Measurements of the holotype (all in mm, taken by SM). SVL 35.7; HW 10.6; HL 9.1; IFE 5.0; IBE 8.2; ED 3.5; TYD 2.2; TYE 2.1; SL 3.0; EN 1.5; NS 1.5; IUE 2.2; IN 3.2; UEW 3.1; FAL 8.0; HAL 8.4; FIL 3.4; FIIL 3.4; FIIIL 5.6; FIVL 3.6; SHL 15.9; TL 15.0; FOL 14.1; TFOL 21.7; IMT 1.6; TIL 1.7; TIIL 3.8; TIIIL 5.5; TIVL 6.7; TVL 3.5.

Distribution. *Ophryophryne gerti* is herein confirmed from three localities on the Langbian Plateau in southern Vietnam, between 700–2000 m a.s.l. (Fig. 1): Cam Ly River and Nui Ba Mt. in environs of Dalat city, Lam Dong Prov., Vietnam (1000–1800 m a.s.l.) (Ohler 2003, this study); Environs of Bidoup Mt. (2000 m a.s.l.), and Giang Ly Ranger Station (1500 m a.s.l.), Bidoup–Nui Ba N.P., Lam Dong Prov., Vietnam (this study); Chu Yang Sin Mt. environs, Krong Kmar Commune, Krong Bong Dist., Dak Lak Prov., Vietnam (700–2000 m a.s.l.) (Orlov et al. 2008; this study). Additional localities reported in literature require confirmation pending further study of voucher material (see Remarks section above).

New records and range extension for *Ophryophryne synoria* Stuart, Sok & Neang, 2006

Chresonymy:

Ophryophryne synoria Stuart et al. 2006:135, fig. 5.

"*Ophryophryne* sp. 3" — Orlov et al. 2008:82 (Chu Yang Sin N.P., Dak Lak Prov., Vietnam; ZISP 12811–12833).

[?]*Ophryophryne* sp. cf. *poilani* — Poyarkov [Paiarkov] and Vassilieva 2011:202 (Bidoup Mt., Bidoup–Nui Ba N.P., Lam Dong Prov., Vietnam; ZMMU A-4713).

Ophryophryne synoria — Vassilieva et al. 2016:54–56, figs. 44–47 (Binh Phuoc Prov. and Dong Nai Prov., Vietnam; ZMMU A-4516, ZMMU A-5003).

Holotype. FMNH 262779, adult male from "O Chung Chry Stream, near 12°17'30"N, 107°03'06"E, 500 m elev., Seima Biodiversity Conservation Area, O'Rang District, Mondolkiri Province, Cambodia", collected by Bryan Lynn Stuart, Sok Ko and Neang Thy (Stuart et al. 2006; FMNH specimen catalogue). Re-examined by SM.

Paratype. FMNH 262778, adult male, collected with the holotype (Stuart et al. 2006).

Measurements of the holotype (all in mm, taken by SM). SVL 48.8; HW 14.2; HL 13.1; IFE 6.1; IBE 10.8; ED 4.7; TYD 3.0; TYE 3.1; SL 4.3; EN 2.5; NS 1.9; IUE 3.5; IN 3.4; UEW 4.1; FAL 12.0; HAL 13.0; FIL 5.1; FIIL 5.1; FIIIL 8.4; FIVL 5.5; SHL 21.8; TL 21.2; FOL 20.0; TFOL 29.4; IMT 2.2.

Distribution and remarks. Stuart et al. (2006) described a large-sized *Ophryophryne* from O'Rang (also spelled as "O'Reang") District in eastern Cambodia, close to the Vietnamese border, as *O. synoria* (Loc. 1, Fig. 1). Subsequently, during field surveys in 2009–2011, the species was reported in southern Vietnam from Bu Gia Map N.P., Binh Phuoc Prov. (Loc. 2, Fig. 1) and Cat Tien N.P. in Dong Nai Prov. (Loc. 3, Fig. 1) based on morphological evidence (Vassilieva et al. 2016). Herein, we confirm the identity of these specimens based on morphological and molecular genetic evidence, and further expand its distribution in southern Vietnam to include medium and low elevation localities in the central and western parts of the Langbian Plateau (Dak Lak, Lam Dong, Dong Nai and Binh Phuoc provinces between 200 and 1500 m a.s.l.; its presence in Dak Nong Prov. is anticipated). We also identify two mtDNA lineages within *O. synoria* with a moderate level of sequence divergence (p = 2.6%: Table 2, Fig. 2): Subclade B inhabits mountain areas in Lam Dong and Dak Lak provinces and was also recorded for the lowland habitat in Dong Nai Prov. (Locs. 3–4, 6 and 11, Fig. 1) whereas Subclade C is only found in Mondolkiri Prov. of Cambodia and adjacent Binh Phuoc Prov. of Vietnam (Fig. 1, Locs 1–2) and corresponds to *O. synoria s. stricto*.

Variation. The studied specimens of *O. synoria* showed substantial variation in morphological characters, including SVL (Fig. 3) and other morphometric characters (Fig. 4), coloration, and degree of development of palpebral projection (Fig. 6D, E and F). Overall morphology, coloration, and skin glands of the newly discovered populations of *O. synoria* are in general agreement with the description of the holotype by Stuart et al. (2006). Young specimens from Bidoup–Nui Ba N.P. in life often

have reddish or orange coloration of thighs and groin, which was not observed in the type specimens from Cambodia (Stuart et al. 2006), nor in the Bu Gia Map population. The degree of development of short dorsolateral glandular folds varied among specimens, but they were always distinct (Fig. 6D, E and F). The holotype and the Bu Gia Map population (Subclade C) have the finger length formula F1 = F2 < F4 < F3, while in populations from Bidoup–Nui Ba N.P. and Chu Yang Sin N.P. (Subclade B), the finger length formula is F1 = F4 < F2 < F3. Subclade B populations also tend to have a slightly larger tympanum (TYD/ED 68.4%–80.1%; TYE/TD 73.3%–80.6%) than the nominative *O. synoria* (TYD/ED 62.0%–71.9%; TYE/TD 83.9%–103.3%). Though the taxonomic status of the two revealed lineages is not completely clear, herein we tentatively regard them as deep intraspecific mtDNA lineages based on observed genetic differentiation and overall morphological similarity.

Vernacular name. English: "*O'Reang Mountain Toad*" (this paper); "*O'Reang horned frog*" (Vassilieva et al. 2016); Vietnamese: "*Cóc Núi O-Reng*" (Vassilieva et al. 2016).

Description of a new species of *Ophryophryne*

Based upon several lines of evidence, including the analyses of diagnostic morphological characters, acoustic analyses of advertisement calls and phylogenetic analyses of mtDNA sequences for the 12S rRNA–16S rRNA genes, the new species of *Ophryophryne* from mid to high elevations of the western Langbian Plateau represents a highly divergent mtDNA lineage, clearly distinct from all other *Ophryophryne* species. These results support our hypothesis that this recently discovered lineage of *Ophryophryne* represents an undescribed species, described below:

***Ophryophryne elfina* sp. n.**
http://zoobank.org/481B0CFA-5428-40E1-A7A3-C132DC840EC0
Figs 6G, H, 8–12, 13C, 14A (right), 14B, C

Chresonymy:

"*Ophryophryne* sp. 1" — Orlov et al. 2008:82 (Chu Yang Sin N.P., Dak Lak Prov., Vietnam; ZISP 12880–12884).

[?] *Ophryophryne gerti* — Stuart et al. 2010:40 (eight uncataloged topotype females from Bidoup–Nui Ba N.P., Langbian Plateau, Vietnam).

"*Ophryophryne* sp." — Poyarkov [Paiarkov] and Vassilieva 2011:174, 202; fig. 5.6 (Bidoup Mt., Bidoup–Nui Ba N.P., Lam Dong Prov., Vietnam; ZMMU A-4716, ZMMU A-4788, ZMMU A-5674, ZMMU A-5675).

Ophryophryne gerti — Nguyen et al. 2014:148–149; fig. 2 (partim—Hon Ba Mt., Hon Ba Nature Reserve [hereafter N.R.], Khanh Hoa Prov., Vietnam; VNMN 983, ZFMK 94220).

Holotype. ZMMU A-5669 (field number NAP-02658), adult male from the northern slope of Chu Pan Fan Mountain, Chu Yang Sin National Park, Bong Krang Commune, Lak District, Dak Lak Province, Vietnam (coordinates 12°22'31.90"N; 108°21'14.10"E, elevation 1725 m a.s.l.), collected along a mountain stream in mixed evergreen montane tropical forest by N.A. Poyarkov on 07 April 2012 (Figs 8 and 9).

Paratypes. ZMMU A-5691 (field numbers ABV-00580; ABV-00581), two juveniles from the north-western slope of Chu Yang Sin Mountain, Chu Yang Sin N.P., Hoa Le Commune, Krong Bong Dist., Dak Lak Prov., Vietnam (12°24'47.70"N; 108°24'25.40"E, 1975 m a.s.l.), collected in leaf litter in mixed evergreen montane tropical forest by N.A. Poyarkov on 25 May 2014; ZMMU A-5692 (field number NAP-00582), adult male from the northern slope of Bidoup Mountain, Bidoup–Nui Ba N.P., Da Chais Commune, Lac Duong Dist., Lam Dong Prov., Vietnam (12°06'5.60"N; 108°39'34.20"E, 2035 m a.s.l.), collected along mountain stream in evergreen montane elfin forest by N.A. Poyarkov on 09 May 2009; ZMMU A-5675 (field numbers NAP-01456; NAP-01459), two adult males from the northern slope of Bidoup Mountain, Bidoup–Nui Ba N.P., Da Chais Commune, Lac Duong Dist., Lam Dong Prov., Vietnam (12°06'5.60"N; 108°39'34.20"E, 2035 m a.s.l.), collected on stones and vegetation along a mountain stream in mixed evergreen montane elfin forest by N.A. Poyarkov and A.B. Vassilieva on 25 June 2010; ZMMU A-4788 (field numbers NAP-01455; NAP-01449; NAP-01450; NAP-01460), four adult males from the southern slope of Hon Giao Mountain Ridge, Bidoup–Nui Ba N.P., Da Chais Commune, Lac Duong Dist., border of Lam Dong Prov. and Khanh Hoa Prov., Vietnam (12°11'33.10"N; 108°42'41.80"E, 1890 m a.s.l.), collected along a mountain stream sitting on stones and tree branches near the water edge, in mixed evergreen montane elfin forest by N.A. Poyarkov and A.B. Vassilieva on 30 June 2010; ZMMU A-5674 (field numbers NAP-01451; NAP-01452), two adult males from the southern slope of Hon Giao Mountain Ridge, Bidoup–Nui Ba N.P., Da Chais Commune, Lac Duong Dist., border of Lam Dong Prov. and Khanh Hoa Prov., Vietnam (12°11'33.10"N; 108°42'41.80"E, 1890 m a.s.l.), collected along a mountain stream on stones and vegetation in evergreen montane elfin forest by N.A. Poyarkov and A.B. Vassilieva on 29 June 2010; ZMMU A-5170 three adult males (field numbers ABV-00454; ABV-00472; ABV-00471), and one adult female (ABV-00455) from the northern slope of Bidoup Mountain, Bidoup–Nui Ba N.P., Da Chais Commune, Lac Duong Dist., Lam Dong Prov., Vietnam (12°06'5.60"N; 108°39'34.20"E, 2035 m a.s.l.), collected along a mountain stream in mixed evergreen montane tropical forest by A.B. Vassilieva on 16 April 2014.

Referred specimens. ITBCZ 2786, ITBCZ 2788, ITBCZ 2792, ITBCZ 2828, three adult females and one adult male collected along a mountain stream in evergreen mountain forest on the summit of Hon Ba Mountain, Hon Ba N.R., Dien Khanh Dist., Khanh Hoa Prov., Vietnam (12°07'10.60"N; 108°56'51.60"E, 1510 m a.s.l.), by Sang Ngoc Nguyen, Luan Thanh Nguyen and Vu Dang Hoang Nguyen on 22–24 December 2015; ITBCZ 2908–2909, ITBCZ 2918–2919, ITBCZ 3502, five adult males collected along a mountain stream in evergreen montane forest on the summit of Hon Ba Mountain, Hon Ba N.R., Dien Khanh Dist., Khanh Hoa Prov., Vietnam (12°07'28.80"N; 108°58'14.20"E, 950 m a.s.l.), by Sang Ngoc Nguyen, Luan Thanh Nguyen, and Vu

Dang Hoang Nguyen on 22–28 March 2016; ZMMU A-5679 (field number NAP-01169), 7 larvae collected in a cascade mountain stream on the northern slope of Bidoup Mountain, Bidoup–Nui Ba N.P., Da Chais Commune, Lac Duong Dist., Lam Dong Prov., Vietnam (12°06'5.60"N; 108°39'34.20"E, 2035 m a.s.l.), by N.A. Poyarkov on 03 May 2009; ZMMU A-5684 (field number NAP-02673), 4 larvae collected in a cascade mountain stream on the northern slope of Chu Pan Fan Mt., Chu Yang Sin N.P., Bong Krang Commune, Lak Dist., Dak Lak Prov., Vietnam (coordinates 12°22'31.90"N; 108°21'14.10"E, elevation 1725 m a.s.l.), by N.A. Poyarkov on 07 April 2012.

Etymology. The specific epithet is an adjective (in agreement with the genus name in feminine gender), derived from "*elf*", the English spelling of "*alfus*" in Latin, referring to usually forest-dwelling supernatural mythological creatures in Germanic mythology and folklore; the name is given in reference both to the funny appearance and small size of the new species, as well as to the their endangered habitat, restricted to wet evergreen montane forests at high elevations of the Langbian Plateau; such forests are often called "*elfin forests*".

Recommended vernacular name. The recommended common name in English is "Elfin Mountain Toad". The recommended common name in Vietnamese is "Cóc Núi Tiểu Yêu Tinh".

Diagnosis. The species is allocated to *Ophryophryne* based on its obvious similarities with its sister taxa, its molecular phylogenetic affinities, and the absence of maxillary teeth considered diagnostic for the genus (previous authors, e.g. Ohler 2003, Delorme et al. 2006 and Fei et al. 2009 also indicated a horizontal pupil and the absence of vomerine teeth as diagnostic for *Ophryophryne*, this is reconsidered by Mahony et al. 2017). *Ophryophryne elfina* sp. n. is distinguished from its congeners by a combination of the following morphological attributes: (1) small adult body size, male SVL 26.9–33.9 mm (N = 29), female SVL 35.1–36.5 mm (N = 6); (2) snout sharply protruding in profile; (3) tympanum diameter approximately half of eye diameter; tympanum to eye distance approximately 70–90% of tympanum diameter; (4) finger length formula: F1 < F4 ≤ F2 < F3, or F1 ≤ F2 < F4 < F3; toe webbing rudimentary, toe length formula: T1 < T5 < T2 < T3 < T4; (5) short dorsolateral glandular ridge present above shoulder; (6) palpebral projection present as a small single tubercle to moderately developed single projection; (7) dermal cloacal protuberance and dermal flaps above cloacal opening absent; (8) skin of dorsal and lateral surfaces of head, body and limbs shagreened with numerous small tubercles, large warts on the flanks; (9) skin on dorsal and lateral surfaces of body, head and limbs with numerous bright orange-red (in life) asperities; (10) males with a red-orange (in life) nuptial pad on F1; (11) dorsal coloration light yellow-brown with dark hourglass-shaped marking on dorsum usually edged with white or beige (in life); (12) posterior suborbital light bar well-defined, usually clearly separated from dark-brown temporal triangular spot, uniformly covering temporal area and tympanum.

The new species is also markedly distinct from all congeners for which comparable sequences are available (16S rRNA mitochondrial gene; uncorrected genetic distance > 8.2%). The advertisement call of the new species consists of whistling notes uttered in series: average 12.84 ± 0.41 calls per series, with an average dominant frequency of

4645.94 ± 4.39 Hz, repetition rate per recording/series 1.18 ± 0.2 calls/s and 3.87 ± 0.07 calls/s, respectively, with average call duration 73 ± 0.23 ms and inter-call interval 207 ± 2.06 ms, also distinguishes the new species from *Ophryophryne* species for which calls are known, including the two species found in sympatry.

Description of holotype. Mature male (SVL 27.2 mm); habitus slender (Figs 8A, B, and 9). Specimen in good state of preservation; median abdomen dissected, dissection length ca. 9.0 mm; liver and testes observable through incision, testes white, enlarged (testes length 3.9 mm; Fig. 8B); ventral right femur dissected for molecular sampling, dissection length 9.8 mm.

Head moderately large (HL/SVL 29.2%; HW/SVL 29.5%), almost as wide as long (HW/HL 101.0%), triangular in dorsal view; top of head flat; snout comparatively short (ES/HL 30.9%), narrow (IFE/HW 39.4%), somewhat truncated in dorsal view (Fig. 8A), sharply protruding in profile, without rostral appendage (Figs 8C, 9); snout projecting significantly beyond margin of lower jaw (Fig. 8C); loreal region slightly concave; canthus rostralis distinct, sharp; dorsal region of snout flat; eyes large (ED/HL 44.8%), slightly protuberant in dorsal view and in profile, eye less than twice as long as maximum tympanum diameter (TYD/ED 53.7%) and half times longer than snout (ED/SL 145.3%); eye-tympanum distance less than maximum tympanum diameter (TYE/TD 83.3%); tympanum distinct, almost circular shaped with vertical diameter slightly exceeding horizontal diameter, tympanum large (TYD/HL 24.1%); pupil in preservation diamond-shaped (Fig. 8C), horizontally orientated; nostril oval-shaped, vertical, laterally orientated, located as far from eye as from snout (EN/NS 100.8%); internarial distance greater than eyelid width (IN/UEW 119.5%), and subequal to narrowest point between upper eyelids (IN/IUE 103.7%); pineal ocellus not visible externally (Fig. 8A); vomerine ridges not absent; maxillary and vomerine teeth absent; vocal sac gular, its openings not discernable; tongue moderately large, with free posterior end, not notched posteriorly, lacking medial lingual process.

Forelimbs slender, forearm moderately long (FAL/SVL 25.7%), slightly enlarged relative to upper forelimb, and shorter than hand (FAL/HAL 96.1%); fingers long and narrow, dorsoventrally flattened, weak lateral fringes present on third and fourth fingers (Fig. 8D), finger length formula F1 < F4 < F2 < F3; fingers completely free of webbing; subarticular tubercles absent, replaced by low callous dermal ridges; supernumerary tubercles absent; outer palmar (metacarpal) tubercle small, round, elevated but with indistinct borders (Fig. 8D); thenar tubercle weak; finger tips in life rounded, weakly expanded relative to digit widths (wider than the distal-most finger articulation), with circular pads (Fig. 8D); terminal grooves absent.

Hindlimbs slender, relatively long, shanks overlap when thighs are held at right angle to body; shank length less than half of snout–vent length (SHL/SVL 48.7%); thighs shorter than shanks (SHL/TL 109.2%), and feet (FOL/TL 110.9%); toes long and slightly dorsoventrally flattened (Fig. 8E), relative toe lengths T1 < T5 < T2 < T3 < T4; toe tips slightly expanded relative to digit widths (wider than the distal-most toe articulation), with circular pads; terminal grooves absent; lateral fringes on toes, outer metatarsal tubercle, subarticular and supernumerary tubercles absent; inner metatarsal

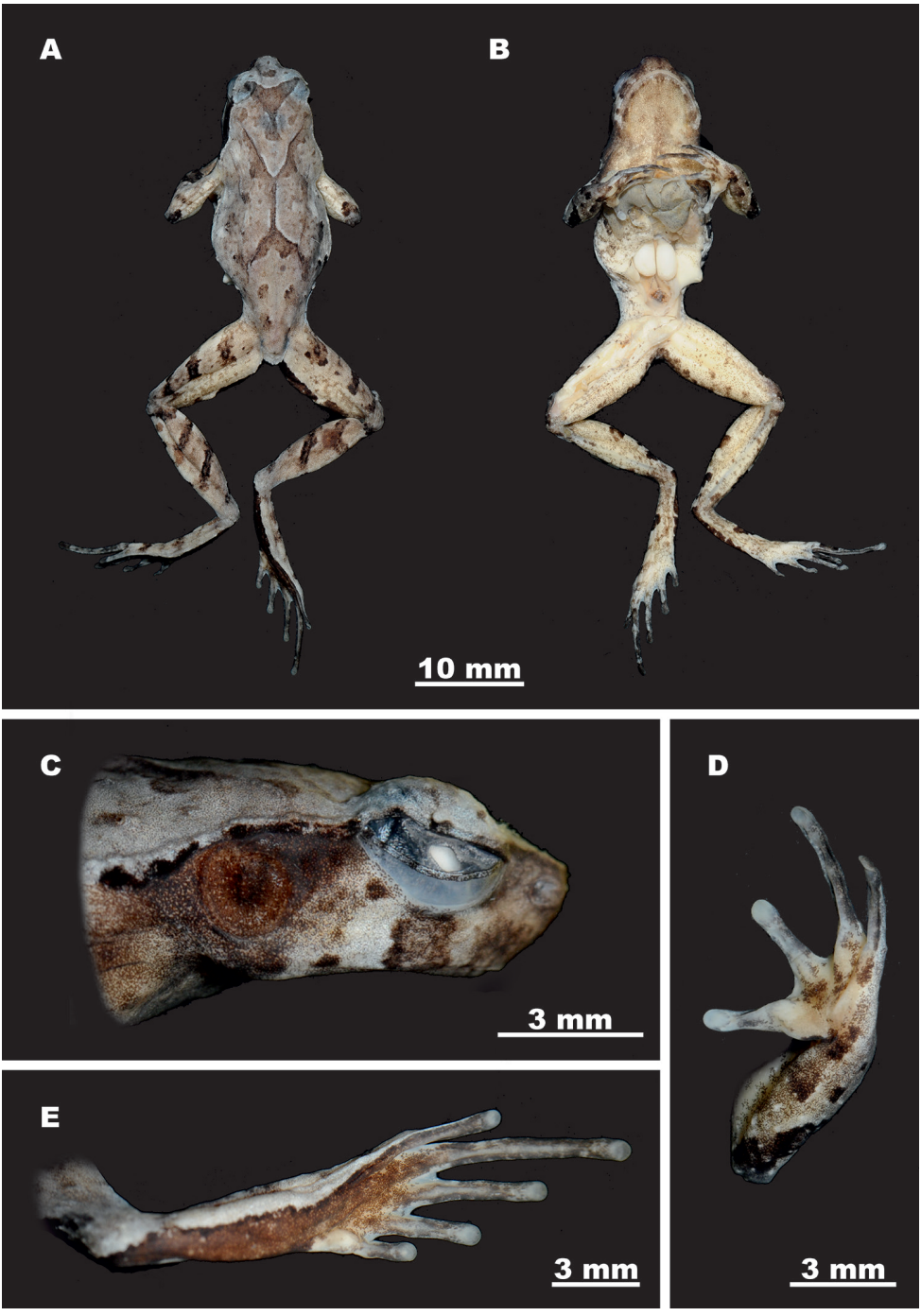


Figure 8. Holotype *Ophryophryne elfina* sp. n. (ZMMU A-5669, male, field number NAP-02658) in preservative: **A** dorsal view **B** ventral view **C** head, lateral view **D** volar view of the left hand **E** plantar view of the left foot. Photos by N.A. Poyarkov.

Figure 9. Holotype of *Ophryophryne elfina* sp. n. in life (ZMMU A-5669, male, field number NAP-02658), dorsolateral view. Photos by N.A. Poyarkov.

tubercle well developed with distinct borders, ca. two times longer than wide, oval-shaped (IMT/FOL 9.3%); well-developed dermal ridge of callous tissue present on ventral surface of all toes and continuing to metatarsus; rudimentary webbing present between all five toes, basal web distinct between toes T2–T3 and T3–T4, but is completely reduced between toes T1–T2 and T4–T5; tarsal fold absent.

Skin texture and skin glands. Skin of dorsal and lateral surfaces of head, body and limbs shagreened, with numerous small skin asperities present on anterior two thirds of dorsum, sparse posteriorly, increasing in density along dermal ridges, densely covering dorsal and lateral surfaces of head, upper eyelids, and dorsal surfaces of thighs, shanks, upper forelimbs, forearms, hands, feet and digits, and absent from all remaining surfaces. Small tubercles finely and relatively evenly scattered on dorsal surfaces of trunk, head and limbs, including maxilla, mandible, eyelids and dorsal surfaces of head, forelimbs and hindlimbs (Figs 8A and 9); small tubercles present on temporal region, tympanum smooth, tympanic rim distinct but not elevated relative to skin of temporal region; on dorsal surfaces tubercles arranged in distinct longitudinal ridges on upper forelimbs, forearms, shanks and thighs, becoming less distinct on dorsal surfaces of hands, feet and digits (Fig. 9); six large tubercles on left flank and seven large tubercles on right flank irregularly scattered from axilla to groin, intermixed with smaller tubercles; central portion of outer edge of upper eyelid slightly thickened, with a distinct small single tubercle on a thickened ridge (Figs 8C, 9); distinct thick glandular supratympanic fold, narrow anteriorly, considerably widening posteriorly, extending from posterior corner of eye gently sloping down towards dorsal margin of tympanum (but not concealing it), where it broadly

curves down, terminating above axilla (Figs 8C, 9); short dorsolateral glandular ridge present above shoulders, on anterior part of dorsum, its length comparable with eye diameter (Fig. 9); a weak " >–< "-shaped glandular dermal parietoscapular-sacral ridge present on dorsum (Figs 8A, 9); transverse fold at head basis absent; dermal projection above cloaca absent; gular region, chest, abdomen and ventral surfaces of limbs smooth to weakly shagreened (especially on the posterior surface of abdomen); nuptial pad present, single, covered with microgranules, covering entire dorsal metacarpal of first finger extending distally to ca. 3/4 basal phalange length; pectoral glands round, flat, of medium size, positioned level with axilla; femoral gland flat, indistinct, on posterior surface of thighs.

Color of holotype in life. Entire dorsum light olive-brown to yellow-brown with large irregular brownish grey spots; dorsal surfaces of head yellowish brown from tip of snout to eyes; small oval-shaped spot with irregular borders on dorsal surface of snout between anterior canthi; a small dark dot on dorsal surface of snout tip; similar single dark dots on anterior parts of upper eyelids; brown "V"-shaped marking on crown between supraorbital horns with apex pointing posteriorly, outlined with thin light-beige edging; round brownish spot at head basis; " >–< "-shaped marking surrounded with dark olive-brown, outlined with thin light-beige edging forming a hourglass-shaped dorsal marking (Fig. 8A); two small roundish brown spots at sacrum (Fig. 8A); supratympanic fold dorsally light yellowish brown, ventrally dark-brown; front and lateral surfaces of snout and lateral canthus rostralis dark reddish brown; lateral surfaces of maxilla dark brown with four distinct orange-brown to yellowish beige bars extending from orbits towards edge of maxilla: smallest anteriormost light band borders nostril ventrally, with two posterior light bands extend from posterior corner of eye towards angle of mouth (Fig. 9); axilla purplish brown; tympanum uniform purplish brown; temporal region uniform dark purplish brown, clearly defined from light beige area on posterior part of maxilla; pupil black, outlined in copper-gold; iris golden dorsally and ventrally, copper-orange at medial part, with tiny dark reticulations spreading from pupil; sclera lemon-yellow; upper surface of limbs yellowish brown with irregular dark-brown spots on forearms and transverse spots forming dark-brown and greyish bands across shanks, thighs and tibio-tarsus (three complete transverse bands on left leg, two complete [both on shank and thigh] and one incomplete [on shank only] transverse bands on right leg: Figs 8 and 9), knee joint dark brown; sides beige-yellow with indistinct greyish white flecking and large black spots with irregular borders: marking location of large warts on each side of body, four smaller spots located dorsally, and two large brown-black spots located ventrally, top of larger flank tubercles brownish cream (Fig. 9); throat brownish to purplish grey with greyish white flecking and irregular dark-brown spots; chest and anterior half of abdomen purplish grey with whitish flecking and grey-brown blotches; posterior half of abdomen lighter greyish pink with irregular dark blotches; lower surface of limbs purplish grey with white and beige flecking; area surrounding vent and posterior surface of thighs dark black-brown with whitish spots, posterior surface of thighs near tibio-tarsal articulation black-brown with sparse whitish dots; dorsal surface of feet and shanks yellowish beige with brown flecking; ventral surface of feet and shanks brown-black. Pectoral and femoral glands creamy white. Nuptial pad, outer metacarpal (palmar) and metatarsal tubercles pink-

red to orange-red. Asperities covering dorsal surfaces of body, head, limbs and digits, lateral sides of head and anterior part of chest in life bright orange-red, forming reddish rows and ridges on dorsal surfaces of limbs as well as on edge of upper eyelid, palpebral projection also with orange-red asperities.

Color of holotype in preservative. In preservative coloration faded to light grey-brown on dorsum and flanks, with slightly paler limbs and greyish beige to whitish on venter; reddish and orange tints, as well as iris coloration, faded completely; dark markings on dorsum, sides and venter and other features remain without significant change (Fig. 8). Banding on limbs is less pronounced. Chest, abdomen, throat, interior portions of forelimbs and thighs are pale greyish brown; formerly brightly colored dorsal asperities and nuptial pads, palmar and metatarsal tubercles turned transparent or creamy white (Fig. 8C, E).

Measurements of the holotype (all in mm, taken by NAP). SVL 27.2; HW 8.0; HL 7.9; IFE 3.2; IBE 6.8; ED 3.6; TYD 1.9; TYE 1.6; SL 2.5; EN 1.3; NS 1.3; IUE 2.3; IN 2.3; UEW 2.0; FAL 7.0; HAL 7.3; FIL 2.4; FIIL 2.8; FIIIL 4.6; FIVL 2.8; SHL 13.2; TL 12.1; FOL 11.9; TFOL 18.9; IMT 1.8; TIL 1.7; TIIL 3.8; TIIIL 5.5; TIVL 6.7; TVL 3.5.

Variation. Morphometric variation within the type series and other referred specimens of the new species is shown in Table 5. Individuals of the type series are similar in morphology and body proportions (Figs 9, 10). There is a clear and significant difference in body size between males and females (Fig. 3): females (SVL 35.1–36.5 mm, $N = 6$) are significantly bigger than males (SVL 26.9–33.9 mm, $N = 29$) (Duncan test, $p < 0.05$); sexual differences were not significant for other mensural characters possibly due to the small sample size of females. Certain variation is observed in finger lengths: most of the examined specimens have the finger length formula F1 < F4 < F2 < F3 ($N = 14$), in some specimens, including the holotype, the second and the fourth fingers are of equal length (F1 < F4 = F2 < F3; $N = 5$), or the fourth finger is longer than the second (F1 < F2 < F4 < F3; $N = 5$); rarely the second finger is as long as the first finger (F1 = F2 < F4 < F3; $N = 2$). Specimens vary in the number and size of black spots and blotches on flanks (Fig. 10A, B). In life, both sexes of the new species have lighter dorsum and belly coloration when nocturnally active. Other in-life variation was observed for throat coloration: throat can be dark brownish with clear dark-grey blotches (Fig. 10C) to almost uniform brown-violet to purple with dark blotches not discernable (Fig. 10D). There is significant variation in dorsal pattern: in some specimens the dorsum looks almost uniform yellowish brown with an indistinct hourglass-shaped figure (Figs 6G, 10A, B, 14 [right]) whereas in other specimens the hourglass-shaped figure is distinct, dark brown and edged with light beige (Figs 6H, 9). There is some variation in the length of palpebral projections, from a small almost indistinct tubercle (Figs 6H, 13C) to a moderately well-developed projection (Figs 6G, 14). Coloration of the lateral surfaces of the head vary, but on all specimens two light suborbital bars are distinct, clearly separated from the uniform dark-brown coloration of the tympanal area. Iris coloration shows insignificant variation: Nui Chua Mt. population appear to have copper-red coloration of the entire iris "(Fig. 14B), somewhat different from the coloration of the holotype (Fig. 9). Recently metamorphosed and

juvenile specimens have numerous bright red-orange tubercles (Fig. 10E, F) which are more conspicuous than in adults. Excluding the presence of nuptial pads on males, the new species shows no significant variation in dermal characters among sexes (Fig. 10); in preservative smaller tubercles become flattened and less distinct.

Tadpole description. Tadpoles were allocated to *Ophryophryne elfina* sp. n. based on the following evidence: (1) morphological features characteristic for megophryine larvae in general; *Ophryophryne* or *Megophrys s. lato* in particular (elliptical shaped body with long muscular tail, oral disk forms a dorsally oriented funnel); (2) collected in the stream where calling males of the new species were recorded; (3) species identification confirmed by mtDNA sequences of short 16S rRNA gene fragment (up to 500 bp) (GenBank Acession numbers: KY515232–KY515233, see Table 1).

Standard tadpoles measurements (mean ± SD, *N* = 5, Stage 25; ZMMU A-5679, field number NAP-01169, collected from Bidoup Mt., 1900 m a.s.l., Bidoup–Nui Ba N.P., Lam Dong Prov.) (all in mm, taken by NAP): TOL = 28.4 ± 1.3 (27.4–30.2); BL = 8.6 ± 0.1 (8.4–8.7); TAL = 19.8 ± 1.2 (18.9–21.5); BW = 4.4 ± 0.4 (3.8–4.6); BH = 3.6 ± 0.2 (3.4–3.7); TH = 4.5 ± 0.4 (4.0–4.8); SVL = 9.2 ± 0.3 (9.0–9.5); SSp = 4.8 ± 0.2 (4.5–4.9); UF = 1.4 ± 0.1 (1.3–1.5); LF = 1.1 ± 0.0 (1.1–1.1); IN = 2.6 ± 0.1 (2.5–2.6); IP = 2.8 ± 0.2 (2.7–3.2); RN = 1.7 ± 0.1 (1.7–1.8); NP = 0.8 ± 0.1 (0.7–0.8); ED = 0.8 ± 0.0 (0.8–0.9).

The following description is based on five tadpoles at stage 25 (ZMMU A-5679, field number NAP-01169). In lateral view (Fig. 11A), body slightly compressed dorsoventrally (BH/BW 83.5 ± 4.09%), especially anteriorly, convex both dorsally and ventrally. Body elliptical in dorsal view (Fig. 11B), with maximum width at middle of body (BW/BL 51.0 ± 4.2%); snout short, rounded, blunt. Eyes of moderate size (ED/BL 9.8 ± 0.3%), not bulging, separated by a distance which equals approximately 1.1 times internarial distance (IP/IN 110.2 ± 6.8%), directed and positioned dorsolaterally, not visible in ventral view; pupils oriented dorsolaterally. Nares tubular, positioned dorsally (near anterior edge of eye), much closer to pupils than to tip of snout; directed laterally. Spiracle sinistral, conical, very short, opening at half of distance from snout tip to vent (SSp/SVL 52.2 ± 3.4%); spiracle attached to body wall for most of its length, extremity is free, positioned at the level of longitudinal axis, oriented dorsoposteriorly, opening varies from rounded to oval. Tail long, more than two times longer than body (TAL/BL 231.3 ± 11.0%), lanceolate; almost equal in height along its length (point of maximum height of tail located just anterior to midlength of tail); tail tip bluntly rounded, without terminal filament; tail musculature strong, gradually tapering, almost reaching tail tip. Tail fins shallow, moderately well developed, not extending onto body: dorsal fin originating almost at body-tail junction, much shorter than lower fin proximally and nearly equal in height to it on middle of tail; dorsal fin slightly higher than ventral fin on distal half of tail (LF/UF 77.1 ± 4.9%); free margin of dorsal fin horizontal and shallow on anterior half of tail; free margin of ventral fin parallel to tail musculature. Vent opening medial, tubular, directed posteriorly, not linked to ventral tail fin. Neither skin glands nor neuromasts visible in preservative, but neuromasts of the lateral line system are distinct in life (Fig. 12A) forming two curved lines running from snout towards orbits and along orbital margins

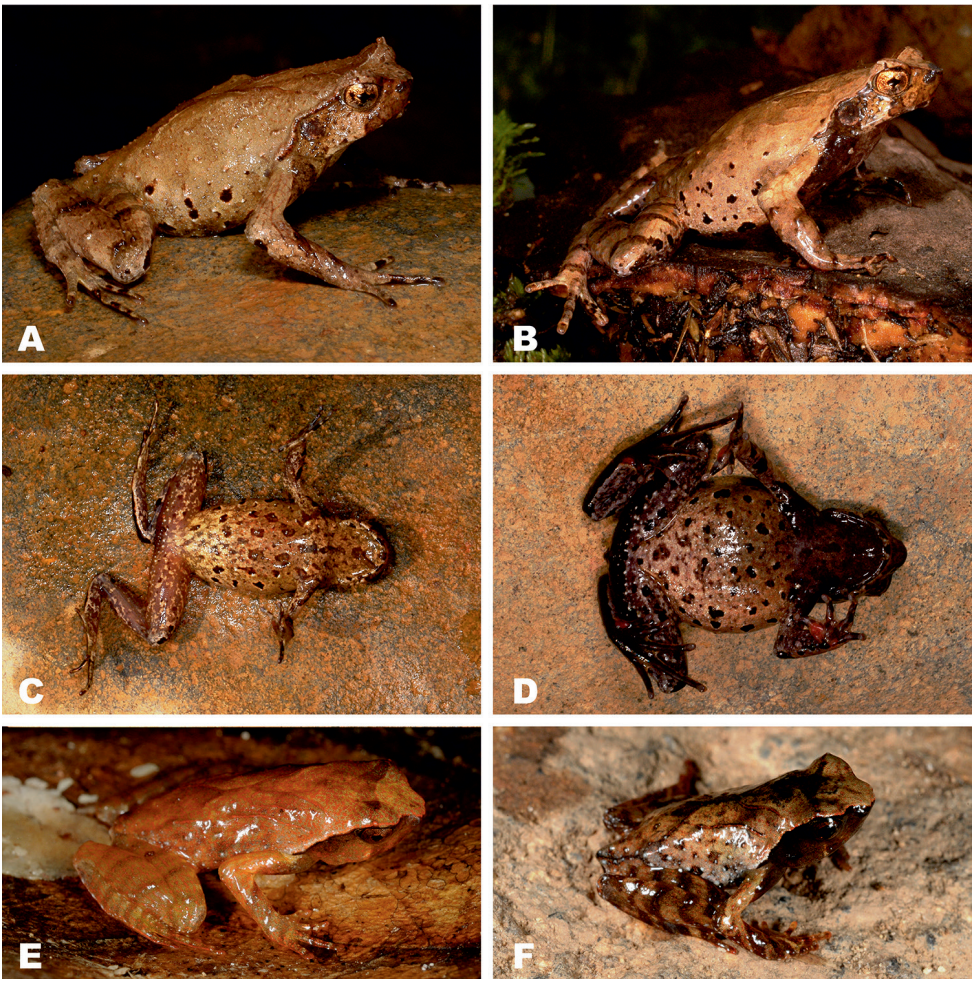


Figure 10. Paratypes of *Ophryophryne elfina* sp. n. in life. **A–D** Bidoup Mt., Bidoup–Nui Ba N.P., Lam Dong Prov., 2000 m a.s.l.: **A** ZMMU A-4788 (field number NAP-01449), male, dorsolateral view **B** ZMMU A-4788 (field number NAP-01455), female, dorsolateral view **C** ZMMU A-4788 (field number NAP-01449), male, ventral view **D** ZMMU A-4788 (field number NAP-01455), female, ventral view **E–F** Chu Yang Sin Mt., Chu Yang Sin N.P., Dak Lak Prov., 1800 m a.s.l.: **E** ZMMU A-5691 (field number ABV-00580), metamorph, dorsolateral view **F** ZMMU A-5691 (field number ABV-00581), metamorph, dorsolateral view. Photos by N.A. Poyarkov.

ventrally. Subterminal oral disk with lips expanded vertically forming a dorsally oriented funnel (Fig. 11B); lateral corners of funnel distinct; upper lip notably smaller than lower; lips lack keratodonts, but bear short, low ridges, more densely arranged on upper than on lower labium, arranged in 18–24 (mean = 22) longitudinal rows and from 2–4 (mean = 3) transverse rows on upper labium to 4–6 (mean = 5) transverse rows on lower labium. Marginal papillae absent. Width of expanded funnel comprises over 75% of body length (Fig. 12A) and just 30% when folded in preservation (Fig. 12B).

Table 5. Measurements of the *Ophryophryne elfina* sp. n. specimens. For museum accession numbers relative to specimen collection numbers (NAP, ABV, HB, ROM, CYS) see Appendix 1. For locality details relative to population number see Table 1 and Fig. 1. For abbreviations see Material and methods. All measurements are given in mm. Table is continued on the next two pages.

Specimen ID	Population	Sex	Type status	FAL	HAL	FIL	FIIL	FIIIL	FIVL	SHL	TL	FOL	TFOL	IMT	IMT
NAP-02658	10	m	holotype	27.2	8.0	7.9	3.6	1.9	1.6	2.5	1.3	1.3	2.3	2.3	2.0
NAP-01455	6	m	paratype	33.9	10.1	9.8	3.8	2.3	1.9	2.7	1.3	1.3	2.8	3.1	2.9
NAP-01449	6	m	paratype	31.0	9.3	9.8	3.8	2.2	1.6	2.5	1.3	1.2	3.1	2.9	3.5
NAP-01450	6	m	paratype	29.2	9.0	9.0	3.7	2.3	1.4	3.0	1.2	1.4	3.2	2.5	3.2
NAP-01460	6	m	paratype	29.6	8.7	8.4	3.4	2.7	1.1	2.1	1.1	1.0	2.5	1.5	2.6
ABV-00316	8	m		28.9	8.8	9.1	3.8	2.6	1.6	2.2	1.3	1.1	2.8	1.8	3.1
HB-36-1	8	m		27.5	8.6	9.2	4.1	2.4	1.3	2.6	1.4	1.4	2.6	2.1	2.6
ROM-36525	11	m		26.9	7.6	8.1	3.6	1.9	1.3	2.9	1.3	1.2	2.3	1.7	2.8
ROM-36523	11	m		27.8	8.2	8.4	4.0	2.2	1.8	2.8	0.9	1.5	2.1	1.6	2.9
ROM-36529	11	m		28.3	7.9	8.6	4.3	2.2	1.6	2.4	1.1	1.5	2.8	2.0	2.7
NAP-01757	6	m		32.0	9.2	9.2	4.0	2.3	1.8	2.6	1.3	1.3	2.3	2.3	2.5
NAP-01782	6	m		31.1	9.3	9.1	3.8	2.2	1.7	2.3	1.1	1.0	2.8	2.6	7.9
NAP-01758	7	m		32.1	9.2	9.3	3.4	2.4	1.7	2.4	0.8	1.3	3.1	2.0	2.4
NAP-01871	7	m		32.8	8.8	9.6	3.7	2.2	1.3	2.7	0.9	1.1	2.9	1.6	2.4
NAP-01783	7	m		31.3	9.0	9.2	4.0	2.2	1.6	2.8	1.2	1.1	3.1	2.1	2.8
ABV-00471	6	m	paratype	30.7	8.5	8.5	3.7	2.3	1.3	2.6	0.9	1.4	2.7	2.4	2.7
ABV-00454	6	m	paratype	31.4	8.8	9.3	3.8	2.1	1.5	2.0	1.0	1.1	2.5	1.9	2.9
ABV-00472	6	m	paratype	30.8	8.9	9.3	3.3	2.3	2.1	2.3	1.6	1.0	3.2	2.1	2.6
ROM-36524	11	m		28.5	8.3	8.5	3.6	2.1	1.2	2.8	0.8	1.6	2.4	2.2	2.5
ROM-36527	11	m		27.7	7.4	7.8	3.1	2.0	1.7	3.0	1.3	1.8	2.7	2.1	2.7
ROM-36522	11	m		28.1	7.9	8.4	3.2	1.7	1.3	2.8	1.2	1.5	2.6	2.1	2.8
ROM-36528	11	m		28.2	7.2	7.4	3.1	1.6	1.5	3.3	1.0	1.8	3.4	1.9	2.9
ROM-36526	11	m		27.1	7.6	8.2	3.6	2.1	1.5	2.9	1.1	2.1	2.6	2.1	2.4
ITBCZ 2828	8	m		27.9	8.2	8.9	3.9	2.2	1.2	2.5	1.1	1.4	2.1	2.8	3.1
ITBCZ 2908	8	m		29.4	9.6	9.1	3.4	2.3	1.4	3.3	1.1	2.2	2.7	3.3	2.7
ITBCZ 2909	8	m		30.1	9.5	9.8	4.1	2.2	1.8	2.9	1.3	1.6	2.4	3.3	2.7

Specimen ID	Population	Sex	Type status	FAL	HAL	FIL	FIIL	FIIIL	FIVL	SHL	TL	FOL	TFOL	IMT	IMT
ITBCZ 2918	8	m		29.6	8.7	9.3	3.4	2.2	1.3	2.8	1.1	1.7	2.2	2.9	2.7
ITBCZ 2919	8	m		30.8	9.2	9.6	3.4	2.1	1.6	3.3	1.4	1.9	2.4	2.6	2.6
ITBCZ 3502	8	m		28.6	8.7	8.9	3.5	2.3	1.8	3.3	1.2	2.1	2.6	2.8	2.5
ABV-00455	6	f	paratype	35.2	9.3	8.9	4.2	2.4	1.7	2.4	1.7	1.2	2.8	1.7	2.6
CYS-10-10	11	f		35.1	9.4	9.1	3.9	1.9	1.9	3.6	1.6	2.1	3.5	2.6	3.0
ROM-36530	11	f		35.7	9.0	8.6	3.6	2.1	1.6	3.3	1.0	1.9	3.6	2.4	2.8
ITBCZ 2786	8	f		36.5	10.0	11.0	4.1	2.4	1.8	2.7	1.2	1.5	2.7	2.8	3.1
ITBCZ 2788	8	f		35.8	10.1	10.9	4.2	3.0	1.6	3.2	1.3	1.9	2.3	2.6	2.8
ITBCZ 2792	8	f		35.3	10.2	10.3	4.1	2.2	1.8	3.3	1.5	1.8	2.8	2.9	3.1

Specimen ID	Population	Sex	Type status	FAL	HAL	FIL	FIIL	FIIIL	FIVL	SHL	TL	FOL	TFOL	IMT
NAP-02658	10	m	holotype	7.0	7.3	2.4	2.8	4.6	2.8	13.2	12.1	18.9	11.9	1.8
NAP-01455	6	m	paratype	8.5	9.4	2.3	4.1	6.0	3.7	16.1	14.8	14.7	28.0	2.0
NAP-01449	6	m	paratype	7.1	7.5	2.8	3.0	4.1	3.0	14.4	13.4	11.8	19.4	1.7
NAP-01450	6	m	paratype	7.5	7.8	2.1	2.9	4.9	2.6	12.9	13.8	11.1	19.7	1.9
NAP-01460	6	m	paratype	6.5	6.7	2.3	2.9	4.4	2.4	13.4	11.3	10.6	17.5	1.3
ABV-00316	8	m		7.3	6.7	2.4	2.9	4.9	2.9	13.2	13.5	11.6	19.2	2.5
HB-36-1	8	m		6.6	7.1	2.1	3.2	4.6	3.0	12.6	13.4	11.6	18.9	1.5
ROM-36525	11	m		6.9	6.6	2.0	2.4	4.5	2.7	11.7	11.9	10.6	17.1	1.2
ROM-36523	11	m		6.4	6.0	2.9	2.9	4.8	3.0	12.8	13.2	11.4	18.3	1.6
ROM-36529	11	m		6.7	6.4	2.9	3.0	4.5	3.0	13.5	13.5	11.0	19.1	1.6
NAP-01757	6	m		8.4	7.4	2.5	2.5	4.5	3.1	13.3	14.5	11.6	19.6	1.2
NAP-01782	6	m		6.1	4.8	1.1	2.0	3.7	1.4	12.7	13.1	11.0	18.7	1.4
NAP-01758	7	m		8.1	6.6	2.1	3.2	4.6	2.5	14.8	15.3	13.3	20.4	1.8
NAP-01871	7	m		6.5	6.8	2.3	2.8	4.6	3.1	14.2	14.6	10.8	19.2	1.2
NAP-01783	7	m		7.6	6.1	2.4	3.4	4.8	3.0	14.0	15.7	10.4	18.1	1.6
ABV-00471	6	m	paratype	8.6	7.1	3.0	3.4	4.9	3.2	14.2	14.5	13.1	19.6	1.2
ABV-00454	6	m	paratype	8.2	7.2	3.0	3.2	5.1	3.2	15.7	15.3	13.1	22.0	1.3
ABV-00472	6	m	paratype	7.9	6.5	2.5	3.0	5.2	3.1	14.7	11.9	12.6	21.4	1.2
ROM-36524	11	m		6.8	6.4	2.2	2.9	4.5	2.4	13.0	13.0	10.8	17.4	1.5

ROM-36527	11	m		6.4	6.7	2.4	2.6	4.9	2.5	12.2	11.9	11.9	19.0	1.5
ROM-36522	11	m		6.8	6.6	2.2	2.7	4.3	2.3	12.3	12.9	11.3	18.7	1.2
ROM-36528	11	m		6.1	5.8	2.2	2.4	4.2	2.5	12.5	13.0	11.7	18.4	1.7
ROM-36526	11	m		6.1	6.3	2.3	2.5	4.3	2.7	12.7	12.4	12.7	18.2	2.2
ITBCZ 2828	8	m		7.8	7.1	3.2	2.9	4.1	3.4	4.6	12.0	11.2	19.6	1.8
ITBCZ 2908	8	m		7.5	8.0	3.5	2.8	5.1	3.9	14.8	14.9	12.7	22.8	1.9
ITBCZ 2909	8	m		7.8	8.2	3.1	3.8	5.2	4.1	16.3	16.1	12.6	21.9	2.1
ITBCZ 2918	8	m		7.0	7.3	3.4	3.3	5.0	3.5	19.6	13.4	12.1	20.0	1.7
ITBCZ 2919	8	m		8.9	8.3	3.4	3.0	6.0	3.6	16.0	15.1	12.3	21.9	2.1
ITBCZ 3502	8	m		7.8	7.7	3.2	3.0	5.5	3.5	15.0	14.7	12.6	20.7	1.8
ABV-00455	6	f	paratype	8.3	7.7	2.9	3.3	4.9	3.1	16.1	16.2	14.2	22.6	1.7
CYS-10-10	11	f		7.9	8.6	3.0	3.4	5.2	3.2	15.3	15.5	13.9	21.8	2.4
ROM-36530	11	f		7.7	8.7	3.3	4.7	5.6	3.6	14.9	15.8	15.1	23.3	1.5
ITBCZ 2786	8	f		9.8	9.2	4.1	3.9	6.3	4.6	19.8	17.8	15.7	27.2	2.3
ITBCZ 2788	8	f		10.1	9.7	4.2	4.1	7.0	4.9	17.7	15.5	15.3	25.0	2.0
ITBCZ 2792	8	f		10.1	9.1	4.5	4.1	5.8	5.2	19.0	17.2	15.1	26.0	2.1

Figure 11. External morphology of the tadpole of *Ophryophryne elfina* sp. n. (ZMMU A-5679, field number NAP-01169; Stage 25, TOL = 30.2 mm). **A** lateral view, mouth funnel closed (in preservative) **B** dorsal view, mouth funnel open (in life). Drawings by V.D. Kretova.

In life tadpoles have dorsal side of body and upper flanks uniform brownish red or brownish orange (Fig. 12A, B). Lower flanks weakly mottled with dark brown, few round blackish spots on tail and dorsum; with orange neuromasts visible on dorsal surface (Fig. 12A). Abdomen light brownish orange, intestine not visible through body wall. Caudal muscles pale; tail fins translucent with a few darker spots (more on upper than on lower fin); dorsally tail with indistinct middorsal orange line (Fig. 12A). Eyes golden with black reticulations. Oral funnel pinkish orange with brownish red papillae (Fig. 12B). In preservative tadpole coloration gets much duller, but the general coloration pattern is still visible after 7 years in ethanol.

Advertisement call characteristics. Refer to the Acoustic differentiation section, Table 4 and Fig. 5, for bioacoustic comparison of the new species with *O. synoria* and *O. gerti*. Refer to Appendix 3 for call variation data in *Ophryophryne elfina* sp. n.

Position in mtDNA phylogeny and sequence divergence. The new species is reconstructed as a member of the *Ophryophryne* Group II (Fig. 2), forming a sister group with respect to the clade joining *O. gerti* and *O. synoria* (see Fig. 2). Uncorrected genetic *p*-distances between *Ophryophryne elfina* sp. n. partial sequences for the 16S rRNA gene and all homologous sequences available on GenBank included in the analysis (see Table 1) varied from 8.2% (with *O. gerti s. stricto*, clade A) to 10.0% (with *O. hansi* and *O. synoria*, clade C) (see Table 2). This degree of pairwise divergence in the 16S rRNA gene is greater than that usually representing differentiation at the species level in anura (Vences et al. 2005a, 2005b, Vieites et al. 2009, Poyarkov et al. 2015a, 2015b). Intraspecific variation in this gene fragment for *Ophryophryne elfina* sp. n. is significant — maximum sequence divergence between Nui Chua Mt. population

Figure 12. Coloration of *Ophryophryne* tadpoles in life: **A** *Ophryophryne elfina* sp. n. "(ZMMU A-5684, field number NAP-02673; Stage 25) from Chu Pan Fan Mt., 1900 m a.s.l. in Chu Yang Sin N.P., Dak Lak Prov., mouth funnel open **B** tadpole from the same locality and collection data (ZMMU A-5684, field number NAP-02673; Stage 25) with mouth funnel closed.. Photos by N.A. Poyarkov.

(Fig. 2, clade D) and populations from the rest of the species range (Fig. 2, clade E) is p = 3.1%. Intraspecific variation in 16S rRNA gene fragment within one geographic population was lower, and ranged from 0.0% to 0.8% of substitutions.

Distribution. *Ophryophryne elfina* sp. n. is found to be endemic to five provinces in (Lam Dong, Dak Lak, Khanh Hoa, Ninh Thuan and Phu Yen) in the northern and eastern part of the Langbian Plateau and its foothills in southern Vietnam (localities 6–12, Fig. 1). The new species is restricted to wet evergreen montane tropical and elfin forests, receiving high precipitation from the sea. Such wet forests are found only on high elevations in the central parts of the Langbian Plateau (e.g. 1900–2100 m a.s.l. on Bidoup Mt., Lam Dong Prov., Fig. 1, Loc. 6) or peripheral mountains remote from the sea (e.g. 1900–2300 m a.s.l. on Chu Pan Fan and Chu Yang Sin Mts., Dak Lak Prov., Fig. 1, Locs 10 and 11), but on the eastern foothills of the plateau which receive more precipitation, the new species is found at lower elevation (from 950 to 1510 m a.s.l. on Hon Ba Mt., Khanh Hoa Prov., Fig. 1, Loc. 8; 780 m a.s.l. on Nui Chua Mt., Ninh Thuan Prov., Fig. 1, Loc. 9; and 700 m in Phu Yen Prov., Fig. 1, Loc. 12).

Ecology. All specimens were collected at night after heavy rains along montane cascade rocky streams, along small waterfalls, or intermittent rocky brooks; or found during the day time under tree-logs and within leaf litter in the limited fragments of

primary montane wet polydominant evergreen tropical forests, with a high abundance of large rocks and fallen trees covered with a thick layer of mosses. This including high montane forests that are composed of the specific floral community known as "elfin" forests, with miniature trees (up to 10 m tall). These areas always have high precipitation and have much milder climate than other tropical forests in southern Vietnam: active breeding of the new species was recorded in February with temperatures of ca. 11–12°C.

On Bidoup Mt. summit (Lam Dong Prov.), *Ophryophryne elfina* sp. n. was recorded from 1890 to 2035 m a.s.l. in montane polydominant high canopy (trees up to 35 m tall) and elfin (trees up to 10 m tall) (sub)tropical forests with the predominance of trees of the family Fagaceae (*Lithocarpus* sp., *Castanopsis* sp.), Elaeocarpaceae (*Elaeocarpus* sp.), Lauraceae (*Machilus* sp.), Magnoliaceae (*Magnolia* sp., *Michelia* sp.), and occasional large trees of *Fokienia hodginsii* (Cupressaceae). These forests have thick leaf litter, numerous fallen logs and rocks covered with mosses, and an undergrowth that is predominated by ferns (mostly *Asplenium* sp., Aspleniaceae) (see Kuznetsov and Kuznetsova 2011) (Fig. 15A). On Hon Giao mountain ridge, the new species was found along mountain streams from 1800 to 1900 m a.s.l. in montane polydominant elfin forests with the predominance of trees of the family Fagaceae (*Castanopsis* sp., *Lithocarpus* sp.), Lauraceae (*Cinnamomum* sp., *Neolitsea* sp.), Ericaceae (*Rhododendron* sp.), Magnoliaceae (*Mangletia* sp., *Michelia* sp.), Elaeocarpaceae (*Elaeocarpus* sp.) and Podocarpaceae (*Podocarpus neriifolius*). This forest had a dense undergrowth of mosses, orchids (*Coelogyne* sp., *Dendrobium* sp.; Orchidaceae) and occasional ferns (*Cyathea* sp., Cyatheaceae) (see Kuznetsov and Kuznetsova 2011) (Fig. 15B). In Chu Yang Sin N.P. (Dak Lak Prov.), the new species was found from 1800 to 2100 m a.s.l. in montane forests with the predominance of trees of the families Pinaceae (*Pinus krempfii*; *Pinus kesiya*) and Fagaceae (*Lithocarpus* sp., *Castanopsis* sp.), with dense undergrowth of ferns, numerous rocks and fallen trees covered with mosses (Fig. 15C). In Hon Ba N.R. (Khanh Hoa Prov.), the new species was found from 950 to 1510 m a.s.l. along mountain streams in forests having polydominant composition including Fagaceae (*Lithocarpus* sp., *Quercus* sp.), Elaeocarpaceae (*Elaeocarpus* sp.), Theaceae (*Thea* sp., *Camellia* sp.), Lauraceae (*Cinnamomum* sp., *Neolitsea* sp.), Araliaceae (*Schefflera* sp.) and Rutaceae (*Euodia* sp.).

On Bidoup Mt. summit (1890–2035 m a.s.l.; Lam Dong Prov.) *Ophryophryne elfina* sp. n. occurs in syntopy with *Leptobrachium pullum* (Smith, 1921), *Leptobrachium leucops* Stuart, Rowley, Tran, Le & Hoang, 2011, *Leptolalax bidoupensis* Stuart, Rowley, Tran, Le & Hoang, 2011, *Leptolalax pallidus* Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016, *Ingerophrynus galeatus* (Günther, 1864), *Hylarana montivaga* (Smith, 1921), *Rhacophorus vampyrus* Rowley, Le, Thi, Stuart & Hoang, 2010, *Theloderma palliatum* Rowley, Le, Hoang, Dau & Cao, 2011 and *Raorchestes gryllus* (Smith, 1924). On Hon Giao Mt. (1900–2000 m a.s.l.; borders of Lam Dong and Khanh Hoa provinces), *Ophryophryne elfina* sp. n. occurs in syntopy with *Leptobrachium leucops*, *Leptolalax bidoupensis*, *Duttaphrynus melanostictus* (Schneider, 1799), *Hylarana montivaga*, *Rhacophorus vampyrus* and *Raorchestes gryllus*. On Chu Pan Fan and Chu Yang Sin Mts. (1900 m a.s.l., Dak Lak Prov.), the new species is found in syntopy with *Xenophrys* cf. *maosonensis* (Bourret, 1937), *Leptobrachium*

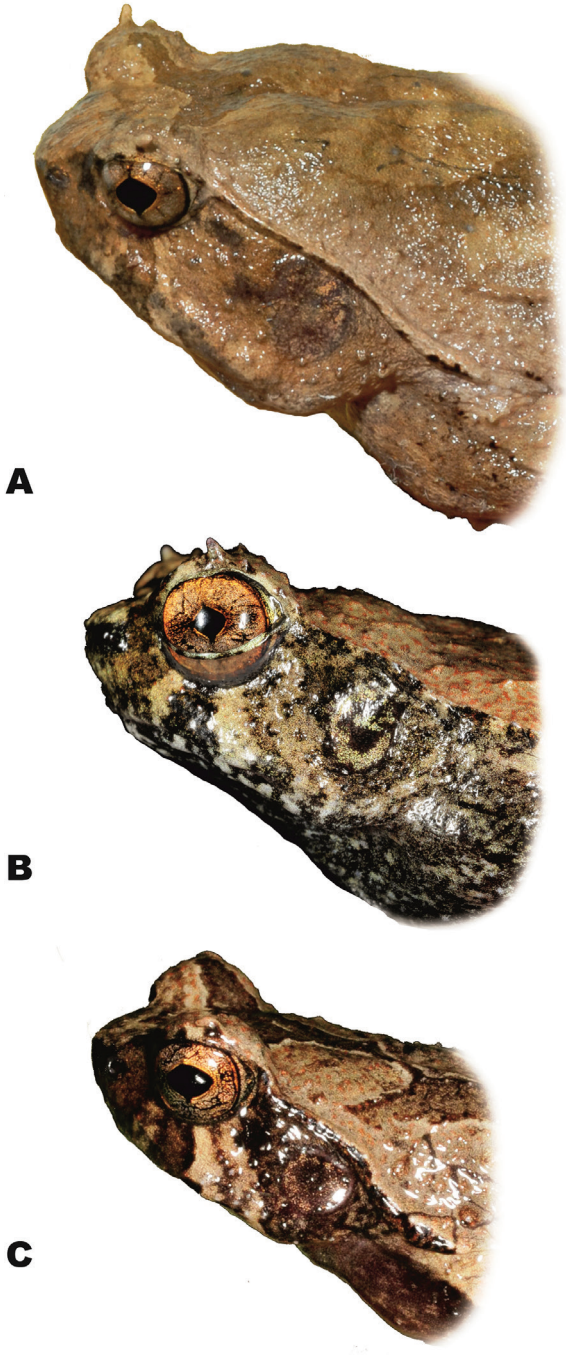


Figure 13. Comparison of the head coloration in life of three *Ophryophryne* species from the Langbian Plateau: **A** *Ophryophryne synoria*, Bu Gia Map N.P., Binh Phuoc Prov., Vietnam **B** *Ophryophryne gerti*, Chu Yang Sin N.P., Dak Lak Prov., Vietnam **C** *Ophryophryne elfina* sp. n., Hon Giao Mt., Bidoup–Nui Ba N.P., Lam Dong Prov. Photos by N.A. Poyarkov and N.L. Orlov.

sp., *Leptolalax* sp., *Hylarana montivaga*, *Rhacophorus vampyrus* and *Raorchestes gryllus*. On Chu Yang Sin Mt. (1700–1800 m a.s.l., Dak Lak Prov.), the new species is also found in syntopy with *Ophryophryne gerti* (Fig. 14A). On Hon Ba Mt. (1500 m a.s.l., Khanh Hoa Prov.), the new species was recorded in syntopy with *Leptobrachium leucops*, *Leptolalax* sp., *Microhyla arboricola* Poyarkov, Vassilieva, Orlov, Galoyan, Tran, Le, Kretova & Geissler, 2014, *Theloderma truongsonense* (Orlov & Ho, 2005) and *Raorchestes gryllus*.

Reproductively active males were found while calling along streams, usually sitting on leaves of ferns or on the stone banks, rarely on rocks or large stones (see Fig. 14B, C). Some specimens were collected hiding amongst fern stems and were difficult to locate. Females were found hiding under tree logs or in the forest litter.

The ovaries of females contained well-developed unpigmented eggs with a diameter of approximately 2.2–2.8 mm (N = 15; measured from ZMMU ABV-00455, gravid female). On Hon Ba Mt., calling males were observed between 22 to 24 December, 22 to 28 March and 15 to 18 October. On Bidoup, Hon Giao and Chu Yang Sin Mts., reproductive activity and calling males were recorded from 10 February until mid-July. Tadpoles were found from April until July in the same streams where calling males were recorded; during the day time tadpoles hide under flat stones or dead leaves on the stream bed, but come out and can be visible in the shallow sandy parts of the stream at night. Metamorphosed individuals were observed in Chu Yang Sin N.P. in May.

Conservation status. The full extent of the distribution of *Ophryophryne elfina* sp. n. is unknown, and the discovery of new localities on mountain ridges at elevations above 1500 m are highly anticipated. To date the species' range includes the following nature conservation areas: Bidoup–Nui Ba N.P. (Lam Dong Prov.), Chu Yang Sin N.P. (Lam Dong Prov.), Hon Ba N.R. (Khanh Hoa Prov.) and Nui Chua N.P. (Ninh Thuan Prov.). However, population size and population dynamics of the new species are unknown. Given the available information, we suggest *Ophryophryne elfina* sp. n. to be considered as a Data Deficient species following IUCN's Red List categories (IUCN Standards and Petitions Subcommittee 2016).

Comparisons. *Ophryophryne elfina* sp. n. is one of the smallest species of its genus, with adult male size (SVL 26.9–33.9 mm) similar to that of *O. pachyproctus* (adult male SVL 28.0–30.0 mm).

Ophryophryne elfina sp. n. differs from allopatric *O. hansi* (central Vietnam and neighboring southeastern Laos and northeastern Cambodia; Ohler 2003, Stuart 2005, Stuart et al. 2006) by its much smaller adult size: *Ophryophryne elfina* sp. n. male SVL 26.9–33.9 mm, N = 29, female SVL 35.1–36.5 mm, N = 6 (vs. *O. hansi* male SVL 33.4–43.1 mm, N = 12, female SVL 45.1–53.9 mm, N = 5; our data), by skin of dorsal and lateral surfaces of head, body and limbs shagreened with numerous small tubercles (vs. notably tubercular and warty skin on dorsal surfaces), and short dorsolateral glandular ridge above each shoulder (vs. dorsolateral glandular ridges absent).

Ophryophryne elfina sp. n. differs from allopatric *O. pachyproctus* (Yunnan Prov. in southern China, central Vietnam and possibly adjacent regions of Laos; Bain et al. 2007, Fei et al. 2009, 2010, 2012) by lacking dermal protuberance with dermal flaps

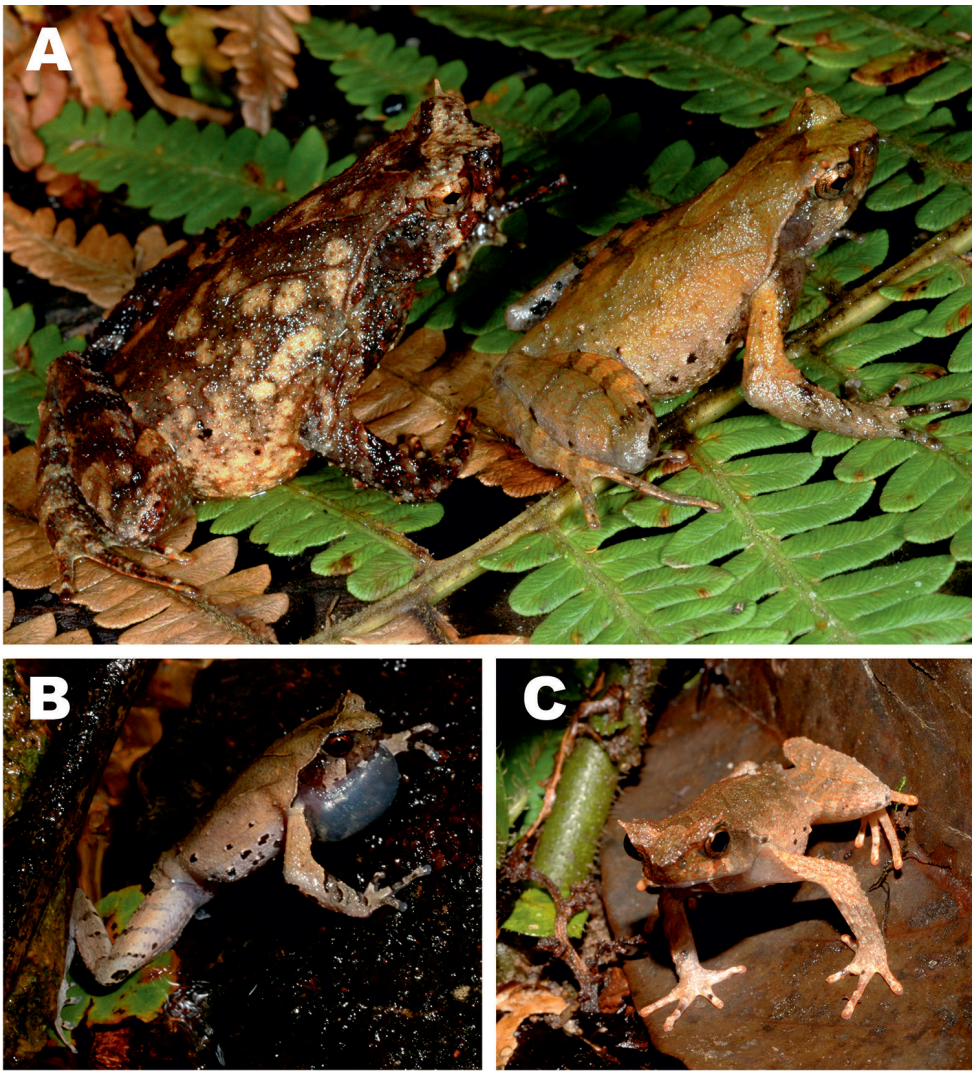


Figure 14. *Ophryophryne elfina* sp. n. in situ: **A** Two syntopically collected males of *Ophryophryne gerti* (left) and *Ophryophryne elfina* sp. n. (right) in Chu Yang Sin N.P., Dak Lak Prov., Vietnam, 1750 m a.s.l., photo by N.L. Orlov **B** calling adult male of *Ophryophryne elfina* sp. n. in Nui Chua Mt., Nui Chua N.P., Ninh Thuan Prov., Vietnam, 780 m a.s.l., photo by S.N. Nguyen **C** adult male of *Ophryophryne elfina* sp. n. in calling position in Hon Ba N.R., Khanh Hoa Prov., Vietnam, 1510 m a.s.l., photo by L.T. Nguyen.

above cloacal opening (vs. present on *O. pachyproctus*), short dorsolateral glandular ridge above each shoulder not connected to posterior tips of " >–< "-shaped glandular parietoscapular-sacral ridge (vs. dorsolateral glandular ridge connected to posterior tips of "H"- or "Y"-shaped glandular parietoscapular-sacral ridge), supratympanic fold light brown dorsally on males (vs. white or light beige dorsally on males), and males with nuptial pad on first finger only (vs. one nuptial pad each on first and second fingers).

Figure 15. Natural habitat of *Ophryophryne elfina* sp. n. on Langbian Plateau, southern Vietnam. **A** Elfin forest on the top of Bidoup Mountain (ca. 2100 m a.s.l.), Bidoup–Nui Ba N.P., Lam Dong Prov. **B** elfin forest on the top of Hon Giao Mountain (ca. 2000 m a.s.l.), Bidoup–Nui Ba N.P., border of Lam Dong and Khanh Hoa provinces **C** typical breeding site along a mountain stream in an evergreen mixed subtropical montane forest on northern slopes of the Chu Pan Fan Mountain, Chu Yang Sin N.P., Dak Lak Prov. (type locality) (ca. 1900 m a.s.l.). Photos by N.A. Poyarkov and O.V. Morozova.

Ophryophryne elfina sp. n. differs from allopatric *O. microstoma* (Guangxi and Yunnan Provs., southern China to northern Vietnam, and northern Thailand; Khonsue and Thirakhupt 2001, Chan-ard 2003, Fei et al. 2009, 2010, 2012, Nguyen et al. 2009), by much smaller adult size: *Ophryophryne elfina* sp. n. male SVL 26.9–33.9 mm, N = 29, female SVL 35.1–36.5 mm, N = 6 (vs. *O. microstoma* male SVL 34.3–44.4 mm, N = 10, female SVL 39.4–57.0 mm, *N* = 7; Ohler 2003, Fei et al. 2009, Stuart et al. 2010; our data), short anterior dorsolateral glandular ridge above each shoulder (vs.

dorsolateral ridges long, ca. 65–90% trunk length), and large tubercles posteriorly on dorsal surface of body (vs. large tubercles absent).

Ophryophryne elfina sp. n. differs from allopatric *O. poilani* (found in the mountains of the Tay Nguyen Plateau of central Vietnam and in adjacent areas of northeastern Cambodia, and, possibly, Laos; Bourret 1937, Stuart et al. 2010) by having smaller adult body size: *Ophryophryne elfina* sp. n. male SVL 26.9–33.9 mm, N = 29, female SVL 35.1–36.5 mm, N = 6 (vs. *O. poilani* male SVL 32.6–38.1 mm, N = 14, female SVL 47.4–50.8 mm, *N* = 2; Stuart et al. 2010), short dorsolateral glandular ridge above the shoulder not connected to posterior tips of " >–< "-shaped parietoscapular-sacral glandular ridge (vs. dorsolateral glandular ridge connected to posterior tips of " >–< "-shaped parietoscapular-sacral glandular ridge), and lacking characteristic dark "mask-like" coloration of temporal region, and supratympanic fold being dark brown ventrally and light brown dorsally on males (vs. temporal region and lateral surfaces of head uniformly dark-colored forming a dark "mask", extending posteriorly towards axilla; supratympanic fold edged with white on males).

Ophryophryne elfina sp. n. differs from sympatric *O. synoria* (found at lower elevation from 200 to 1500 m a.s.l. in the foothills of the Langbian Plateau in southern Vietnam and adjacent easternmost hilly Cambodia) by much smaller adults body size: *Ophryophryne elfina* sp. n. male SVL 26.9–33.9 mm, N = 29, female SVL 35.1–36.5 mm, N = 6 (vs. *O. synoria* male SVL 38.2–53.7 mm, N = 14, female SVL 51.4–70.7 mm, N = 3; our data; Fig. 3), red-orange nuptial pad (in life) on first finger only (vs. two nuptial pads, covered in brown microgranules, large on first finger, covering entire dorsal metacarpal extending to 3/4 basal phalange length, on second finger medium sized on metacarpal extending to mid basal phalange on inner dorsal side), numerous bright red-orange asperities (in life) on dorsal and lateral surfaces of body, head and dorsal surfaces of limbs (vs. black and white asperities, small sized, spinular, moderately dense in narrow band along lower jaw, and on posterior upper jaw, few on tympanic region [exlcuding tympanum], along supratemporal folds and on posterior upper eyelids; some on anterior dorsum, becoming moderately dense posteriorly, above and surrounding cloaca, few on dorsal shanks, and absent on remaining surfaces on holotype of *O. synoria*), and smaller tympanum/eye diameter ratio, TYD/ED 48.9%–62.6%, N=29 (vs. TYD/ED 64.8%–85.2%, N=14).

Ophryophryne elfina sp. n. differs from sympatric *O. gerti* (found at mid-elevations from 700 to 2000 m a.s.l. in the central and northern parts of the Langbian Plateau in southern Vietnam) by typically smaller adults body size: *Ophryophryne elfina* sp. n. male SVL 26.9–33.9 mm, N = 29, female SVL 35.1–36.5 mm, N = 6 (vs. *O. gerti* male SVL 31.7–42.2 mm, N = 15, female SVL 43.1–47.4 mm, *N* = 3; our data; Fig. 3), bright red-orange nuptial pads on males in life (vs. grey or black-brown nuptial pads on males in life), short dorsolateral glandular ridge above each shoulder, not connected to posterior tips of " >–< "-shaped parietoscapular-sacral glandular ridge, see Figs 6G–H, and 8 (vs. strong dorsolateral glandular ridge from above each shoulder to approximately 4/5 distance between axilla and groin, connecting with posterior tips of " >–< "-shaped parietoscapular-sacral glandular ridge; see Figs 6A–C, 7), skin on dor-

sal and lateral surfaces of body shagreened with numerous small tubercles (vs. skin on dorsum and sides of body granular, with numerous small and medium-sized tubercles and larger warts, see Fig. 6A–C), dark hourglass-shaped markings on dorsum normally edged with white, see Figs 6H and 8 (vs. dark hourglass-shape on dorsum indistinct or, if present, unclear and not edged with white, Fig. 6A–C), and throat, chest and abdomen having generally lighter coloration than in *O. gerti*.

Though available information on tadpole morphology of *Oprhyophryne* is very limited (Liu and Hu 1962, Huang et al. 1991, Grosjean 2003, Fei et al. 2009), the tadpoles assigned to the new species based on the analysis of short 16S rRNA gene sequences (Table 1) have certain morphological characteristics that could be useful for distinguishing the larval stage of *Ophryophryne elfina* sp. n. from other *Ophryophryne* species. From tadpoles of *O. microstoma*, described in detail by Grosjean (2003), tadpoles of *Ophryophryne elfina* sp. n. differ mainly by possessing a longer tail: TOL/BL ratio 231.3 ± 11%, $N = 5$ (vs. TOL/BL < 210%, $N = 52$ on *O. microstoma*), and tail tip rounded (vs. tail tip bluntly pointed), mean = 22 longitudinal rows of papillae and from 2–4 (mean = 3) transverse rows of papillae on the upper labium and 4–6 (mean = 5) transverse rows of papillae on the lower labium, $N = 5$ (vs. mean = 20 longitudinal rows of papillae, and two upper labium and four lower labium transverse rows of papillae [without clear limits], $N = 52$); however, some of these differences may relate to the fact that Grosjeans' description was based on later developmental stages (Gosner stage 37) than our sampling (Gosners' stage 25).

DNA-barcoding using short sequences for 16S rRNA (Table 1) also enabled us to identify tadpoles of sympatric *Ophryophryne* species from the Langbian Plateau, and though our sampling is not big enough to provide detailed morphological descriptions of larval morphology for *O. gerti* and *O. synoria*, we found some differences in coloration of tadpoles which may be useful for preliminary diagnostics of the three sympatric *Ophryophryne* species in the wild. Despite overall morphological similarity, both *O. gerti* and *O. synoria* show the presence of light golden to copper blotches on dorsal surfaces of the body and tail, whereas *Ophryophryne elfina* sp. n. tadpoles always have distinctive uniform brownish coloration with small coppery dots (Fig. 12A–B).

Despite overall similarity, advertisement calls of each *Ophryophryne* species inhabiting the Langbian Plateau are easily diagnosable based on acoustic parameters. Some parameter values clearly differ between all of the studied species (Fig. 5). For example, the highest values of the repetition rate per recording and per series are found for *O. synoria* (3.07 ± 0.13 calls/s, $N = 3$, and 5.34 ± 0.15 calls/s, $N = 15$, respectively) while the lowest are reported for *O. gerti* (0.35 ± 0.14 calls/s, $N = 3$, and 2.33 ± 0.03 calls/s, $N = 108$, respectively). *Ophryophryne elfina* sp. n. occupies an intermediate position between these two species (1.18 ± 0.2 calls/s, $N = 5$, and 3.87 ± 0.07 calls/s, $N = 140$, respectively; differences significant, $F_{2.8} = 46.7$, $p < 0.001$ and $F_{2.260} = 220.7$, $p < 0.001$, respectively, one-way ANOVA).

The call temporal parameters for *Ophryophryne elfina* sp. n. compared to sympatric *O. synoria* and *O. gerti*, also differ for series duration, which is the highest in calls of *Ophryophryne elfina* sp. n., comprising 3.42 ± 0.11 s, $N = 140$ (see Table 4) (differences

with *O. gerti* significant, $F_{2.267} = 40.4$, $p < 0.001$, one-way ANOVA; differences with *O. synoria* not significant, see Table 4 for details). The advertisement call of the new species is further significantly different from calls of *O. gerti* for values of a number of acoustic parameters (see Table 4), such as the number of calls per series (12.84 ± 0.41, $N = 140$, versus 4.64 ± 0.16, N = 115, for *O. gerti*; differences significant, $F_{2.267} = 151.4$, $p < 0.001$, one-way ANOVA), the call duration (73 ± 0.23 ms, $N = 1797$, versus 104 ± 0.5 ms, $N = 533$, for *O. gerti*; differences significant, $H_{2.2530} = 1345.1$, $p < 0.001$, Kruskal-Wallis ANOVA) and inter-calls interval (207 ± 2.06 ms, $N = 1657$, versus 421.54 ± 4.17 ms, $N = 418$, for *O. gerti*; differences significant, $H_{2.2260} = 1008.5$, $p < 0.001$, Kruskal-Wallis ANOVA). The advertisement call of *Ophryophryne elfina* sp. n. is further different from calls of *O. synoria* in the frequency of maximum amplitude (4645.94 ± 4.39 Hz, $N = 1797$, versus 3798.9 ± 4.87 Hz, $N = 200$; differences significant, $H_{2.2530} = 1030.2$, $p < 0.001$ (U = 0), Kruskal-Wallis ANOVA).

Finally, the new species is markedly distinct from all other congeners for which comparable sequences are available, including it closest relatives *O. gerti* and *O. synoria*, by relatively large genetic distances in 16S rRNA mtDNA gene fragment ($p \geq 8.2\%$).

Discussion

The data presented here provide the most extensive molecular sampling for the elucidation of phylogentic relationships within the genus *Ophryophryne*. According to our data, genetic variation within *Ophryophryne* appears to be strongly geographically structured. Thus, our results indicate the division of the genus *Ophryophryne* into two major reciprocally monophyletic groups: one corresponding to species found on the Langbian Plateau (Group II, Fig. 2), and another joining species found outside the plateau from central and northern Truong Son and adjacent areas (Group I, Fig. 2). Our data support the hypothesis that eastern Indochina, including the central and southern parts of the Truong Son Mountains (known also as Tay Nguyen Plateau), host the highest diversity of *Ophryophryne*, and was the center of radiation for this genus (Orlov and Ananjeva 2007, Mahony et al. 2017). Similar patterns of geographic structuring of mtDNA lineages were reported for the genus *Leptolalax*, another megophryid genus inhabiting the Truong Son Mountains (Poyarkov et al. 2015a, Rowley et al. 2016).

A hidden diversity of *Ophryophryne* frogs is revealed in the mountains of the Langbian Plateau, where previously only one species, *O. gerti*, was correctly reported (Ohler 2003, Nguyen et al. 2009, Stuart et al. 2010). In our study it is shown that the previous records of *O.* cf. *gerti* from central Vietnam and Laos (Ohler 2003, Bain et al. 2007) actually belong to different species of *Ophryophryne* and thus we clarify the range of *O. gerti* showing that this species is likely endemic to the Langbian Plateau. The known distribution of *O. synoria* is also extended, previously known exclusively from Cambodia (Stuart et al. 2006) and adjacent provinces of Vietnam (Vassilieva et al. 2016), and demonstrate that this species has a considerably wider range encompassing the central, northern and western edges of the Langbian Plateau. Finally, we describe the new spe-

cies *Ophryophrye elfina* sp. n., which is endemic to the northern and eastern edges of the plateau. Thus, the Langbian Plateau was a center of *Ophryophryne* radiation and cradles three endemic species of these frogs; all of them are sympatric in eastern and northern parts of the plateau and often can be recorded in synbiotopy.

Ophryophrye elfina sp. n. represents one of the smallest known species of the genus *Ophryophryne*. We found that the three *Ophryophryne* species of the Langbian Plateau are differentiated in body size with the largest species *O. synoria* preferring lowland and foothill monsoon forests at elevations from 200 to 1500 m a.s.l., medium-sized *O. gerti* found in evergreen montane tropical forests at mid-elevations from 700 to 2000 m a.s.l. and the smallest species *Ophryophryne elfina* sp. n. being restricted to wet montane subtropical forests at elevations from 700 to 2100 m a.s.l., including elfin forests at high elevations. It is probably not surprising that advertisement calls of the three occasionally sympatric *Ophryophryne* species show significant differences both in call structure and frequency parameters (see Table 4, Fig. 5), and the three studied species are characterized by relatively high values of the frequency parameters (as compared to several other Megophryidae species studied acoustically, especially of the genera *Leptolalax*, see review in Rowley et al. 2016, and *Leptobrachium*, see e.g. Stuart et al. 2010). The high frequency parameters may be related with their tendency to vocalize in close proximity to mountain cascade streams, which would create a low-frequency background noise (Preininger et al. 2007). It was shown that low background noise may induce frogs to call at higher frequency rates than expected from their body size, thereby improving the signal-to-noise ratio of their calls (Penna et al. 2005, Wells 2007, Goutte et al. 2016). Our study also recorded that the values of some temporal call parameters of *Ophryophryne elfina* sp. n. significantly differ between February (average temperature 11.3°C) and April recordings (average temperature 17.5°C; see Appendix 3 for details). Our results correspond with previous reports that intraspecific variation of temporal parameters of anuran calls can depend upon temperature (e.g., Gerhardt and Huber 2002).

The frequency of maximum amplitude coincides with the fundamental frequency for all *Ophryophryne* species, and have almost equal values for *Ophryophryne elfina* sp. n. and *O. gerti* (4645.94 ± 4.39 Hz, N = 1797, and 4845.99 ± 4.22 Hz, N = 533, respectively). The frequency of maximum amplitude of *O. synoria* is significantly lower (3798.9 ± 4.87 Hz, N = 200; see Table 4 for details), which may be related to the larger body size of the latter species (Stuart et al. 2006, Wells 2007). Further studies on acoustic communication of Langbian *Ophryophryne* species in areas of allopatry and sympatry would be valuable for better understanding the bioacoustic patterns observed here.

The Langbian Plateau is known for its high herpetofaunal diversity and endemism, a significant portion of which has been discovered only recently (e.g., Orlov et al. 2008, 2012, Rowley et al. 2010c, 2011a, 2011b, 2016, Stuart et al. 2011, Poyarkov [Paiarkov] and Vassilieva 2011, Nazarov et al. 2012, Chan et al. 2013, Hartmann et al. 2013, Geissler et al. 2014, 2015, Vassilieva et al. 2014, Poyarkov et al. 2014, 2015a, 2015b). Despite this increase in species discoveries, many areas of the Annamites have received little scientific attention and are very likely to host further

previously unknown diversity. The need for biological exploration in this region is made more urgent given the ongoing loss of natural habitats due to logging, road construction, increasing agricultural pressure and other human activities (Meijer 1973, De Koninck 1999, Laurance 2007, Meyfroidt and Lambin 2008, Kuznetsov and Kuznetsova 2011).

Habitat loss is the greatest threat to amphibians in southeast Asia, and the amphibians of the region appear to be particularly vulnerable to habitat alterations (Rowley et al. 2010b). Frogs of the genus *Ophryophryne* depend on fast-flowing clean mountain streams for reproduction, and appear to be restricted to relatively undisturbed broadleaf evergreen forests: such habitat specialist range-restricted species are likely to be most at risk (Poyarkov et al. 2012, Rowley et al. 2010b, 2016). Deforestation, habitat loss and modification are continued threats in southern Indochina (Meyfroidt and Lambin 2008), and further studies on herpetofaunal biodiversity in this region are urgently required for elaboration of effective conservation measures.

Addendum (added post manuscript acceptance)

Due to the simultaneous review period of the present paper, and the now recently published Mahony et al. (2017), we chose to preliminarily use *Ophryophryne* at the genus level (following Chen et al. 2017; published online 1 December 2016), pending the publication of the taxonomic justification by Mahony et al. (2017) which supports a subgenus level classification of *Ophryophryne* within *Megophrys*. Mahony et al. (2017) also provided the replacement name *Megophrys* (*Ophryophryne*) *koui* Mahony, Foley, Biju & Teeling, 2017 for *Ophryophryne pachyproctus* Kou, 1985. We suggest that the new species combination *Ophryophryne elfina* sp. n. should hereafter be referred to as *Megophrys* (*Ophryophryne*) *elfina* (Poyarkov, Duong, Orlov, Gogoleva, Vassilieva, Nguyen, Nguyen, Nguyen, Che & Mahony) to reflect this revised taxonomy.

Author contributions

NA Poyarkov envisioned the original idea of the manuscript, collected material and data in the field and in the lab, executed this study and wrote the manuscript; TV Duong performed morphometric, molecular and phylogenetic analyses; NL Orlov collected material in the field; SS Gogoleva collected data in the field and performed acoustic analyses and wrote the relevant parts of the manuscript; AB Vassilieva collected material and data in the field; LT Nguyen collected material in the field and assisted with morphological analysis; VDH Nguyen, J Che and SN Nguyen collected material in the field and provided additional molecular data; S Mahony examined type and comparative specimens, performed molecular analysis, provided redescription of types, and edited the manuscript. All authors contributed to this paper sufficiently.

Acknowledgements

Fieldwork was funded by the Joint Russian-Vietnamese Tropical and Technological Center (JRVTTC) and was conducted under permission of the Bureau of Forestry, Ministry of Agriculture and Rural Development of Vietnam (permits Nos. 170/ TCLN–BTTN of 07/02/2013; 400/TCLN-BTTN of 26/03/2014; 831/ TCLN–BTTN of 05/07/2013) and of local administration (Lam Dong Prov.: No. 5832/UBND-LN of 22/10/12; Khanh Hoa Prov.: No. 522/SngV-TTDN&HTQT of 13/07/2013; fieldwork was conducted in accordance to the Agreement No. 37/HD on the scientific cooperation between Cat Tien N.P. and the JRVTTC and the Agreement № 137/HD NCKH of 23.06.2010 on the scientific cooperation between Bu Gia Map N.P. and the JRVTTC). The authors are grateful to Andrei N. Kuznetsov, Leonid P. Korzoun and Vitaly L. Trounov for support and organization of fieldwork. We sincerely thank our Vietnamese colleagues Nguyen Dang Hoi, Hoang Minh Duc, Nguyen Ngoc Hung, Nguyen Thien Tao, Pham Thi Ha Giang, Tran Tien and Le Xuan Son for help and continued support. Many thanks to Eduard A. Galoyan, Igor V. Palko, Evgeniy S. Popov, Olga V. Morozova, Alina V. Alexandrova, Valentina D. Kretova and Evgeniya N. Solovyeva for their help during the fieldwork, assistance in the laboratory and continued support of this project. We thank Ilya A. Volodin for help with acoustic analyses. For permission to study specimens under their care, we thank Valentina F. Orlova (ZMMU) and Natalia B. Ananjeva (ZISP). We are grateful to Andrey N. Kuznetsov for help with plant identification. We are sincerely grateful to Angelica Crottini, Annemarie Ohler and an anonymous reviewer for their kind help and useful comments which helped us to improve the previous version of the manuscript. The study was completed under government theme AAAA-A16-116021660078-0 with financial support from the Russian Foundation for Basic Research (grants RFBR Nos. 15-04-02029a, 15-29-02771) to NAP. Specimen preservation and examination were completed with financial support from the Russian Science Foundation (RSF grant No. 14-50-00029). SNN would like to thank Mr. Thi, Hanh, Phuong, Trang, Lam, Nong, Hieu for their permission and organization of fieldwork in Hon Ba NR and thank The Rufford Foundation for financial support. JC was supported by the programs of the National Natural Science Foundation of China (31622052), Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences (Y4ZK111B01: 2015CASEABRI002), the Youth Innovation Promotion Association CAS, and the Animal Branch of the Germplasm Bank of Wild Species, Chinese Academy of Sciences (the Large Research Infrastructure Funding). SM was supported by a Field Museum of Natural History Science Visiting Scholarship, for FMNH specimen study, and would like to thank the following for providing access to specimens and tissues: Barry Clark and Jeffrey Streicher (NHMUK), Darrel Frost and David Kizirian (AMNH), Julie Feinstein (AMCC), and Alan Resetar (FMNH). SM thanks Emma Teeling for laboratory support and resources.

References

Bain RH, Nguyen TQ, Doan KV (2007) New herpetofaunal records from Vietnam. Herpetological Review 38: 107–117.

Bonaparte CLJL (1850) Conspectus Systematum. Herpetologiae et Amphibiologiae. Editio altera reformata. Brill EJ, Lugdini Batayorum, 8 pp. [In Latin]

Boulenger GA (1903) Descriptions of three new batrachians from Tonkin. Annals and Magazine of Natural History. Series 7 12: 186–188. https://doi.org/10.1080/00222930308678835

Bourret R (1937) Notes herpétologiques sur l'Indochine française. XIV. Les batraciens de la collection du Laboratoire des Sciences Naturelles de l'Université. Descriptions de quinze espèces ou variétés nouvelles. Annexe au Bulletin Général de l'Instruction Publique. Hanoi 1937: 5–56. [In French]

Bourret R (1942) Les Batraciens de l'Indochine. Institut Océanographique de l'Indochine, Hanoi, 453 pp. [In French]

Chan KO, Blackburn DC, Murphy RW, Stuart BL, Emmett DA, Ho CT, Brown RM (2013) A new species of narrow-mouthed frog of the genus *Kaloula* from eastern Indochina. Herpetologica 69: 329–341. https://doi.org/10.1655/HERPETOLOGICA-D-12-00094

Chan-ard T (2003) A Photographic Guide to Amphibians in Thailand. Darnsutha Press Co., Ltd., Bangkok, Thailand, 176 pp. [In Thai]

Chen JM, Zhou WW, Poyarkov Jr. NA, Stuart BL, Brown RM, Lathrop A, Wang YY, Yuan ZY, Jiang K, Hou M, Chen HM, Suwannapoom C, Nguyen NS, Duong VT, Papenfuss TJ, Murphy RW, Zhang YP, Che J (2017) A novel multilocus phylogenetic estimation reveals unrecognized diversity in Asian horned toads, genus *Megophrys* sensu lato (Anura: Megophryidae). Molecular Phylogenetics and Evolution 106: 28–43. https://doi.org/10.1016/j.ympev.2016.09.004

De Koninck R (1999) Deforestation in Viet Nam. International Research Centre, Ottawa, 101 pp.

Delorme M, Dubois A, Grosjean S, Ohler A (2006) Une nouvelle ergotaxinomie des Megophryidae (Amphibia, Anura). Alytes, Paris 24: 6–21. [In French]

Dubois A (1980) Notes sur la systematique et la répartition des amphibiens anoures de Chine et des régions avoisinantes IV. Classification générique et subgénérique des Pelobatidae Megophryinae. Bulletin Mensuel de la Société Linnéenne de Lyon 49: 469–482. https://doi.org/10.3406/linly.1980.10444 [In French]

Dubois A (1987) "1986"Miscellanea taxinomica batrachologica (I). Alytes, Paris 5: 7–95.

Fei L, Ye C-y, Huang Y-a, Liu M-y (1999) [Atlas of Amphibians of China]. Henan Press of Science and Technology, 432 pp. [In Chinese]

Fei L, Ye C-y, Huang Y-z, Jiang J-p, Xie F (2005) An Illustrated Key to Chinese Amphibians. Sichuan Publishing House of Science and Technology, Chongqing, 341 pp. [In Chinese]

Fei L, Hu S-q, Ye C-y, Huang Y-z (2009) Fauna Sinica. Amphibia. Volume 2. Anura. Chinese Academy of Science; Science Press, Beijing, 957 pp. [In Chinese]

Fei L, Ye C-y, Jiang J-p (2010) Colored Atlas of Chinese Amphibians. Sichuan Publishing Group/Sichuan Publishing House of Science and Technology, 519 pp. [In Chinese]

Fei L, Ye C-y, Jiang J-p (2012) Colored Atlas of Chinese Amphibians and Their Distributions. Sichuan Publishing House of Science and Technology, Sichuan, China, 619 pp. [In Chinese]

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39(4): 783–791. https://doi.org/10.2307/2408678

Frost DR [Ed.] (1985) Amphibian Species of the World. A Taxonomic and Geographical Reference. Association of Systematics Collections and Allen Press, Lawrence, Kansas, USA, 732 pp.

Frost DR, Grant T, Faivovich J, Bain RH, Haas A, Haddad CFB, de Sá RO, Channing A, Wilkinson M, Donnellan SC, Raxworthy CJ, Campbell JA, Blotto BL, Moler PE, Drewes RC, Nussbaum RA, Lynch JD, Green DM, Wheeler WC (2006) The amphibian tree of life. Bulletin of the American Museum of Natural History 297: 1–370. https://doi.org/10.1206/0003-0090(2006)297[0001:TATOL]2.0.CO;2

Geissler G, Poyarkov Jr. NA, Grismer LL, Nguyen TQ, An HT, Neang T, Kupfer A, Ziegler T, Böhme W, Müller H (2014) New *Ichthyophis* species from Indochina (Gymnophiona, Ichthyophiidae): 1. The unstriped forms with descriptions of three new species and the redescriptions of *I. acuminatus* Taylor, 1960, *I. youngorum* Taylor, 1960 and *I. laosensis* Taylor, 1969. Organisms Diversity and Evolution, 1–32. https://doi.org/10.1007/s13127-014-0190-6

Geissler P, Hartmann T, Ihlow F, Rödder D, Poyarkov Jr. NA,, Nguyen TQ, Ziegler T, Böhme W (2015) The Lower Mekong: an insurmountable barrier for amphibians in southern Indochina. Biological Journal of the Linnean Society 114(4): 905–914. https://doi.org/10.1111/bij.12444

Gerhardt HC, Huber F (2002) Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions. University of Chicago Press, Chicago, 531 pp.

Gorham SW (1974) Checklist of World Amphibians up to January 1, 1970. Saint-John, The New Brunswick Museum, 173 pp.

Gosner KL (1960) A simplified table for staging Anura embryos and larvae with notes on identification. Herpetologica 16: 183–190.

Goutte S, Dubois A, Howard SD, Marquez R, Rowley JJ, Dehling JM, Grandcolas P, Rongchuan X, Legendre F (2016) Environmental constraints and call evolution in torrent-dwelling frogs. Evolution 70(4): 811–826. https://doi.org/10.1111/evo.12903

Gray JE (1825) A synopsis of the genera of reptiles and Amphibia, with description of some new species. Annals of Philosophy. Series 2 10: 193–217.

Grosjean S (2001) The tadpole of *Leptobrachium* (*Vibrissaphora*) *echinatum* (Amphibia, Anura, Megophryidae). Zoosystema 23: 143–156.

Grosjean S (2003) A redescription of the external and buccopharyngeal morphology of the tadpole of *Ophryophryne microstoma* Boulenger, 1903 (Megophryidae). Alytes 21(1–2): 45–58.

Guibé J (1950) Catalogue des types d'Amphibiens du Muséum National d'Histoire Naturelle. Paris, 71 pp. [In French]

Günther A (1864) The Reptiles of British India. The Ray Society, London, 452 pp + I–XXVII + 1–26 pl.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nuclear Acids Symposium. Series 41: 95–98.

Hamidy A, Matsui M, Nishikawa K, Belabut DM (2012) Detection of cryptic taxa in *Leptobrachium nigrops* (Amphibia, Anura, Megophryidae), with description of two new species. Zootaxa 3398: 22–39.

Hartmann T, Geissler P, Poyarkov Jr. NA,, Ihlow F, Galoyan EA, Rödder D, Böhme W (2013) A new species of the genus *Calotes* Cuvier, 1817 (Squamata: Agamidae) from southern Vietnam. Zootaxa 3599(3): 246–260. https://doi.org/10.11646/zootaxa.3599.3.3

Hedges SB (1994) Molecular evidence for the origin of birds. Proceedings of the National Academy of Sciences of the United States of America 91: 2621–2624.

Hillis DM, Moritz C, Mable BK (1996) Molecular Systematics. 2nd edition. Sinauer Associates, Inc., Sunderland, Massachusetts, xvi + 655 pp.

Huang Y, Fei L, Ye C (1991) Studies on internal oral structures of tadpoles of Chinese Pelobatidae. Acta Biologica Sinica 10: 71–99. [In Chinese]

Huelsenbeck JP, Hillis DM (1993) Success of phylogenetic methods in the four-taxon case. Systematical Biology 42: 247–264. https://doi.org/10.1093/sysbio/42.3.247

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Inger RF, Orlov NL, Darevsky IS (1999) Frogs of Vietnam: A report on new collections. Fieldiana. Zoology. New Series 92: 1–46.

IUCN Standards and Petitions Subcommittee (2016) Guidelines for Using the IUCN Red List Categories and Criteria. Version 12. Prepared by the Standards and Petitions Subcommittee, 101 pp. http://www.iucnredlist.org/documents/RedListGuidelines.pdf

Jiang K, Yan F, Suwannapoom C, Chomdej S, Che J (2013) A new species of the genus *Leptolalax* (Anura: Megophryidae) from northern Thailand. Asian Herpetological Research 4: 100–108. https://doi.org/10.3724/SP.J.1245.2013.00100

Jobb G, Von Haeseler A, Strimmer K (2004) TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. BMC Evolutionary Biology 4: 18. https://doi.org/10.1186/1471-2148-4-18

Khonsue W, Thirakhupt K (2001) A checklist of the amphibians of Thailand. Natural History Journal of Chulalongkorn University 1: 69–82.

Kou Z-t (1985) A new species of *Ophryophryne* from Yunnan. Acta Herpetologica Sinica/ Liangqi baxing dongwu yanjiu. New Series, Chengdu 4(1): 41–43. [In Chinese]

Kuhl H, Van Hasselt JC (1822a) Uittreksels uit breieven van de Heeren Kuhl en van Hasselt, aan de Heeren C. J. Temminck, Th. van Swinderen en W. de Haan. Algemeene Konst-en Letter-Bode 7: 99–104. [In Dutch]

Kuznetsov AN, Kuznetsova SP (2011) Chuong 2. Thực Vật VQG Bidoup–Núi Bà [Chapter 5. Reptiles and Amphibians of Bidoup–Nui Ba National Park]. In: Nguyen DH, Kuznetsov AN (Eds) Da Dang Sinh Hoc Va Dac Trung Sinh Thai Vuon Quoc Gia Bidoup–Nui Ba, Nha Xuat Ban Hoa Hoc Tu Nhien Va Cong Nghe Publishing House, Hanoi, 37–105. [In Vietnamese]

Laurance WF (2007) Forest destruction in tropical Asia. Current Science 93(11): 1544–1550.

Liu C-c, Hu S-q (1962) A herpetological report of Kwangsi. Acta Zoologica Sinica/ Dong wu xue bao, Beijing 14(Supplement): 73–104. [In Chinese]

Mahony S (2011a) Two new species of *Megophrys* Kuhl and Van Hasselt (Amphibia: Megophryidae), from western Thailand and southern Cambodia. Zootaxa 2734: 23–39.

Mahony S (2011b) Erratum: Mahony, Stephen (2011) Two new species of *Megophrys* Kuhl and Van Hasselt (Amphibia: Megophryidae), from western Thailand and southern Cambodia. Zootaxa 2734: 23–39. Zootaxa 2924: 68.

Mahony S, Teeling EC, Biju SD (2013) Three new species of horned frogs, *Megophrys* (Amphibia: Megophryidae), from northeast India, with a resolution to the identity of *Megophrys boettgeri* populations reported from the region. Zootaxa 3722(2): 143–169.

Mahony S, Foley NM, Biju SD, Teeling EC (2017) Evolutionary history of the Asian Horned Frogs (Megophryinae): integrative approaches to timetree dating in the absence of a fossil record. Molecular Biology and Evolution 34(3): 744–771. https://doi.org/10.1093/molbev/msw267

Manthey U, Grossmann W (1997) Amphibien and Reptilien Südostasiens. Natur und Tier, Münster, 512 pp. [In German]

Matsui M, Shimada T, Liu WZ, Maryati M, Khonsue W, Orlov N (2006) Phylogenetic relationships of Oriental torrent frogs in the genus *Amolops* and its allies (Amphibia, Anura, Ranidae). Molecular Phylogenetics and Evolution 38: 659–666. https://doi.org/10.1016/j.ympev.2005.11.019

Matsui M, Hamidy A, Murphy RW, Khonsue W, Yambun P, Shimada T, Norhayati A, Daicus BM, Jiang JP (2010) Phylogenetic relationships of megophryid frogs of the genus *Leptobrachium* (Amphibia, Anura) as revealed by mtDNA gene sequences. Molecular Phylogenetics and Evolution 56: 259–272. https://doi.org/10.1016/j.ympev.2010.03.014

Matsui M, Nishikawa K, Yambun P (2014a) A new *Leptolalax* from the mountains of Sabah, Borneo (Amphibia, Anura, Megophryidae). Zootaxa 3753: 440–452. https://doi.org/10.11646/zootaxa.3753.5.3

Meijer W (1973) Devastation and Regeneration of Lowland Dipterocarp Forests in Southeast Asia. BioScience 23(9): 528–533. https://doi.org/10.2307/1296481

Meyfroidt P, Lambin EF (2008) Forest transition in Vietnam and its environmental impacts. Global Change Biology 14(6): 1319–1336. https://doi.org/10.1111/j.1365-2486.2008.01575.x

Murphy WJ, O'Brien SJ (2007) Designing and optimizing comparative anchor primers for comparative gene mapping and phylogenetic inference. Nature Protocols 2(11): 3022–3030. https://doi.org/10.1038/nprot.2007.429

Nazarov R, Poyarkov NA, Orlov NL, Phung TM, Nguyen TT, Hoang DM, Ziegler T (2012) Two new cryptic species of the *Cyrtodactylus irregularis* complex (Squamata: Gekkonidae) from southern Vietnam. Zootaxa 3302: 1–24.

Nguyen TQ, Phung TM, Schneider N, Botov A, Tran DTA, Ziegler T (2014) New records of amphibians and reptiles from southern Vietnam. Bonn zoological Bulletin 63(2): 148–156.

Nguyen SV, Ho CT, Nguyen TQ (2005) Danh Luc Ech Nhai Va Bo Sat Viet Nam [A Checklist of the Amphibians and Reptiles of Vietnam]. Hanoi: Nha Xuat Ban Hong Nghiep, 180 pp. [In Vietnamese]

Nguyen VS, Ho TC (1996) Danh luc bò sát và êch nhái Viêt Nam. HàNôi, Nhà xuât ban khoa hoc và ky thuât: 1–264. [In Vietnamese]

Nguyen VS, Ho TC, Nguyen QT (2009) Herpetofauna of Vietnam. Edition Chimaira, Frankfurt am Main, 768 pp.

Noble GK (1926) An analysis of the remarkable cases of distribution among the Amphibia, with descriptions of new genera. American Museum Novitates 212: 1–24.

Ohler A (2003) Revision of the genus *Ophryophryne* Boulenger, 1903 (Megophryidae) with description of two new species. Alytes. Paris 21: 23–42.

Ohler A, Wollenberg KC, Grosjean S, Hendrix R, Vences M, Ziegler T, Dubois A (2011) Sorting out *Lalos*: description of new species and additional taxonomic data on megophryid frogs from northern Indochina (genus *Leptolalax*, Megophryidae, Anura). Zootaxa 3147: 1–83.

Orlov NL, Poyarkov Jr. NA,, Nguyen TT (2015) Taxonomic notes on *Megophrys* frogs (Megophryidae: Anura) of Vietnam, with description of a new species. Russian Journal of Herpetology 22(3): 206–218.

Orlov NL, Poyarkov Jr. NA,, Vassilieva AB, Ananjeva NB, Nguyen TT, Sang NV, Geissler P (2012) Taxonomic notes on rhacophorid frogs (Rhacophorinae: Rhacophoridae: Anura) of southern part of Annamite Mountains (Truong Son, Vietnam), with description of three new species. Russian Journal of Herpetology 19: 23–64.

Orlov NL, Murphy RW, Ananjeva NB, Ryabov SA, Ho CT (2002) Herpetofauna of Vietnam, a checklist. Part 1. Amphibia. Russian Journal of Herpetology 9: 81–104.

Orlov NL, Nguyen SN, Ho CT (2008) Description of a new species and new records of *Rhacophorus* genus (Amphibia: Anura: Rhacophoridae) with the review of amphibians and reptiles diversity of Chu Yang Sin National Park (Dac Lac Province, Vietnam). Russian Journal of Herpetology 15: 67–84.

Orlov NL, Ananjeva NB (2007) Amphibians of South-East Asia. St. Petersburg University Press, St. Petersburg, 270 pp. [In Russian]

Orlov NL, Ho CT (2005) A new species of *Philautus* from Vietnam (Anura: Rhacophoridae). Russian Journal of Herpetology 12: 135–142.

Palumbi SR (1996) Nucleic acids II: the polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK (Eds) Molecular systematics. Second Edition. Sinauer Associates, Sunderland, 205–247.

Penna M, Pottstock H, Velasquez N (2005) Effect of natural and synthetic noise on evoked vocal responses in a frog of the temperate austral forest. Animal Behaviour 70(3): 639–651. https://doi.org/10.1016/j.anbehav.2004.11.022

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics 14(9): 817–818. https://doi.org/10.1093/bioinformatics/14.9.817

Poyarkov Jr. NA,, Rowley JJL, Gogoleva SS, Vassilieva AB, Galoyan EA, Orlov NL (2015a) A new species of *Leptolalax* (Anura: Megophryidae) from the western Langbian Plateau, southern Vietnam. Zootaxa 3931(2): 221–252. https://doi.org/10.11646/zootaxa.3931.2.3

Poyarkov Jr. NA,, Orlov NL, Moiseeva AV, Pawangkhanant P, Ruangsuwan T, Vassilieva AB, Galoyan EA, Nguyen TT, Gogoleva SS (2015b) Sorting out moss frogs: mtDNA data on taxonomic diversity and phylogenetic relationships of the Indochinese species of the genus *Theloderma* (Anura, Rhacophoridae). Russian Journal of Herpetology 22(4): 241–280.

Poyarkov Jr. NA,, Che J, Min M-S, Kuro-o M, Yan F, Li Ch, Iizuka K, Vieites DR (2012) Review of the systematics, morphology and distribution of Asian Clawed Salamanders, genus *Onychodactylus* (Amphibia, Caudata: Hynobiidae), with the description of four new species. Zootaxa 3465: 1–106.

Poyarkov Jr. NA,, Vassilieva AB, Orlov NL, Galoyan EA, Tran TAD, Le DTT, Kretova VD, Geissler P (2014) Taxonomy and distribution of narrow-mouth frogs of the genus *Microhyla* Tschudi, 1838 (Anura: Microhylidae) from Vietnam with descriptions of five new species. Russian Journal of Herpetology 21(2): 89–148.

Poyarkov [Paiarkov] NA, Vasilieva AB (2011) Chuong 5. Bò Sát — Lương củ VQG (Vườn Quốc Gia) Bidoup–Núi Bà [Chapter 5. Reptiles and Amphibians of Bidoup–Nui Ba National Park]. In: Nguyen DH, Kuznetsov AN (Eds) Da Dang Sinh Hoc Va Dac Trung Sinh Thai Vuon Quoc Gia Bidoup–Nui Ba, Hanoi, Nha Xuat Ban Hoa Hoc Tu Nhien Va Cong Nghe Publishing House, pp 169–220. [In Vietnamese]

Preininger D, Böckle M, Hödl W (2007) Comparison of anuran acoustic communities of two habitat types in the Danum Valley Conservation Area, Sabah, Malaysia. Salamandra 43(3): 129–138.

Puillandre N, Lambert A, Brouillet S, Achaz G (2012) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Molecular Ecology 21: 1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x

Pyron RA, Wiens JJ (2011) A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of advanced frogs, salamanders, and caecilians. Molecular Phylogenetics and Evolution 61: 543–583. https://doi.org/10.1016/j.ympev.2011.06.012

Rao D-q, Yang D-t (1997) The karyotypes of Megophryinae (Pelobatinae) with a discussion on their classification and phylogenetic relationships. Asiatic Herpetological Research 7: 93–102. https://doi.org/10.5962/bhl.part.18858

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Rowley JJL, Hoang DH, Le TTD, Dau QV, Cao TT (2010a) A new species of *Leptolalax* (Anura: Megophryidae) from Vietnam and further information on *Leptolalax tuberosus*. Zootaxa 2660: 33–45.

Rowley J, Brown R, Kusrini M, Inger R, Stuart B, Wogan G, Chan-ard T, Cao TT, Diesmos A, Iskandar DT, Lau M, Ming LT, Makchai S, Neang T, Nguyen QT, Phimmachak S (2010b) Impending conservation crisis for Southeast Asian amphibians. Biology Letters 6: 336–338. https://doi.org/10.1098/rsbl.2009.0793

Rowley JJL, Le TTD, Tran TAD, Stuart BL, Hoang DH (2010c) A new tree frog of the genus *Rhacophorus* (Anura: Rhacophoridae) from southern Vietnam. Zootaxa 2727: 45–55.

Rowley JJL, Le DTT, Tran DTA, Hoang DH (2011a) A new species of *Leptolalax* (Anura: Megophryidae) from southern Vietnam. Zootaxa 2796: 15–28.

Rowley JJL, Le TTD, Hoang DH, Dau QV, Cao TT (2011b) Two new species of *Theloderma* (Anura: Rhacophoridae) from Vietnam. Zootaxa 3098: 1–20.

Rowley JJL, Hoang HD, Dau VQ, Le TTD (2012) A new species of *Leptolalax* (Anura: Megophryidae) from central Vietnam. Zootaxa 3321: 56–68.

Rowley JJL, Tran DTA, Le DTT, Dau VQ, Peloso PLV, Nguyen TQ, Hoang HD, Nguyen TT, Ziegler T (2016) Five new, microendemic Asian Leaf-litter Frogs (*Leptolalax*) from the southern Annamite mountains, Vietnam. Zootaxa 4085: 63–102. https://doi.org/10.11646/zootaxa.4085.1.3

Schneider JG (1799) Historia Amphibiorum Naturalis et Literarariae. Fasciculus Primus. Continens Ranas, Calamitas, Bufones, Salamandras et Hydros in Genera et Species Descriptos Notisque usis Distinctos. Friederici Frommanni, Jena, 304 pp.

Shimada T, Matsui M, Yambun P, Sudin A (2011) A taxonomic study of Whitehead's torrent frog, *Meristogenys whiteheadi*, with descriptions of two new species (Amphibia: Ranidae).

Zoological Journal of the Linnean Society 161: 157–183. https://doi.org/10.1111/j.1096-3642.2010.00641.x

Smith MA (1921) New or little-known reptiles and batrachians from southern Annam (Indo-China). Proceedings of the Zoological Society of London 1921: 423–440.

Smith MA (1924) New tree-frogs from Indo-China and the Malay Peninsula. Proceedings of the Zoological Society of London 1924: 225–234. https://doi.org/10.1111/j.1096-3642.1924.tb01499.x

Stuart BL (2005) New frog records from Laos. Herpetological Review 36: 473–479.

Stuart BL, Rowley JJL, Neang T, Emmett DA, Sitha S (2010) Significant new records of amphibians and reptiles from Virachey National Park, northeastern Cambodia. Cambodian Journal of Natural History 2010: 38–47.

Stuart BL, Sok K, Neang T (2006) A collection of amphibians and reptiles from hilly eastern Cambodia. Raffles Bulletin of Zoology 54: 129–155.

Stuart BL, Phimmachak S, Seateun S, Sivongxay N (2012) A new *Leptobrachium* (Anura: Megophryidae) from the highlands of southeastern Laos. Zootaxa 3155: 29–37.

Stuart BL, Rowley JJL, Tran DTA, Le DTT, Hoang DH (2011) The *Leptobrachium* (Anura: Megophryidae) of the Langbian Plateau, southern Vietnam, with description of a new species. Zootaxa 2804: 25–40.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Taylor EH (1962) The amphibian fauna of Thailand. The University of Kansas Science Bulletin, XLIII, 255–599. https://doi.org/10.5962/bhl.part.13347

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876–4882. https://doi.org/10.1093/nar/25.24.4876

Tian W, Hu Q (1983) Taxonomic study on genus *Megophrys*, with descriptions of two new genera. Acta Herpetologica Sinica. New Series 2(2): 41–48. [In Chinese]

Tian W, Hu Q (1985) Taxonomical studies on the primitive anurans of the Hengduan mountains, with descriptions of a new subfamily and subdivision of *Bombina*. Acta herpetologica Sinica 4: 219–224. [In Chinese]

Tschudi JJv (1838) Classification de Batrachier mit Berücksichtigung der fossilen Thiere dieser Abtheilung der Reptilien. Neuschâtel, Petitpierre.

Vassilieva AB, Galoyan EA, Poyarkov NA, Geissler P (2016) A Photographic Field Guide to the Amphibians and Reptiles of the Lowland Monsoon Forests of Southern Vietnam. Frankfurt Contributions to Natural History, Vol. 36. Chimaira Buchhandelsgesellschaft mbH, Frankfurt-am-Main, 319 pp.

Vassilieva AB, Galoyan EA, Gogoleva SI, Poyarkov NA Jr (2014) Two new species of *Kalophrynus* Tschudi, 1838 (Anura: Microhylidae) from the Annamite mountains in southern Vietnam. Zootaxa 3796(3): 401–434. https://doi.org/10.11646/zootaxa.3796.3.1

Vences M, Thomas M, Bonett RM, Vieites DR (2005a) Deciphering amphibian diversity through DNA barcoding: chances and challenges. Philosophical Transactions of the Royal Society London, Ser. B 360: 1859–1868.

Vences M, Thomas M, Van der Meijden A, Chiari Y, Vieites DR (2005b) Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. Frontiers in Zoology 2: 5. https://doi.org/10.1186/1742-9994-2-5

Vieites DR, Wollenberg KC, Andreone F, Kohler J, Glaw F, Vences M (2009) Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. Proceedings of the National Academy of Sciences of the United States of America 106: 8267–8272. https://doi.org/10.1073/pnas.0810821106

Wang Y, Zhang T-d, Zhao J, Sung Y-h, Yang J, Pang H, Zhang Z (2012) Description of a new species of the genus *Xenophrys* Günther, 1864 (Amphibia: Anura: Megophryidae) from Mount Jinggang, China, based on molecular and morphological data. Zootaxa 3546: 53–67.

Wells KD (2007) The Ecology and Behaviour of Amphibians. Chicago University Press, 1148 pp. https://doi.org/10.7208/chicago/9780226893334.001.0001

Wilkinson JA, Drewes RC, Tatum OL (2002) A molecular phylogenetic analysis of the family Rhacophoridae with an emphasis on the Asian and African genera. Molecular Phylogenetics and Evolution 24: 265–273. https://doi.org/10.1016/S1055-7903(02)00212-9

Xu Q, Liu S, Wan R, Yue B, Zhang X (2014) The complete mitochondrial genome of the *Vibrissaphora boringii* (Anura: Megophryidae). Mitochondrial DNA 27(1): 758–759. https://doi.org/10.3109/19401736.2014.915527

Yang D-t (Ed.) (1991) Yunnan liang qi lei zhi [Amphibian Fauna of Yunnan]. China Forestry Publishing House, Beijing, China, 259 pp. [In Chinese]

Yang D-t (2008) Amphibia. In: Yang D-t, Rao D-q (Eds) Amphibia and Reptilia of Yunnan. Yunnan Publishing Group Corporation, Yunnan Science and Technology Press, Kunming, 12–152. [In Chinese]

Ye C-y, Fei L, Hu S-q (1993) Rare and Economic Amphibians of China. Chengdu, China: Sichuan Publishing House of Science and Technology, 412 pp. [In Chinese]

Zhang P, Liang D, Mao R-L, Hillis DM, Wake DB, Cannatella D (2013) Efficient Sequencing of Anuran mtDNAs and a Mitogenomic Exploration of the Phylogeny and Evolution of Frogs. Molecular Biology and Evolution 30(8): 1899–1915. https://doi.org/10.1093/molbev/mst091

Zhang P, Papenfuss TJ, Wake MH, Qu L, Wake DB (2008) Phylogeny and biogeography of the family Salamandridae (Amphibia: Caudata) inferred from complete mitochondrial genomes. Molecular Phylogenetics and Evolution 49(2): 586–597. https://doi.org/10.1016/j.ympev.2008.08.020

Zhang Y-x, Wen Y-t (2000) Amphibians in Guangxi. Guangxi Biodiversity Studies. Guilin, China, Guangxi Normal University Press, 183 pp. [In Chinese]

Zheng Y, Zeng X, Yuan Y, Liu Z (2004) Phylogenetic positions of *Ophryophryne* and four *Leptobrachium* group genera in Megophryidae (Anura). Sichuan Dong Wu 23(3): 290–295. [In Chinese]

Appendix I

Examined material, museum IDs given in bold.

Ophryophryne elfina sp. n.: **ZMMU A-5669** (Vietnam, Dak Lak Prov., Chu Yang Sin N.P.; NAP-02658; 1 adult male; holotype); **ZMMU A-5170** (Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Bidoup Mt.; ABV-00455; ABV-00454; ABV-00472; ABV-00471; 4 adults; paratypes); **ZMMU A-4788** (Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Hon Giao Mt., Bidoup Mt.; NAP-01455; NAP-01449; NAP-01450; NAP-01460; 4 adults; paratypes); **ZMMU A-5674** (Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Hon Giao Mt.; NAP-01451; NAP-01452; 2 adults; paratypes); **ZMMU A-5675** (Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Bidoup Mt.; NAP-01456; NAP-01459; 2 adults; paratypes); **ZMMU A-5691** (Vietnam, Dak Lak Prov., Chu Yang Sin N.P.; ABV-00580, ABV-00581; 2 juveniles; paratypes); **ZMMU A-5650** (Vietnam, Dak Lak Prov., Chu Yang Sin N.P.; 10 adults); **ZISP 12836–12879** (Vietnam, Dak Lak Prov., Chu Yang Sin N.P.; 44 adults; tentative identification); **ZMMU A-5665** (Vietnam, Dak Lak, Chu Yang Sin N.P.; ROM 36348; ROM 36289; ROM 36554; ROM 36470; 4 adults); **ZISP 12880–12884** (Vietnam, Dak Lak Prov., Chu Yang Sin N.P.; ROM 36471; ROM 36472; ROM 36469; ROM 36366; ROM 36349; 5 adults); **ZMMU A-3937** (Vietnam, Khanh Hoa Prov., Hon Ba N.R.; 1 adult); **ZMMU A-4935** (Vietnam, Khanh Hoa Prov., Hon Ba N.R.; ABV-00316; 1 adult); **ZMMU A-4716** (Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Hon Giao Mt., Bidoup Mt.; NAP-01871; NAP-01782; NAP-01758; NAP-01757; NAP-01783; 5 adults); **ITBCZ 2786, 2788, 2792, 2828, 2908–09, 2918–19, 3502** (Vietnam, Khanh Hoa Prov., Hon Ba N.R.; 9 adults); **ZMMU A-5679** (Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Bidoup Mt.; NAP-01169; 7 larvae); **ZMMU A-5684** (Vietnam, Dak Lak Prov., Chu Yang Sin N.P., Chu Pan Fan Mt.; NAP-02673; 4 larvae).

Ophryophryne gerti: **BMNH 1921.4.1.324** (Vietnam, Lam Dong Prov., sout-east of Da Lat, Cam Ly river; 1 adult male; holotype); **BMNH 1921.4.1.323** (Vietnam, Lam Dong Prov., Dran; 1 immature female; paratype); **ZMMU A-4714** (Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Giang Ly; NAP-01878; 1 adult); **ZMMU A-4715** (Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Giang Ly; NAP-01790; NAP-01788; 2 adults); **ZMMU A-4718** (Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Giang Ly; NAP-01789; 1 adult); **ZMMU A-4843** (Vietnam, Dak Lak Prov., Chu Yang Sin N.P.; ABV-00067; 1 adult); **ZMMU A-5670** (Vietnam, Dak Lak Prov., Chu Yang Sin N.P.; NAP-02758; NAP-02759; NAP-02760; NAP-02761; 4 adults); **ZMMU A-5673** (Vietnam, Dak Lak Prov., Chu Yang Sin N.P.; ABV-00530; ABV-00577; 2 adults); **ZISP 12740–12747** (Vietnam, Dak Lak Prov., Chu Yang Sin N.P.; ROM 36522–36529; 8 adults).

Ophryophryne hansi: **FMNH 252879, FMNH 252880, FMNH 252882, FMNH 252884** (Vietnam, Gia-Lai Prov., Ankhe Dist., 60 km to the northwest from Kannack; 1 adult male; holotype; 2 adult males and 1 adult female; paratypes); **FMNH 252873** (Vietnam, Gia-Lai Prov., Ankhe Dist., 20 km to the northwest from Kan-

nack; 1 adult male); **AMNH 163680** (Vietnam, Quang Nam Prov., Tra My Dist., Tra Don Commune; 1 adult male); **AMNH 169284** (Vietnam, Thua Tien Hue Prov., A Luoi Dist., Tram Tra Ve (Forestry station of Huong Giang State Forestry Enterprise); 1 adult male); **AMNH 169286** (Vietnam, Thua Tien Hue Prov., Huong Thuy Dist., Khe Dau Station; 1 adult male); **AMNH 161353** (Vietnam, Ha Tinh Prov., Huong Son, Huong Son Reserve; 1 adult male); **FMNH 258008–258009, FMNH 258046–258051** (Lao P.D.R., Xe Kong Prov., Kaleum Dist., Xe Sap National Biodiversity Conservation Area; 5 adult males and 4 adult females).

Ophryophryne miscrostoma: **BMNH 1947.2.22.50, BMNH 1947.2.22.52** (Vietnam, Mau Son Mts.; 2 adult females; lectotype & paralectotype); **AMNH 168682** (Vietnam, Lao Cai Prov., Van Ban Dist., Nam Tha Commune; 1 adult male).

Ophryophryne pachyproctus: **YU A8311032** (China, Yunnan Prov., Xishuangbanna Pref., Mengla Co., Yiwu, Zhoushihe river; 1 adult male; holotype); **YU A8311033–A8311037, YU A845099– A845100** (China, Yunnan Prov., Xishuangbanna Pref., Mengla Co., Yiwu, Zhoushihe river; 8 adult males; paratypes).

Ophryophryne cf. *poilani*: **AMNH 169287** (Vietnam, Thua Tien Hue Prov., A Luoi Dist., A Pat Forestry Protection Department Range Station; 1 adult female); **AMNH 163668** (Vietnam, Quang Nam Prov., Tra My Dist., Tra Don Commune; 1 adult female).

Ophryophryne synoria: **FMNH 262779** (Cambodia, Mondolkiri Prov., O'Rang Dist., O Chung Chry stream; 1 adult male; holotype); **FMNH 262778** (Cambodia, Mondolkiri Prov., O'Rang Dist., O Chung Chry stream; 1 adult male; paratype); **ZMMU A-5003** (Vietnam, Binh Phuoc Prov., Bu Gia Map N.P.; ABV-00379; ABV-00380; ABV-00376; ABV-00381; ABV-00377; 5 adults); **ZMMU A-4516** (Vietnam, Binh Phuoc Prov., Bu Gia Map N.P.; NAP-00729; NAP-00727; NAP-00728; NAP-00730; NAP-00731; 5 adults); **ZMMU A-4864** (Vietnam, Lam Dong Prov., Loc Bac forestry; ABV-00159; ABV-00209; 2 adults); **ZMMU A-5676** (Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Giang Ly; NAP-01438; NAP-01437; 2 adults).

Ophryophryne sp.: **BMNH 1972.1524** (Lao P.D.R., "Pak Maat"; 1 adult male; paratype of *O. gerti*).

Appendix 2

Factor coordinates of the morphometric characters used in PCA analysis, based on correlations (factors 1 to 3).

Character	factor 1	factor 2	factor 3
SVL	-0.969013	-0.052119	-0.061729
HW	-0.972214	-0.094198	-0.099264
HL	-0.963376	-0.112316	-0.089300
ED	-0.872338	-0.060587	0.293135
TYD	-0.853903	-0.302433	0.054377
TYE	-0.869868	-0.053510	-0.256344
SL	-0.817708	0.454058	-0.126722
EN	-0.774425	-0.018511	-0.529049
NS	-0.302259	0.915825	0.017344
IUE	-0.866068	-0.171158	0.204049
IN	-0.818811	0.248915	0.129748
UEW	-0.848819	0.181381	0.241783
FAL	-0.937173	-0.162120	-0.073309
HAL	-0.957549	0.054374	-0.001476
FIL	-0.931528	-0.047484	0.090017
FIIL	-0.939026	-0.049085	0.087852
FIIIL	-0.963650	0.030269	-0.041650
FIVL	-0.902097	0.102388	-0.091818
SHL	-0.950468	-0.104557	0.046962
TL	-0.921529	-0.091021	0.019328
FOL	-0.918076	0.080433	0.100931
TFOL	-0.971401	0.003596	0.002474
IMT	-0.761261	-0.079858	0.068715

Appendix 3

Measurements of advertisement call temporal parameters and one-way ANOVA/Kruskal-Wallis results for comparison (*p < 0.001) between April and February sets of call recordings for *Ophryophryne elfina* sp. n. Seconds (s), milliseconds (ms).

Parameters	***O. elfina* sp. n.** 10–15 April 2014	***O. elfina* sp. n.** 10 February 2015	**Tukey/ Mann-Whitney U post hoc tests**	**ANOVA/ Kruskal-Wallis results**
Temperature of recording	11.3–11.4°C	17.0–17.5°C	–	–
Number of males	2	1	–	–
Number of recordings	3	2	–	–
Number of series	93	47	–	–
Number of calls	1301	496	–	–
Call repetition rate per recording (calls/s)	1.25 ± 0.35 (0.77–1.95) *N* = 3	1.07 ± 0.06 (1.01–1.12) *N* = 2	p = 0.71	$F_{1.3} = 0.2$
Number of calls per series	14 ± 0.45 (2–22) *N* = 93	10.53 ± 0.72 (3–21) *N* = 47	p < 0.001	$F_{1.138} = 18.2$*
Series duration (s)	3.37 ± 0.1 (0.43– 5.05) *N* = 93	3.52 ± 0.27 (0.61–9) *N* = 47	p = 0.52	$F_{1.138} = 0.4$
Call repetition rate per series (calls/s)	4.22 ± 0.08 (2.92–5.49) *N* = 93	3.18 ± 0.1 (1.33–4.91) *N* = 47	p < 0.001	$F_{1.138} = 63.9$*
Call duration (ms)	70 ± 0.27 (25–112) *N* = 1301	79 ± 0.32 (48–102) *N* = 496	p < 0.001 (U = 131097)	$H_{1.1798} = 380.2$*
Inter-calls interval (ms)	184 ± 1.72 (96–621) *N* = 1209	271.17 ± 4.92 (102–942) *N* = 448	p < 0.001 (U = 109162)	$H_{1.1657} = 349.2$*
Inter-series interval (s)	6.72 ± 0.54 (1.26–31.65) *N* = 90	6.09 ± 0.56 (1.42–22.14) *N* = 45	p = 0.83 (U = 2022)	$H_{1.136} = 0.05$

ZooKeys 672: 121–133 (2017)
doi: 10.3897/zookeys.672.10078
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

The selection by the Asiatic black bear (*Ursus thibetanus*) of spring plant food items according to their nutritional values

Shino Furusaka[1], Chinatsu Kozakai[2,4], Yui Nemoto[1], Yoshihiro Umemura[1], Tomoko Naganuma[1], Koji Yamazaki[3,5], Shinsuke Koike[1]

1 *Tokyo University of Agriculture and Technology, 3-5-8 Saiwai, Fuchu, Tokyo 183-8509, Japan* **2** *Kanagawa Prefectural Museum of Natural History, 499 Iryuda, Odawara, Kanagawa 250-0031, Japan* **3** *Ibaraki Nature Museum, 700 Osaki, Bando, Ibaraki 306-0622, Japan* **4** *Present address: National Agriculture and Food Research Organization, 2-1-18 Kannondai, Tsukuba, Ibaraki, 305-8518, Japan* **5** *Present address: Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya, Tokyo 156-8502, Japan*

Corresponding author: *Shinsuke Koike* (koikes@cc.tuat.ac.jp)

Academic editor: *J. Maldonado* | Received 3 August 2016 | Accepted 12 April 2017 | Published 4 May 2017

http://zoobank.org/00A65F31-5E93-49BD-A549-C02E6D9B108B

Citation: Furusaka S, Kozakai C, Nemoto Y, Umemura Y, Naganuma T, Yamazaki K, Koike S (2017) The selection by the Asiatic black bear (*Urses thibetanus*) of spring plant food items according to their nutritional values. ZooKeys 672: 121–133. https://doi.org/10.3897/zookeys.672.10078

Abstract

The present study aimed to investigate the nutritional aspects of the bear diet quantitatively, in order to understand plant food selection in spring. Bears were observed directly from April to July in 2013 and 2014, to visually recognize plant species consumed by bears, and to describe the foraging period in the Ashio-Nikko Mountains, central Japan. Leaves were collected from eight dominant tree species, regardless of whether bears fed on them in spring, and their key nutritional components analyzed: crude protein (CP), neutral detergent fiber (NDF), and total energy. Bears tended to consume fresh leaves of specific species in May, and nutritional analysis revealed that these leaves had higher CP and lower NDF than other non-food leaves. However, CP in consumed leaves gradually decreased, and NDF increased from May to July, when the bears' food item preference changed from plant materials to ants. Bears may consume tree leaves with high CP and low NDF after hibernation to rebuild muscle mass.

Keywords

Direct observation, feeding ecology, feeding strategy, food habits, nutritional analysis

Copyright *Shino Furusaka et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

The composition of diets selected by wildlife has long been of interest to range and wildlife biologists. Understanding the reasons underlying food choices is useful when developing and revising habitat management plans (e.g., Aryal et al. 2015a, b, Panthi et al. 2015). Mammals select food types based on relative abundance, seasonal availability, palatability, and nutritional content (e.g., Hanley 1982, Lambert 2010, Aryal et al. 2015a, b). The palatability and nutrient content of plant foods can show rapid temporal variations (Panthi et al. 2015). Therefore, understanding the nutritional basis of dietary selection can help explain feeding phenology.

The feeding habits of bears before and after hibernation (autumn and spring, respectively) are of particular interest (e.g., Noyce and Garshelis 1998, McLellan 2011). Bears show a hyperphagia in autumn for key foods that help build fat reserves for hibernation. In contrast, spring feeding must provide nutrients to rebuild muscle mass lost during hibernation and support lactation in females that are nourishing cubs. American black bears (*Ursus americanus* Pallas, 1780) consume higher protein foods in spring than in summer or fall (Mclellan 2011). This diet facilitates gains in bone and muscle mass in both adult and juvenile bears, with a concurrent loss in fat mass (McLellan 2011, Noyce et al. 1997, Noyce and Garshelis 1998). Because bears are non-cecal monogastric mammals, they are unable to efficiently digest fiber. Thus, the digestibility of plant foods is inversely proportional to their fiber content (Bunnel and Hamilton 1983, Pritchard and Robbins 1990).

Current knowledge suggests that Asiatic black bears (*U. thibetanus* G. Cuvier, 1823) mainly consume green vegetation during spring, such as newly emerged leaves or grasses (Hashimoto and Takatsuki 1997, Hwang et al. 2001). Despite the high volume of green vegetation in the spring diet, vegetative plant remains in feces are difficult to quantify and identify. Most of the previous spring diet studies of Asiatic black bears have relied on fecal analyses, so detailed information on the species consumed is lacking (Hashimoto and Takatsuki 1997). Thus, it also remains unclear what nutritional factors influence the use of food items by Asiatic black bears during spring. Further, bears consume this green vegetation during a limited spring period and then change to other food items quickly (Koike 2010). However, it is unknown why bears select this green vegetation for a short period.

In this study, we aimed to clarify the spring feeding behavior of Asiatic black bears, with a focus on the nutritional factors that contribute to their consumption of spring plant food items. Based on previous studies of American black bears (McLellan 2011, Noyce et al. 1997), we hypothesized that their spring feeding habits are affected by the nutritional components of food items, particularly protein, fiber, and energy. We hypothesized that bears consume plant food items with high protein, high energy, and low fiber to maximize their feeding efficiency during spring. We used direct observations to determine which food items bears consumed and when they consumed them. We then analyzed those food items to determine their nutritional content and elucidated the relationship between nutrient contents and food usage.

Methods

Study area

This study was conducted in the Ashio area of the Ashio-Nikko Mountains (36°54'–36°80'E, 139°22'–139°49'N; Fig. 1). The main study area was approximately 60 km^2, with an annual precipitation of 2,236 mm and an annual mean temperature of 7.2°C (Tochigi Prefecture 2011). The natural vegetation of the Ashio-Nikko Mountains up to 1,600 m is deciduous broad-leaved forest composed of *Quercus crispula* Blume, *Acer* spp., and *Fagus crenata* Blume. Mixed forests of *Tsuga* spp. and *Betula* spp. exist above this altitude. Since 1956, the Japanese government has undertaken major tree planting operations in the area, and today, there are scattered stands of *Clethra barbinervis* Sieb. et Zucc., *Alnus firma* Sieb. et Zucc., *Robinia pseudoacacia* Linn., and *Pinus densiflora* Sieb. et Zucc.. However, the landscape remains partly open, with a mosaic of grasslands, rough bare land, planted forest, and natural vegetation.

To clarify the dominant tree species in this area and to select the tree species for analysis, the abundance of tall trees was calculated by a vegetation survey in Ashio study area. Thirty random points were generated in the central Ashio area and set transects (20 × 20 m) in each of the selected points. A handheld GPS receiver (eTrex Legend HCx, Garmin Ltd., Kansas, USA) was used to establish the location where transects were measured. The diameter was measured at chest height of all tall trees and then the total basal area of each tree species was calculated within the transects. The most abundant tall trees in the central Ashio area were *R. pseudoacacia*, *C. barbinervis*, *A. firma*, *Betula ermanii* Cham., and *Q. crispula.*

Foraging behavior of bears

To identify the food items and foraging period for each food item, bears were directly observed feeding during the daytime from April–July in 2013 and 2014. Most bears showed a diurnal feeding pattern during this season (Kozakai et al. 2013) and, therefore, did little foraging at night. We experienced two types of challenges while using direct observation: it was difficult to locate target animals in this temperate broad-leaved forest and observations had to be made without disturbing the animals or changing their behavior. The steep topography and mosaic grasslands of the Ashio study area enabled us to circumvent these problems; we located bears by visually scanning open grasslands and made our observations from the opposite side of the valley using a video camera with a powerful telescopic lens.

To avoid biases toward certain time frames or individuals, we recorded the feeding behavior for as many different bears as was possible. We were able to identify most of the bears by their physical characteristics including body size, chest markings, and the presence of cubs.

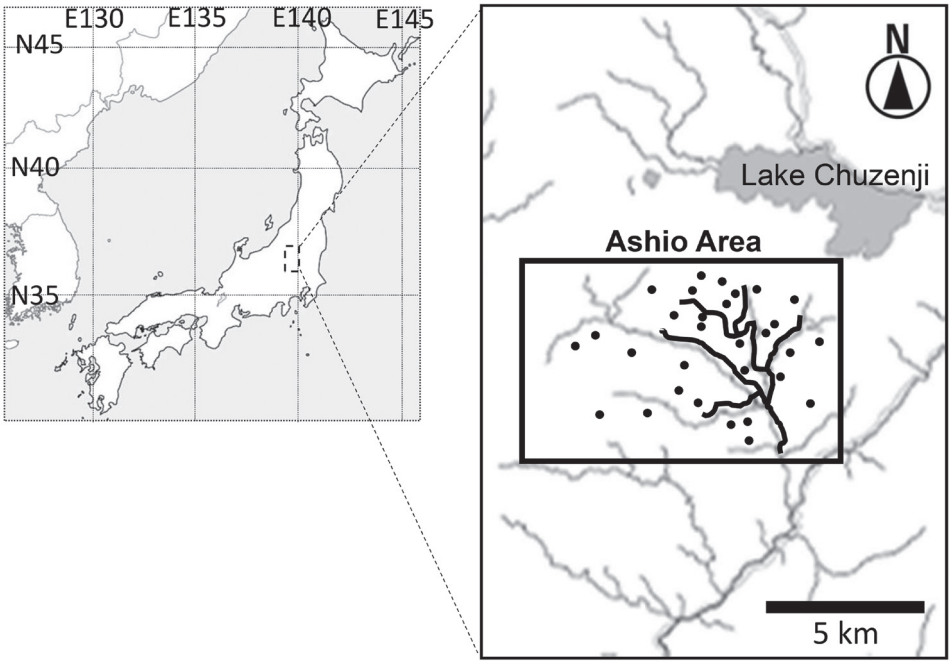


Figure 1. Map of the study area, located in the Ashio-Nikko Mountains range in Tochigi and Gunma Prefectures, central Japan. Black lines were trails to observe bears and black circles were the points of vegetation surveyed in the Ashio area.

The observation frequency was more than once a week (average ± SD: 1.3 ± 0.5 days/week) depending on weather conditions. We walked along trails in the study area and searched for bears using binoculars (Kenko Skymate 8 × 40 mm) as quietly as possible. When bears were located, their behaviors were recorded using a video camera (Panasonic Lumix GH2) with a telescopic lens. In the laboratory, we reviewed the video recordings to identify each food item by tree shape, tree color, or other characteristics, and to calculate the foraging period for each food item. If we could not identify food items from the video recordings, we returned to the feeding location and directly identified the food items based on the signs of feeding. In addition, particularly during 2014, when observation points were proximate to feeding sites and access to accurate feeding location data (via GPS collars fitted on the bears) was available, we download the GPS location data from GPS collars. This allowed us to visit the feeding locations and assess the food items based on signs of feeding such as bear shelves (broken branches on trees made by bears when eating leaves) or claw marks on tree trunks. We defined "ant-feeding" as occurring when the following conditions were met: a bear turned over a stone, put its nose close to the ground, moved its face up and down, moved its front paws, and these actions had to continue for more than 5 s (Fujiwara et al. 2013). During the survey, to protect from attack by bears, we stayed on mountain slopes opposite from the mountain slopes on which the bears were foraging, generally across the valley, and keeping a minimum distance of 200 m between us and bears.

Nutritional analysis of food items

From previous studies, we know that Asiatic black bears mainly consume green vegetation, particularly fresh leaves of woody plants, grasses, and herbs in spring (e.g., Hashimoto and Takatsuki 1997). Therefore, we targeted the leaves of woody plants, grasses, and herbs for nutritional analyses during 2013 samplings.

Six food items were identified by direct observation. For the buds or leaves of all tree species (total of four species), the species could be accurately identified by video data based on confirmation (visiting the feeding site and assessing the feeding signs). However, the species of herbs and grasses could not be identified by video data and only used the data of two species that could be confirmed.

The nutritional contents of tree species consumed by bears were compared to those not consumed by bears. We targeted the leaves of woody plants because a previous study indicated that bears in the study area exclusively fed on tree leaves in spring (Koike et al. 2016). We collected leaves from eight tree species (four consumed species and four non-consumed species) with ten individual trees for each species on two separate occasions (early May and late May). The target non-consumed tree species were dominant species (*R. pseudoacacia*, *C. barbinervis*, *A. firma*, and *B. ermanii*), selected based on quantitative data. On the other hand, we observed bears feeding on the leaves of four of these species (*Malus toringo* Sieb., *Q. crispula*, *Salix bakko* Kimura, and *Elaeagnus umbellata* Thund.), and we selected these species as consumed tree species.

Second, to assess the phenologic change in the nutritional value of the three main tree species consumed by bears (*M. toringo*, *S. bakko*, and *Q. crispula*), we collected leaves from ten individual trees of each species once every 2 weeks from leaf flush (early May) to late June (total of four times: early May, late May, early June, and late June). Because feeding on *E. umbellata* leaves was observed less frequently than feeding on other species, *E. umbellata* was excluded from this analysis. Third, whenever we confirmed any other plant species being consumed by bears during our 2013 observations, we collected samples from the foraging site as soon as possible.

After collection, samples (10 g (dry weight) for each tree) were kept in paper envelopes and brought to the Tokyo University of Agriculture and Technology where they were dried at 60°C for 48 h. The dried items were milled, placed in plastic tubes, and stored in desiccators until analysis. For each sample, we determined crude protein (CP, % of dry matter), neutral detergent fiber (NDF, % of dry matter), and total energy content (EN, kcal/gdw) according to the methodology prescribed by the Association of Official Analytical Chemists (2003). We determined the nitrogen content (%) using a CN CODER MT-700 analyzer (Yanaco New Science Inc., Kyoto, Japan) and calculated CP using the following formula: CP = nitrogen × 6.25 (Robbins 1993). We analyzed NDF using an Ankom fiber determination system (Ankom^{200} Fiber Analyzer; Ankom Technology Corp., NY, USA) and analyzed EN using a bomb calorimeter (IKA-Calorimeter C200; IKA-Werke GmbH and Co. KG, Staufen, Germany). All analyses were conducted by the NARO Institute of Livestock and Grassland Science at Tsukuba city, Japan.

After performing nutritional analysis, we used right-angled mixture triangle (RMT; Raubenheimer 2011) analysis to examine the balance of macronutrients and fiber in plant samples. The RMT is a geometric approach used to investigate multidimensional data on the ratios (or balance) of food components in individual foods or food mixtures and is especially relevant to field-based nutritional ecology studies where proportional compositions (as opposed to accurate intake amounts) are the only metric available (Raubenheimer 2011; Raubenheimer et al. 2014). We used a 3-dimensional RMT, where macronutrients were expressed as percentage of total macronutrients (i.e., CP + EN + NDF) on a dry matter basis; CP was shown on the implicit z-axis, the value of which is inversely related with distance from the origin.

Data analysis

We compared the nutritional content of leaves from four tree species not consumed by bears and from four tree species that bears did consume in early and late May by the Bartlett test and used the Kruskal–Wallis test (KW) to determine the differences in nutritional values between tree species. We also compared the nutritional values of *M. toringo*, *S. bakko*, and *Q. crispula* leaves over time and determined the differences in nutritional values related to phenological change by Bartlett test and KW test. We used a single chi-square test to compare the frequency differences from video recordings of bear foraging times for each food item in each half-month period between 2013 and 2014.

Results

We recorded bear foraging behavior for 7 h 25 min during 2013 and 8 h 10 min during 2014 (45 individual and 30 individual foraging behavior events during 2013 and 2014, respectively). The number of observed feeding bouts where we could not identify the bear was 68 and 48 during 2013 and 2014, respectively. The minimum number of identifiable individuals was 10 and nine during 2013 and 2014, respectively. We recorded three bears with a GPS collar in 2013 and two in 2014.

During early April, we did not observe any bear feeding behavior, and the leaf flush had not occurred yet. During late April, the leaf flush had still not occurred, and bears mainly ate *Miscanthus sinensis* Andersson. Grass (overwintered culms that had stopped growing in November of the preceding year) [74.7% ± 0.5% (mean ± SD) of video-recorded bear foraging time] and *S. bakko* buds (25.3% ± 0.5%). By early May, the leaf flush had occurred in all tree species except *B. ermanii*. During this period, bears ate newly emerged leaves of *S. bakko* (20.7% ± 0.9%), *M. toringo* (20.3% ± 0.4%), *Q. crispula* (16.6% ± 2.2%), *E. umbellata* (16.1% ± 1.6%), *Eragrostis curvula* (Schrad.) Nees herbs (13.2% ± 2.4%), and unknown grasses (13.1% ± 2.7%). In late May, bears ate the leaves of *S. bakko* (4.7% ± 0.4%) and *Q. crispula* (14.7% ± 0.5%) and the flowers and leaves of *M. toringo* (80.6% ± 0.9%). From June to July, the bears mainly ate

Table 1. Nutritional contents of tree leaves in early and late May.

Species	Early May			Late May		
	EN (kcal/dgw)	NDF (%)	CP (%)	EN (kcal/dgw)	NDF (%)	CP (%)
Bear consumed plants						
Quercus crispula	**5.2 ± 0.1**	**37.0 ± 5.6**	**31 .8 ± 0.3**	**5.2 ± 0.0**	**26.0 ± 1.4**	**28.4 ± 1.5**
Salix bakko	**5.0 ± 0.1**	**20.2 ± 1.0**	**33 .2 ± 4.6**	**5.0 ± 0.2**	**20.3 ± 1.9**	**33.8 ± 0.6**
Malus toringo	**5.0 ± 0.1**	**27.9 ± 4.8**	**31.0 ± 2.3**	**4.9 ± 0.8**	**26.7 ± 2 .8**	**27.8 ± 1.6**
Elaeagnus umbellata	**5.0 ± 0.0**	**37.4 ± 2.8**	**39.2 ± 1.7**	4.8 ± 0.1	46.2 ± 3.5	38.6 ± 0.8
No consumed plants						
Alnus firma	5.4 ± 0.1	40.9 ± 2.3	23.1 ± 4.5	5.3 ± 0.1	43.0 ± 2.3	22.9 ± 1.1
Clethra barbinervis	4.9 ± 0.4	50.9 ± 19.6	28.3 ± 8.0	4.9 ± 0.3	53.2 ± 1.9	21.1 ± 1.9
Robinia pseudoacacia	5.2 ± 0.3	40.5 ± 13.5	27.6 ± 5.6	5.1 ± 0.2	47.6 ± 3.5	25.6 ± 3.5
Betula ermanii	–	–	–	5.1 ± 0.3	41.6 ± 0.7	26.3 ± 3.9

Bold type: we can observe that bears consume the food items in that period.
EN, energy content; CP%, crude protein; NDF%, neutral detergent fiber.

ants (96.5% ± 2.1%) and unknown grasses. In early June, bears ate ants (95.0%) and unknown grasses (5.0%) in 2013 and 2014. In late June, bears ate ants (93.9%) and bees (6.1%) in 2013; however, they ate ants (91.5%), unknown grasses (4.5%), and sika deer (*Cervus nippon* Temminck) carcasses (4.0%) in 2014. In early July, bears ate ants (97.2%) and *Cirsium* spp. (2.8%) in 2013; however, they ate ants (94.5%) and unknown grasses (5.5%) in 2014. In late July, bears ate ants (98.0%) and unknown grasses (2.0%) in 2013; however, they ate ants (93.5%), unknown grasses (2.5%), and sika deer carcasses (4.0%) in 2014. There were no large differences in leaf flush timing for any plant species between 2013 and 2014 (all <1 week). There were also no differences between 2013 and 2014 frequencies of video-recorded bear foraging times for each food item during each half-month period, except for late June ($\chi^2 = 6.586$, $P < 0.05$).

During early and late May, EN of food items had no significant effect on consumption; however, bears were significantly more likely to consume food items with a higher CP (KW test: early May: $df = 6$, $P < 0.05$ and late May: $df = 7$, $P < 0.05$) and a lower NDF (KW test: early May: $df = 6$, $P < 0.05$ and late May: $df = 7$, $P < 0.05$). These results indicate that bears use spring plant food items with at least 27% CP and <38% NDF (Table 1).

In CP, *Q. crispula* leaves decreased from May to late June and was significantly higher in early and late May than in early and late June (KW: $df = 3$, $P < 0.01$; Figs 2, 3). In contrast, NDF increased from May to late June and was significantly lower in early and late May than in early and late June (KW: $df = 3$, $P < 0.05$). EN was significantly lower during early May and early June than during late June and early July (KW: $df = 3$, $P < 0.01$). Bears only consumed the leaves of *M. toringo* in early May (Fig. 1). CP in *M. toringo* leaves significantly decreased for each period from early May to late June (KW: $df = 3$, $P < 0.01$). NDF was significantly lower during early and late May than during early and late June and early July (KW: $df = 3$, $P < 0.01$). EN in *M. toringo* leaves decreased from early May to early July (KW: $df = 3$, $P < 0.01$) and was

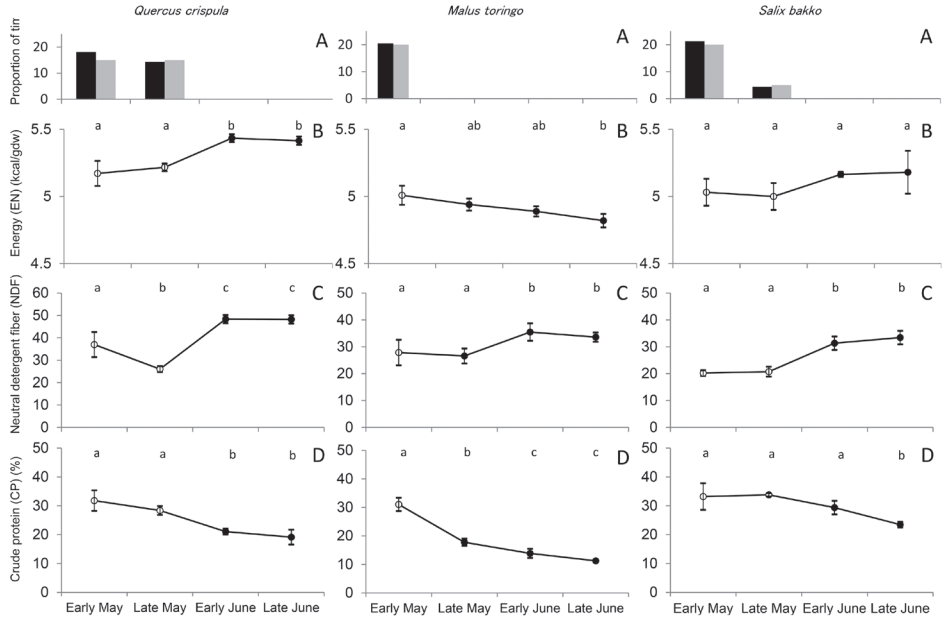


Figure 2. Proportion of observed time for each food item and seasonal changes in the nutritional values of *Quercus crispula* (left) leaves, *Malus toringo* (center) leaves, and *Salix bakko* (right) leaves from early May (leaf flash) to late June 2013. **A** The proportion of time for which bears were observed consuming (2013: black and 2014: gray) **B** total energy **C** neutral detergent fiber, and **D** crude protein. Different lower case letters within each graph indicate significant differences (Kruskal–Wallis test, $P < 0.05$). White circles indicate when bears were observed consuming *Q. crispula* leaves (early May and late May), *M. toringo* leaves (early May), and *S. bakko* (early May and late May).

probably related to the phenology of the leaves. Bears only consumed *S. bakko* leaves in early and late May (Fig. 1). CP in *S. bakko* leaves decreased from May to June and was significantly higher in early and late May and early June than in late June (KW: $df = 3$, $P < 0.01$). NDF was significantly lower during early and late May than during early and late June and early July (KW: $df = 3$, $P < 0.01$). During May and June, EN in *S. bakko* leaves was not significantly different.

Other food items were identified and collected for analysis. In late April, bears consumed dead *M. sinensis* (EN, 4.4 kcal/dgw; NDF, 86.0%; CP, 3.2%) and *S. bakko* buds (EN, 5.0 kcal/dgw; NDF, 24.8%; CP, 45.0%). In late May, bears consumed *M. toringo* flowers (EN, 4.2 kcal/dgw; NDF, 28.3%; CP, 28.4%). The herb *E. curvula* was consumed in early May; however, we were unable to collect samples for analysis.

Discussion

We found that in spring, Asiatic black bears consumed plant foods with high CP and low NDF. Bears stopped eating the leaves of each of the three tree species when NDF

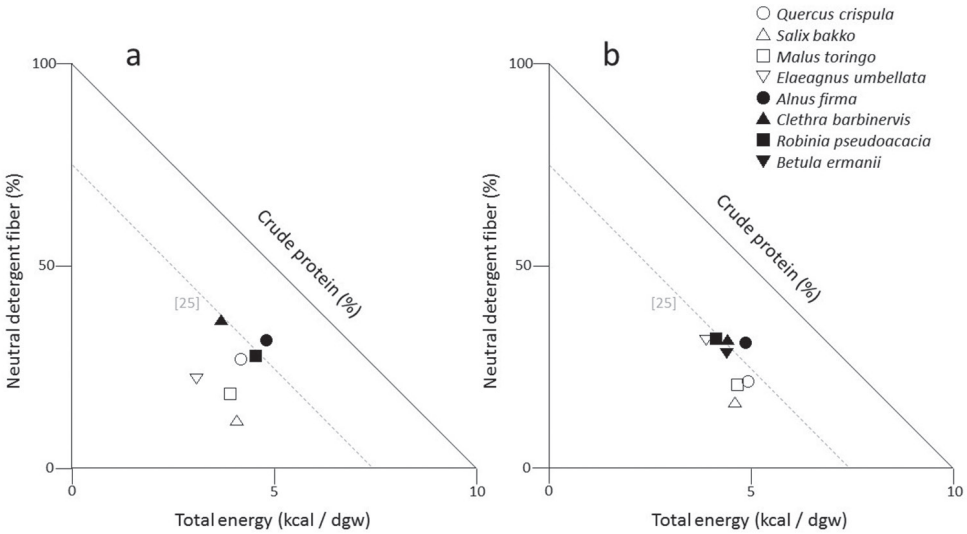


Figure 3. Right-angled mixture triangles (RMT) depicting the macronutrient balance of *Quercus crispula*, *Salix bakko*, *Malus toringo*, *Elaeagnus umbellata*, *Alnus firma*, *Clethra barbinervis*, *Robinia pseudoacacia,* and *Betula ermanii* leaves in early and late May. The RMT on the left **a** is early May, while the RMT on the right **b** is late May. Crude protein is represented on the implicit axis which varies inversely with distance from the origin (the dashed gray line indicates 25% protein content).

increased above approximately 38%, or CP decreased to less than approximately 27%, which was typical of late spring to early summer. This suggests that CP and NDF are key factors driving the usage of plant food items. EN of foods, per se, was not related to the bears' choice of foods, suggesting again that a high protein content is the key factor driving bears to eat certain foods, as long as the fiber content is low enough for good digestibility. In contrast, the total energy of a food item may be not an appropriate indicator to explain the diet selectivity of bears.

Adult brown bears (*U. arctos*) accumulate lean mass reserves mostly during spring and early summer (Hilderbrand et al. 2000, McLellan 2011), and rich protein diets during spring enhance this body mass gain (Swenson et al. 2007). Previous studies found that American black bears also gained weight from winter to early summer, suggesting that the protein content of spring food items is of vital importance (Noyce and Galshelis 1998, McDonald and Fuller 2005). In contrast, bears in Japan consume a large quantity of fruits from summer to autumn, many of which contain high levels of available energy, but very low levels of protein and other nutrients (Masaki et al. 2012). Therefore, spring foods containing high levels of protein may be needed to sustain growth throughout the year. However, during late April, bears consumed dead *M. sinensis* that are low in protein and high in fiber. There were exceptions, for example, when limited food items were available and the bears had to consume food items with relatively poor nutritional value. However, the number of these observations was few, and this might be a rare behavior.

Another reason for diet-switching in bears was because more nutritious foods became available. From June to July, the post-season for consuming green vegetation, Asiatic black bears mostly switched to a diet of ants (Fujiwara et al. 2013), which was also seen in our study. A previous study indicated that bears at Ashio could not get sufficient energy from a diet of ants (Yamazaki et al. 2012), thus, the probability of presenting more nutritious foods after June was low in this area, and it is during this period of the year that Asiatic black bears in Japan strip and damage the bark of plantation conifers (Oi and Yamazaki 2006, Kobashikawa and Koike 2016). Although the reasons for this are unclear, research on American black bears suggests that this behavior occurs when their nutritional status is compromised (Flowers 1986). Because the bones and mass of American black bears increase during spring, although there is a loss of fat (Noyce and Garshelis 1998), bears may not be able to replenish lost fat stores on a diet of leaves alone, even if leaves are plentiful during spring. Brown bears in other Asian regions consumed animal materials primarily (Xu et al. 2006, Aryal et al. 2012); therefore, if the bears can get animal materials, they may want to eat more animal materials with higher nutritional quality. In general, when body fat reserves are high, fat is utilized as the main source of energy; however, when fat reserves are low, lean mass is increasingly used as an energy source (Dunn et al. 1998, Caolin 2004, McCue 2010). However, to efficiently maximize the protein in spring plant foods for growth, bears may use their body fat stores remaining after winter to "fuel" their growth, as it takes energy as well as protein to construct new muscle and bone. Future work should focus on how seasonal changes in the availability and nutritional content of food items affect physical conditions such as fat stores.

We recognize the limitations of the present study. First, some food items are easier to observe than others, so focal sampling may be biased toward foods that are easier to observe. Previous food habit research showed that bears ate deer in spring (Koike et al. 2016). We did not observe this behavior, although we may have missed these observations when bears ate under a heavy canopy cover. In contrast, for the first time, most of the vegetative plants were identified and quantified. Second, the results may have been biased toward individual bears, sex, or age classes. However, we observed multiple bears over multiple years; thus, our results may be generally applicable to the bear population in this area. Third, we could not separate the availability of protein and fiber contents in terms of food preferences. Future research should consider other aspects such as changes in biomass (availability) and should also include other food items and other nutritional components.

Acknowledgments

We would like to thank H. Yokota who provided valuable advice on observing bears in the field and S. Haneo and S. Fujiwara who provided advice on conducting research in the Ashio area. We would also like to thank the many people who helped with the fieldwork, particularly A. Nakajima. We wish to thank O. Enishi for advice on nutrient

analysis and the Gunma and Nikko district forest offices of the Ministry of Agriculture for use of their facilities. We gratefully acknowledge several helpful discussions on statistics and comments by M. Soga. We wish to thank K. Noyce for her helpful advice. This study was partly funded by grants-in-aid for Scientific Research (Nos. 24380088, 25241026, and 25850103).

SK and SF conceived the idea. KM, SF and KY contributed to the study design. SF, CK. YN, YU and TN collected the data. SF and SK analyzed the data and wrote the manuscript with input from all authors.

References

Aryal A, Hopkins J, Ji W, Raubenheimer D, Brunton D (2012) Distribution and diet of brown bear in the upper Mustang region, Nepal. Ursus 23: 231–236. http://dx.doi.org/10.2192/URSUS-D-11-00015.1

Aryal A, Coogan SCP, Ji W, Rothman JM, Raubenheimer D (2015a) Foods, macronutrients and fibre in the diet of blue sheep (*Psuedois nayaur*) in the Annapurna Conservation Area of Nepal. Ecology and Evolution 5: 4006–4017. http://dx.doi.org/10.1002/ece3.1661

Aryal A, Brunton D, Ji W, Rothman J, Coogan SCP, Adhikari B, Juhnu S, Raubenheimer D (2015b) Habitat, diet, macronutrient, and fiber balance of Himalayan marmot (*Marmota himalayana*) in the Central Himalaya, Nepal. Journal of Mammalogy 96: 308–316. Http://dx.doi.org/10.1093/jmammal/gyv032

Association of Official Analytical Chemists (2003) Official methods of analysis. Seventeenth edition. Association of Official Analytical Chemists. Arlington, Virginia, USA, 2000 pp.

Bunnel FL, Hamilton T (1983) Forage digestibility and fitness in grizzly bears. International Conference on Bear Research and Management 5: 179–185. https://doi.org/10.2307/3872536

Caolin M (2004) Modeling of lipid and protein depletion during total starvation. American Journal of Physiology - Endocrinology and Metabolism 287: 790–798. http://dx.doi.org/10.1152/ajpendo.00414.2003

Dunn MA, Houts SK, Hartsook EW (1998) Maternal investment and factors affecting offspring size in polar bears (*Ursus maritimus*). Journal of Zoology 245: 253–260. http://dx.doi.org/10.1111/j.1469-7998.1998.tb00099.x

Flowers RH (1986) Supplemental feeding of black bear in tree damaged areas of western Washington. In: Baumgartner DM, Mahoney R, Evans J, Caslick J, Brewer D (Eds) Proceedings of animal damage management in Pacific Northwest forest. Washington State University, Pullman, USA, 147–148.

Fujiwara S, Koike S, Yamazaki K, Kozakai C, Kaji K (2013) Direct observation of bear myrmecophagy: Relationship between bears' feeding habits and ant phenology. Mammalian Biology 78: 34–40. http://dx.doi.org/10.1016/j.mambio.2012.09.002

Hanley TA (1982) The nutritional basis for food selection by ungulates. Journal of Range Management 35: 146–151.

Hashimoto Y, Takatsuki S (1997) Food habits of Japanese black bear: a review. Mammalian Science 37: 1–19. [In Japanese with English abstract]

Hilderbrand GV, Schwartz CC, Robbins CT, Hanley TA (2000) Effect of hibernation and reproductive status on body mass and condition of coastal brown bears. Journal of Wildlife Management 64: 178–183. http://dx.doi.org/10.2307/3802988

Horwitz W, Latimer GW (2005) Official Methods of Analysis of AOAC 18, AOAC International, Gaithersburg, Md.

Hwang MH, Garshelis DL, Wang Y (2002) Diets of Asiatic black bears in Taiwan, with methodological and geographical comparisons. Ursus 13: 111–125.

Kobashikawa S, Koike S (2016) Spatiotemporal factors affecting bark stripping of conifer trees by Asiatic black bears (*Ursus thibetanus*) in Japan. Forest Ecology and Management 380: 100–106. http://doi.org/10.1016/j.foreco.2016.08.042

Koike S (2010) Long-term trends in food habits of Asiatic black bears in the Misaka Mountains on the Pacific coast of central Japan. Mammalian Biology 75: 17–28. http://dx.doi.org/10.1016/j.mambio.2009.03.008

Koike S, Nakashita R, Kozakai C, Nakajima A, Nemoto Y, Yamazaki K (2016) Baseline characterization of the diet and stable isotope signatures of bears that consume natural foods in central Japan. European Journal of Wildlife Research 62: 23–31. http://dx.doi.org/10.1007/s10344-015-0969-6

Kozakai C, Yamazaki K, Nemoto Y, Nakajima A, Umemura Y, Koike S, Goto Y, Kasai S, Abe S, Masaki T, Kaji K (2013) Fluctuation of daily activity time budgets of Japanese black bears: relationship to sex, reproductive status, and hardmast availability. Journal of Mammalogy 94: 351–360. http://dx.doi.org/10.1644/11-MAMM-A-246.1

Lambert JE (2010) Primate nutritional ecology: feeding biology and diet at ecological and evolutionary scales. In: Campbell C, Fuentes A, MacKinnon KC, Panger M, Bearder S, Stumpf R (Eds) Primates in Perspective 2nd Edition. Oxford University Press, 512–522.

Masaki T, Takahashi K, Sawa A, Kado T, Naoe S, Koike S, Shibata M (2012) Fleshy fruit characteristics in a temperate deciduous forest of Japan: how unique are they? Journal of Plant Research 125: 103–114. http://dx.doi.org/10.1007/s10265-011-0423-0

McCue MD (2010) Starvation physiology: reviewing the different strategies animals use to survive a common challenge Comp. Biochemistry and Physiology 156: 1–18. http://dx.doi.org/10.1016/j.cbpa.2010.01.002

McDonald JE, Fuller TK (2005) Effects of spring acorn availability on black bear diet, milk composition, and cub survival. Journal of Mammalogy 86: 1022–1028. http://dx.doi.org/10.1644/1545-1542(2005)86[1022:EOSAAO]2.0.CO;2

McLellan BN (2011) Implications of a high-energy and low-protein diet on the body composition, fitness, and competitive abilities of black (*Ursus americanus*) and grizzly (*Ursus arctos*) bears. Canadian Journal of Zoology 89: 546–558. http://dx.doi.org/10.1139/z11-026

Noyce KV, Kannowski PB, Riggs MR (1997) Black bears as ant-eaters: seasonal associations between bear myrmecophagy and ant ecology in north-central Minnesota. Canadian Journal of Zoology 75: 1671–1686. http://dx.doi.org/10.1139/z97-794

Noyce KV, Garshelis DL (1998) Spring weight changes in black bears in northcentral Minnesota: the negative foraging period revisited. Ursus 10: 521–531.

Oi T, Yamazaki K (2006) The status of Asiatic black bears in Japan. In: Japan bear network (Ed.) Understanding Asian bears to secure their future, Japan Bear Network, Ibaraki, Japan, 122–133.

Panthi S, Coogan SCP, Aryal A, Raubenheimer D (2015) Diet and nutrient balance of red panda in Nepal. The Science of Nature 102: 54. http://dx.doi.org/10.1007/s00114-015-1307-2

Pritchard GT, Robbins CT (1990) Digestive and metabolic efficiencies of grizzly and black bears. Canadian Journal of Zoology 68: 1645–1651. http://dx.doi.org/10.1139/z90-244

Raubenheimer D (2011) Toward a quantitative nutritional ecology: the right-angled mixture triangle. Ecological Monograph 81: 407–427. http://dx.doi.org/10.1890/10-1707.1

Robbins CT (1993) Wildlife Feeding and Nutrition. 2nd ed. Academic Press, New York, NY, 352 pp.

Swenson JE, Adamic M, Huber D, Stokke S (2007) Brown bear body mass growth in northern and southern Europe. Oecologia 153: 37–47. http://dx.doi.org/10.1007/s00442-007-0715-1

Tochigi Prefecture (2011) Management of sika deer in Tochigi Prefecture: Monitoring report 2010. Tochigi Prefecture, Utsunomiya, Japan, 24 pp. [In Japanese]

Xu A, Jiang Z, Li C, Guo J, Wu G, Cai P (2006) Summer food habits of brown bears in Kekexili Nature Reserve, Qinghai–Tibetan plateau, China. Ursus 17: 132–137. http://dx.doi.org/10.2192/1537-6176(2006)17[132:SFHOBB]2.0.CO;2

Yamazaki K, Kozakai C, Koike S, Morimoto H, Goto Y, Furubayashi K (2012) Myrmecophagy of Japanese black bear in the grasslands of the Ashio area, Nikko National Park, Japan. Ursus 23: 52–64. http://dx.doi.org/10.2192/URSUS-D-10-00012.1

ZooKeys 672: 135–144 (2017)
doi: 10.3897/zookeys.672.11885
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Overview and new records of the species of the tribes Dyschiriini and Clivinini from Iraq (Coleoptera, Carabidae, Scaritinae)

Petr Bulirsch[1], Mieczysław Stachowiak[2]

1 *Milánská 461, CZ-109 00 Praha 111, Czech Republic* **2** *UTP University of Science and Technology; ul. Sucha 9, 85-796 Bydgoszcz, Poland*

Corresponding authors: *Petr Bulirsch* (p.bulirsch@seznam.cz); *Mieczysław Stachowiak* (pogonus@utp.edu.pl)

Academic editor: *A. Casale* | Received 25 January 2017 | Accepted 16 March 2017 | Published 9 May 2017

http://zoobank.org/B410117B-8902-4B9A-9B3B-7C72F8C45C1F

Citation: Bulirsch P, Stachowiak M (2017) Overview and new records of the species of the tribes Dyschiriini and Clivinini from Iraq (Coleoptera, Carabidae, Scaritinae). ZooKeys 672: 135–144. https://doi.org/10.3897/zookeys.672.11885

Abstract

The genera *Clivinopsis* Bedel, 1895 and *Torretassoa* Schatzmayr & Koch, 1933 have been recorded in Iraq for the first time. New records of several species of *Dyschirius* Bonelli, 1810, *Dyschiriodes* Jeannel, 1941 (Dyschiriini W. Kolbe, 1880), and *Clivina* Latreille, 1802 (Clivinini Rafinesque, 1815) are given. The identification key to the species of Dyschiriini from Iraq is provided.

Keywords

Carabidae, *Clivina*, Clivinini, *Clivinopsis*, Coleoptera, Dyschiriini, *Dyschiriodes*, *Dyschirius*, Iraq, key, Scaritinae, *Torretassoa*

Introduction

The scaritine tribes Clivinini Rafinesque, 1815 and Dyschiriini W. Kolbe, 1880 are distributed almost worldwide. The former includes several genera and several hundred species, and the latter includes several genera and few hundred species. The knowledge about the Iraqi species of the two tribes is incomplete. The species were reported from Iraq, without exact localities provided (Ali 1966, Balkenohl 2003). Fedorenko (1996) listed exact localities of some dyschiriine species, while only mapped them for some others.

Copyright *Petr Bulirsch and Mieczysław Stachowiak*. This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Material and methods

This article is based on the collections listed below. The specimens collected by Z. Stebnicka and J. Pawłowski during the 1977–1978 expedition of ISEA to Iraq were identified by the second author and the remaining specimens by the first author (mostly placed in his collection). The acronyms used are as follows:

ISEA The Institute of Systematics and Evolution of Animals of the Polish Academy of Sciences (Instytut Systematyki i Ewolucji Zwierząt Polskiej Akademii Nauk), Kraków, Poland;
HNHM Hungarian Natural History Museum (Magyar Természettudományi Múzeum), Budapest, Hungary;
PBPC collection of Petr Bulirsch, Prague, Czech Republic.

Abbreviations used in the key are as follows:

B basal setiferous puncture;
D dorsal setiferous punctures;
A apical setiferous punctures;
H (post)humeral setiferous punctures,
SP setiferous punctures;
L length of body.

Results

Tribe *Clivinini* Rafinesque, 1815

Genus *Clivina* Latreille, 1802

Note. This genus comprises approximately 650 species distributed almost worldwide.

Clivina (*Clivina*) *ypsilon* Dejean, 1830

Clivina (*Clivina*) *ypsilon* Dejean, 1830; Ali 1966: 15.

New records. 1 specimen: Hatra, 3 v 1978, at light in a hotel, leg. Z. Stebnicka, (ISEA); 1 specimen: S. E. Iraq, Amara, vi.1956, K. Khalaf coll., (PBPC).

Comments. A widespread, common and variable species reported from exact localities in Iraq for the first time.

***Clivina* (*Clivina*) *euphratica* Putzeys, 1866**

Clivina (*Clivina*) *euphratica* Putzeys, 1866; Ali 1966: 15; Balkenohl 2003: 219.

Comment. A poorly known species described from Iraq.

***Clivina* (*Leucocara*) *laevifrons* Chaudoir, 1842**

Clivina (*Leucocara*) *laevifrons* Chaudoir, 1842; Ali 1966: 15; Balkenohl 2003: 220.

Comment. A common species widespread in the Mediterranean area to the Middle Asia. Ali (1966) properly keyed its characters, but by mistake omitted the species name in the key.

Tribe *Dyschiriini* W. Kolbe, 1880

Genus *Dyschirius* Bonelli, 1810

Note. This genus in the sense of Fedorenko (1996) comprises nearly 20 mostly Palearctic taxa; one of them is known also from Iraq.

***Dyschirius beludscha ganglbaueri* Znojko 1927**

Dyschirius beludscha ganglbaueri Znojko 1927; Fedorenko 1996: 80: (Iraq, Baghdad, Abu-Ghraib, v.1984, Ing. Smatana).

New record. 1 specimen: Iraq, Kirkuk, lgt. W. Schors, (PBPC).
Comment. A common subspecies widespread in NW Africa to the Middle Asia.

Genus *Dyschiriodes* Jeannel, 1941

Note. Unlike Balkenohl (2003), Fedorenko (1996) considers this taxon as independent genus, not subgenus of *Dyschirius*. It is very largely distributed and includes five subgenera with over 300 species and subspecies, including ten hitherto reported from Iraq.

***Dyschiriodes* (*Dyschiriodes*) *agnatus* (Motschulsky, 1844)**

Dyschiriodes (*Dyschiriodes*) *agnatus* (Motschulsky, 1844); Ali 1966: 15 (as *D. lucidus* Putzeys, 1866); Fedorenko 1996: 157 (geographical distribution map); Balkenohl 2003: 224.

New record. 1 specimen: Iraq, Tekrit, ii-v.1979, (PBPC).

Comment. A very common species widespread from NW Africa to W Kazakhstan.

***Dyschiriodes* (*Dyschiriodes*) *auriculatus* (Wollaston, 1867)**

Dyschiriodes (*Dyschiriodes*) *auriculatus* (Wollaston, 1867); Fedorenko 1996: 173 (Tekrit); Balkenohl 2003: 224.

New record. 5 specimens: Iraq, Tekrit, ii-v.1979, (PBPC).

Comment. A rather common species widespread from NW Africa to the Middle Asia.

***Dyschiriodes* (*Dyschiriodes*) *cariniceps* (Baudi di Selve, 1864)**

Dyschiriodes (*Dyschiriodes*) *cariniceps* (Baudi di Selve, 1864); Mařan 1935: 211 (*Dyschirius kalalae*; Baghdad); Fedorenko 1996: 185; Balkenohl 2003: 225.

New records. 1 specimen (ab. *kalalae*): Iraq, Mosul, 28.vii.1956; 2 specimens (non-aberrant): Iraq, 80 km SW Baghdad, Shitatha oasis, creek bed, (PBPC).

Comment. A rather common species populating Sardinia, Sicilia, North Africa and extending eastward as far as Iraq and Iran.

***Dyschiriodes* (*Dyschiriodes*) *clypeatus* (Putzeys, 1866)**

Dyschiriodes (*Dyschiriodes*) *clypeatus* (Putzeys, 1866); Ali 1966: 16; Fedorenko 1996: 189 (as *D. clypeatus perlongus* (Müller, 1937); Baghdad); Balkenohl 2003: 225.

***Dyschiriodes* (*Dyschiriodes*) *euphraticus* (Putzeys, 1846)**

Dyschiriodes (*Dyschiriodes*) *euphraticus* (Putzeys, 1846); Ali 1966: 16 (also as *D. tuberculifer* Müller, 1922); Fedorenko 1996: 176 ('Mesopotamien' and 'Euphrates'); Balkenohl 2003: 225.

Comment. Not a frequent species in Turkey, Iraq and Iran.

***Dyschiriodes* (*Dyschiriodes*) *jedlickai* (Kult, 1940)**

Dyschiriodes (*Dyschiriodes*) *jedlickai* (Kult, 1940); Kult 1946: 1 (Mesopotamie, Euphrat); Fedorenko 1996: 157 (as *D. agnatus*, part.); Balkenohl 2003: 226 (as *D. agnatus*, part.); Bulirsch and Fedorenko 2007: 5.

Revised material. 1 specimen: Iraq, Euphrat, (PBPC).

Comment. A rare species in Turkey and Iraq (one old record).

***Dyschiriodes* (*Dyschiriodes*) *mesopotamicus* (Müller, 1922)**

Dyschiriodes (*Dyschiriodes*) *mesopotamicus* (Müller, 1922); Ali 1966: 16; Fedorenko 1996: 184 (geographical distribution map); Balkenohl 2003: 226.

Comment. Not a common species, distributed from Turkey to Middle Asia.

***Dyschiriodes* (*Dyschiriodes*) *pusillus pusillus* (Dejean, 1825)**

New records. 1 specimen: Iraq, Tekrit, ii-v.1979, (PBPC); 15 specimens: Iraq: Tharthar Lake, vii–viii. 1977, leg. J. Pawłowski, 21 specimens: 15 km W Kerbala, 14.v.1978, leg. Z. Stebnicka, (ISEA).

Comment. Some specimens of *D. pusillus pusillus* are very similar to those of *D. clypeatus* and its differentiation is difficult. It is recorded in Iraq for the first time.

***Dyschiriodes* (*Dyschiriodes*) *salinus striatopunctatus* (Putzeys, 1846)**

Dyschiriodes (*Dyschiriodes*) *salinus striatopunctatus* (Putzeys, 1846); Balkenohl 2003: 227.

New record. 1 specimen: Iraq: Razeza Lake, 15 km W Kerbala, 14 v 1978, leg. Z. Stebnicka, (ISEA).

Comment. A very common subspecies, very largely distributed from CE Europe, N Africa to Mongolia.

***Dyschiriodes* (*Eudyschirius*) *importunus importunus* (Putzeys, 1857)**

Dyschiriodes (*Eudyschirius*) *importunus importunus* (Putzeys, 1857); Fedorenko 1996: 128 (geographical distribution map); Balkenohl 2003: 229

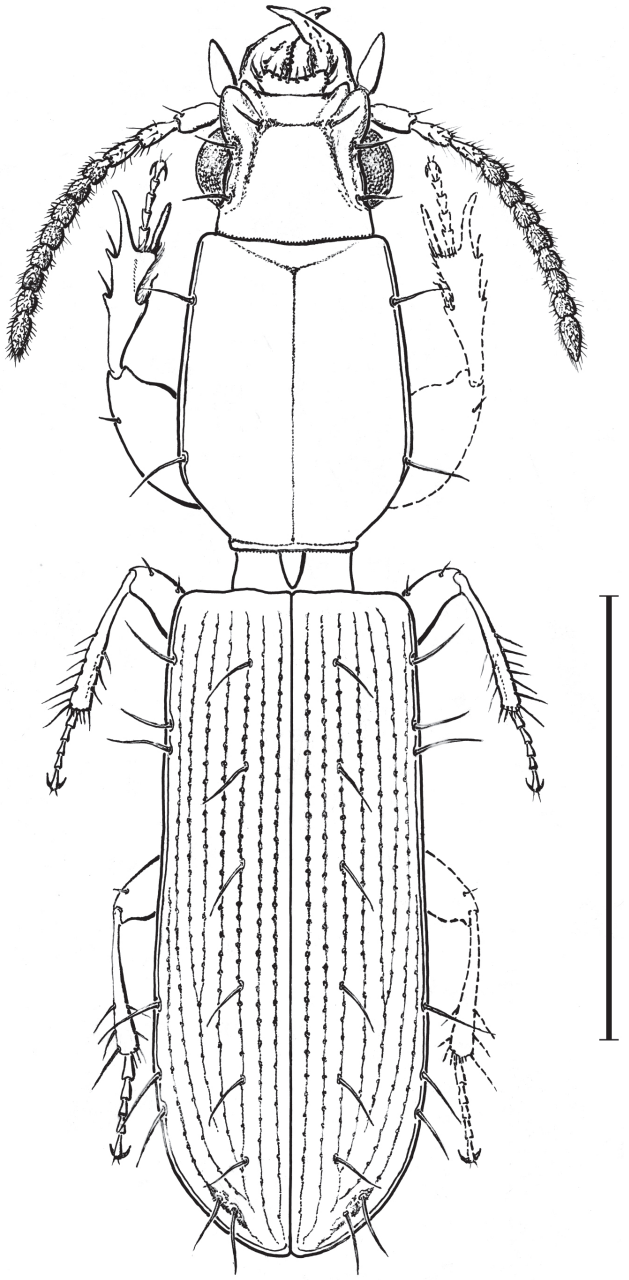


Figure 1. Habitus of *Torretassoa alfierii* Schatzm. & Koch from Iraq. Scale bar 1 mm.

New records. 1 specimen: Iraq: Hatra, 3.v.1978, leg. Z. Stebnicka, (ISEA); 1 specimen: Iraq, Baguba [= Baqubah], (PBPC).

Comment. A common subspecies distributed from Italy to Mesopotamia.

Genus Torretassoa Schatzmayr & Koch, 1933

Torretassoa alfierii Schatzmayr & Koch, 1933

Studied material. Paratypus, Egitto, Helwan, 18.ii.1935, W. Wittmer; 5 specimens: Iran Prov. Markazi, Kavir Desert, Houz-e Soltan, 830m, 3 km S Kushk-e Nosrat; 35°5'14"N, 50°55'26"E, at light, 28.vi.2000, leg. Kálmán Székely, (HNHM, PBPC);

New record. 1 specimen: Iraq: Tharthar Lake, vii-viii.1977, leg. J. Pawłowski, on banks of lake, in detritus, together with *D. clypeatus*, (ISEA) – Fig. 1., the first record in Iraq.

Comment. The genus includes a single species described from Heluan in Egypt and then reported from Karaman Island in Yemen (Fedorenko 1996), Iran and Saudi Arabia (Gueorguiev 2011).

Genus *Clivinopsis* Bedel, 1895

Note. The genus is recognized as including one rare species from North Africa (with two subspecies) and the other one from the Middle Asia.

Clivinopsis strigifrons (Fairmaire, 1874)

Studied material. *Clivinopsis strigifrons*: 1 specimen: Tunisie, Tozeur, iv.1954, R. Demoflys; (PBPC); *Clivinopsis conicicollis* (Reitter, 1909): 1 specimen: Turkmenistan, Mary, Badchyz NSG, Eroyulenduz, 19–20.iv.1993, Cate & Dostal leg., (PBPC).

New record. 1 specimen: Iraq: Hatra, 3.v.1978, leg. Z. Stebnicka, (ISEA).

Comments. The locality in Iraq is subequally distant from those of *C. conicicollis* (Turkmenistan, Kazakhstan) and *C. strigifrons* (Algeria, Tunis, Mauretania). We have found no significant difference between all examined specimens of these two species. Unlike of Fedorenko (1996) and Balkenohl (2003) who treated both taxa separately we follow Müller (1937: 130) in recognizing these taxa as conspecific. The validity of *C. strigifrons bonifacei* Bruneau de Miré, 1952 (Mauretania) is unclear.

Key to Iraqi Dyschiriini species

(including possible species in square brackets)

1 Head tumid; body cylindrical, redish to testaceous; pronotum distinctly longer than broad **2**

– Head not tumid. Other combination of characters. **3**

2(1) Head and pronotum anteriorly coarsely rugose. B1, D3, A1, H1. L. about 5 mm .. .. ***Clivinopsis strigifrons*** **(Fairmaire, 1874)** [= *C. conicicollis* Reitter, 1909]

– Head and pronotum smooth. B0, D5-7, A2, H3. L. about 3 mm .... ***Torretassoa alfierii*** **Schatzmayr & Koch, 1933** (Fig. 1)

3(1') Clypeus tridentate; elytra with basal ridge, fore tibia strongly dentate. B1, D3, A2, H2. L. 3.0 – 4.2 mm.....***Dyschirius beludscha ganglbaueri*** **Znojko, 1927**

– Clypeus not tridentate, at most broadly vaulted in middle; elytra without, rarely with gently basal ridge, fore tibia less strongly dentate..... ..... **Genus *Dyschiriodes* Jeannel, 1941...4**

4(3') Fore tibia with indistinct lateral teeth and with distinctly curved apical spine. B1, D2-3, A2, H1. L. mostly over 4 mm **5**

– Fore tibia with distinct marginal teeth (at least lower one) **6**

5(4) Pronotum and elytra broader, elytral striae finer. D2. L. 4.0–5.4 mm..... ..... ***D. agnatus*** **(Motschulsky, 1844)**

– Pronotum and elytra narrower, elytral striae coarser. D3. L. 3.9–4.3 mm..... ..... ***D. jedlickai*** **(Kult, 1940)**

6(4') Basal SP present..... **7**

– Basal SP missing **10**

7(6) Elytral striae not weakened apically; lateral channel of pronotum not shortened. D3, H3, A2..... **8**

– Elytral striae weakened to obliterated apically **9**

8(7) Head with frons coarsely rugose; elytral base with small tubercle. L. 2.8–3.7 mm..... [***D. chalybeus gibbifrons*** **(Apfelbeck, 1899)**]

– Head with straight or obsolete clypeofrontal suture; elytral base with two distinct tubercles. L. 3.5–4.9 mm .... ***D. salinus striatopunctatus*** **(Putzeys, 1846)**

9(7') Lateral channel of pronotum not shortened. D3. L. 2.6–3.3 mm .... [***D. schaumi*** **(Putzeys, 1866)**]

– Lateral channel of pronotum strongly shortened. D2. L. 3.1–4.2 mm .... [***D. syriacus*** **(Putzeys, 1866)**]

10(6') Lateral channel of pronotum shortened, not reaching posterior SP; elytra non cylindric..... **11**

– Lateral channel of pronotum not shortened, at least reaching posterior SP.... **13**

11(10) Clypeofrontal suture V-shaped, elytral striae obliterated apically..... **12**

– Clypeofrontal suture straight; lateral channel of pronotum strongly shortened, disappeared just below anterior SP; elytral striae strongly weakened apically. L. 3.1–4.0 mm..... ***D. importunus importunus*** **(Schaum, 1857)**

12(11) Lateral channel of pronotum strongly shortened, disappeared just below anterior SP. D3, A1-2. L. 2.3–3.1 mm ..... [***D. luticola luticola*** **(Chaudoir, 1850)**]

– Lateral channel of pronotum slightly shortened, disappeared just above posterior SP. D3, A1. L. 2.6–3.0 mm ... ***D. cariniceps*** **(ab. *kalalae* Mařan, 1935)**

13(10') Elytra cylindric; striae deep apically **14**

– Elytra shorter, ovate to elliptic **17**

14(13) Each elytron with two distinct, often fused tubercles at base. L. 3.7–5.0 mm..... .....[***D. cylindricus hauseri*** **(Fleischer, 1898)**]
– Elytra without or with a small basal tubercle.....**15**
15(14) Larger species. L. 3.9–5.0 mm. Elytral base without tubercles..... ..... ***D. auriculatus*** **(Wollaston, 1867)**
– Smaller species. L. 2.3–3.4 mm. Elytral base with one small tubercle.....**16**
16(15) Clypeofrontal suture prolonged posteriorly by distinct keel; pronotum and elytra in average narrower. L. 2.1–3.3 mm..... ***D. clypeatus*** **(Putzeys, 1866)**
– Clypeofrontal suture not prolonged posteriorly by distinct keel; pronotum and elytra in average broader. L. 2.3–3.4 mm... ***D. pusillus*** **(Dejean, 1825)**
17(13') Elytral base with two tubercles; striae deep throughout. A2. L. 2.9–3.7 mm ....***D. euphraticus*** **(Putzeys, 1846)**
– Elytral base with 0-1 tubercles. A1.....**18**
18(17) Elytral base with one tubercle; elytra longer, with striae not to slightly weakened apically. D0-3. L. 2.2–3.2 mm..... ***D. mesopotamicus*** **(Müller, 1922)**
– Elytral base without distinct tubercle; elytra shorter, with striae disappeared to strongly weakened apically. D3. L. 2.5–3.0 mm..... ..... ***D. cariniceps*** **(Baudi di Selve, 1864)**

Acknowledgements

We would like to thank Prof. Jerzy Pawlowski for supplying material for this publication and for additional information about the circumstances of its collection.

References

Ali HA (1966) A Key to the Carabidae (Insecta, Coleoptera) of Iraq. Iraq National History Museum Publication No. 23: 1–38.

Balkenohl M (2003) Scaritinae. In: Löbl I, Smetana A (Eds) Catalogue of Palearctic Coleoptera, Volume 1 Archostemata - Myxophaga - Adephaga. Apollo Books, Stenstrup, 219–234.

Bruneau de Miré P (1952) Contribution a l´étude des Dyschiriinae (Col. Carabiques) du Sahara méridional et de ses confins Sahéliens. Annales de la Société Entomologique de France 121: 49–60.

Bulirsch P, Fedorenko D (2007) Species of the tribe Dyschiriini (Coleoptera: Carabidae: Scaritinae) from Turkey, Syria and Cyprus. Studies and reports of District Museum Prague-East. Taxonomical series 3: 1–16.

Fairmaire L (1874) Note sur quelques coléoptères des environs de Tuggurt. Petites Nouvelles Entomologiques 1: 405-409.

Fedorenko DN (1996) Reclassification of world Dyschiriini, with a revision of the Palearctic fauna (Coleoptera, Carabidae). Pensoft Publishers, Sofia-Moscow-St. Petersburg, 224 pp.

Guéorguiev B (2011) New and interesting records of Carabid Beetles from South-East Europe, South-West and Central Asia, with taxonomic notes on Pterostichini and Zabrini (Coleoptera, Carabidae). Linzer biologische Beitrage 43: 501–547.

Jedlička A (1966) Neue Carabiden aus Kazakhstan (Coleoptera, Carabiden). Reichenbachia 8: 21–26.

Kult K (1940) Genus Dyschirius Bon. (Col., Carab.). Časopis Československé Společnosti Entomologické 37: 55–58.

Kult K (1946) New Palearctic Dyschirius species (Carab. Col.) (13*). Časopis Československé Společnosti Entomologické 43: 1–8.

Mařan J (1935) De novis Carabidarum speciebus formisque faunae Bohemiae (Coleoptera). Sborník Entomologického Oddělení Národního Muzea v Praze 13: 211–216.

Müller G (1937) Note su alcuni carabidi della Balcania e della regione mediterranea. Atti del Museo Civico di Storia Naturale di Trieste 13: 135–139.

Reitter E (1909) Einige neue Coleopteren aus der paläarktischen Fauna. Wiener Entomologische Zeitung 28: 53–58.

Schatzmayr A, Koch C (1933) Descrizione di un nuovo genere appartenente alla sezione dei Clivinina. (Coleotteri: Carabidae-Scaritinae). Bulletin de la Société Royale Entomologique d´Égypte 17: 121–125.