

A new species of the genus *Hyaella* (Crustacea, Amphipoda) from northern Mexico

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Abstract

A new species, *Hyaella tepehuana* **sp. nov.**, is described from Durango state, Mexico, a region where studies on *Hyaella* have been few. This species differs from most species of the North and South American genus *Hyaella* in the number of setae on the inner plate of maxilla 1 and maxilla 2, characters it shares with *Hyaella faxoni* Stebbing, 1903. Nevertheless, *H. faxoni*, from the Volcan Barva in Costa Rica, lacks a dorsal process on pereonites 1 and 2. Also, this new species differs from other described *Hyaella* species in Mexico by the shape of the palp on maxilla 1, the number of setae on the uropods, and the shape of the telson.

Keywords

Durango, freshwater amphipod, Nearctic region, scud, taxonomy

Introduction

Covich et al. (2009) recorded seven species of the amphipod *Hyaella* Smith, 1874 for North America (including northern Mexico) and emphasized that there were probably one or more undescribed species. Until now, there are 12 formally described species in North America, nine of them from the United States of America: *Hyaella texana* Stevenson & Peden, 1973; *H. montezuma* Cole & Watkins, 1977; *H. longicornis* Bous-

field, 1996; *H. muerta* Baldinger, Shepard & Threlhoff, 2000; *H. sandra* Baldinger, Shepard & Threlhoff, 2000; *H. meraspinosa* Baldinger, 2004; *H. spinicauda* Soucek & Lazo-Wasem, 2015; *H. wellborni* Soucek & Lazo-Wasem, 2015; *H. wakulla* Drumm & Knight-Gray, 2019; and *Hyaella cheyennis* Bueno, Oliveira & Wellborn, 2019.

Mexico is in the transition zone between two biogeographic regions: the Nearctic and the Neotropical regions. In the Neotropical region, the genus has been found to be highly diverse, with three species in Mexico, three species in the Caribbean region, two species in Central America, and more than 60 species in South America (Marrón-Becerra et al. 2018; Horton et al. 2019). Of the three species in Mexico, the type locality of *Hyaella azteca* (De Saussure, 1858) is in Veracruz, and the type localities of *Hyaella cenotensis* Marrón-Becerra, Hermoso-Salazar & Solis-Weiss, 2014 and *Hyaella maya* Marrón-Becerra, Hermoso-Salazar & Solis-Weiss, 2018 are in the Yucatan Peninsula. In northern Mexico, the taxonomic status of *Hyaella* populations is unknown, and the few records of *H. azteca*, identified by Rodríguez-Almaraz et al. (2014), from Parque Nacional Cumbres de Monterrey in Nuevo Leon state requires morphological confirmation. Most of the studies on amphipods in northern Mexico are focused on the stygobitic environment (e.g. Holsinger 1973). Herein, we record and formally describe for the first time an epigeic amphipod, *Hyaella tepehuana* sp. nov., from Durango state. This new species is the first epicontinental freshwater amphipod described in the Nearctic Region of Mexico.

Materials and methods

The material was collected using a net with fine, 250 µm mesh on aquatic vegetation in the Tunal River in Durango state, Mexico (Fig. 1). This river belongs to San Pedro hydrological basin and flows to the Pacific Ocean.

The body parts of the collected material were dissected and mounted: semi-permanent slides were mounted on glycerol, and permanent slides on Entellan, a synthetic resin. The terminology used for the setae follows that of Zimmer et al. (2009). The morphological description includes intraspecific variation.

The type material was deposited in the Colección Nacional de Crustáceos (CNCR), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM).

Scanning electron micrographs were taken from paratypes (one female and one male) with a Hitachi SU1510 scanning electron microscope at the Laboratory of Microscopy and Photography of Biodiversity I, Instituto de Biología, UNAM.

We compared our specimens with the lectotype and syntype material (now paralectotype) of *Amphitoe aztecus*, originally collected by De Saussure (1858) and in the Muséum d'Histoire Naturelle, Geneva, Switzerland. This material had been redescribed as *H. azteca* lectotype (no catalogue number assigned) by Gonzalez and Watling (2002a).

We present a key of the species from North America, Central America, and Caribbean region. However, *Hyaella sapropelica* Brehm, 1939 is excluded because the short description and incomplete drawings, make it is necessary to redescribe this species. In the key, we retain the subgenus *Hyaella* (*Hyaella*), proposed by Bousfield (1996);

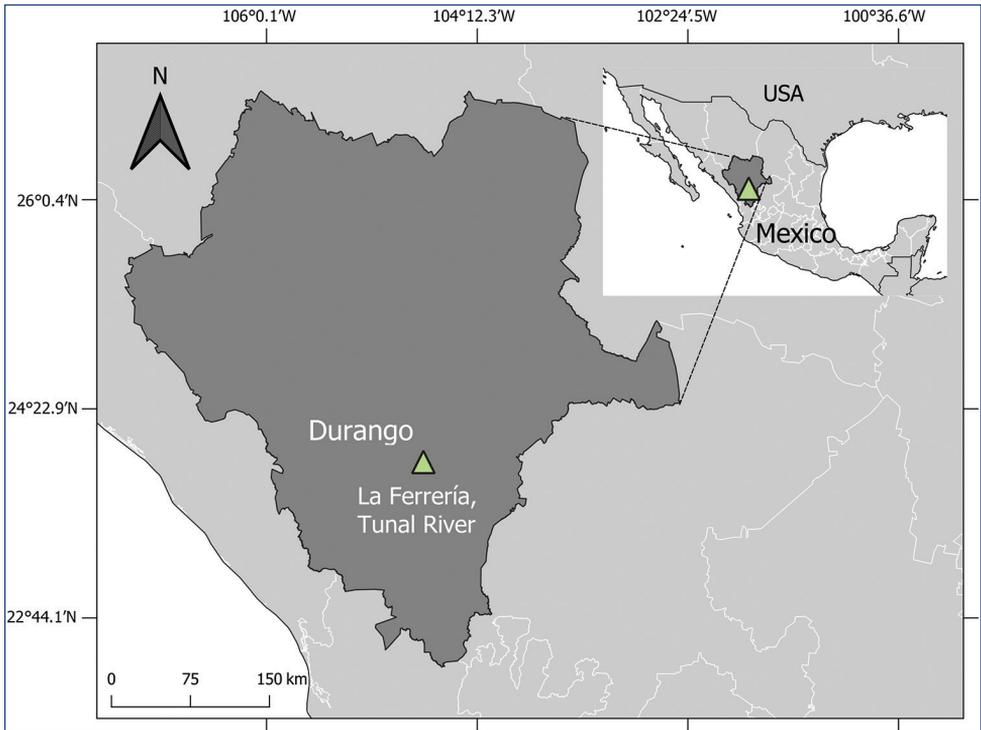


Figure 1. Type locality. Tunal River, La Ferrería, Durango state, Mexico (23°57.905'N, 104°39.817'W).

Baldinger (2004) considered Bousfield's classification to be an artificial grouping of species with no curved spine in the male uropod 1, the propodus in gnathopod 1 mainly hammer-shaped, five pairs sternal gills, the ramus of uropod 3 "elongated", and the telson mainly with paired setae. Nevertheless, we emphasize this classification is no longer accepted, and a revision of the characters and relationships proposed by Bousfield (1996) need revision.

Taxonomy

Order Amphipoda Latreille, 1816
 Family Hyaellidae Bulycheva, 1957
 Genus *Hyaella* Smith, 1874

Hyaella tepehuana sp. nov.

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Figures 2–8

Etymology. The specific epithet *tepehuana* refers to the great Tepehuan ethnic group, whose current settlement is in southern Durango. The name Tepehuan comes from

Náhuatl and has two meanings: “owners of the hills”, *tepetl* (hill) and *huan* (possessive), and “winner of battles”, *tepehuani*.

Material examined. Holotype male, body length 5.4 mm (CNCR 35295), from Tunal River in La Ferrería Durango, Mexico (23°57.905'N, 104°39.817'W) 12 June, 2016. Collectors: A. Marrón-Becerra and G. Rivas. Paratypes ($n = 10$ males, $n = 10$ females): males mean size 5.7 ± 0.6 mm, female body length 5.6 ± 0.6 mm (CNCR 35296, permanent slides and CNCR 35297 SEM preparations), same locality, date and collectors as holotype.

Type locality. Mexico, Durango, Tunal River in La Ferrería: 23°57.905'N, 104°39.817'W (Fig. 1), above 1874 m a.s.l., scarce water but with high density of macroalgae.

Diagnosis. Pleonite 1 and 2 with dorsoposterior carina. Coxa 4 excavated posteriorly. Eyes pigmented. Antenna 1 shorter than Antenna 2 without accessory flagellum. Antenna 2 less than one-half body length. Maxilla 1 palp short, reaching less than half distance between base of palp and tip of setae of outer plate, with one stout distal seta; inner plate slender with three or four strong pappose distal setae. Maxilla 2 inner plate with two or three strong pappose setae on inner margin. Gnathopod 1, propodus hammer-shaped, palm slope transverse, inner face with three or four pappose setae, comb scales on distoposterior and distoanterior border. Gnathopod 2, basis hind margin with two setae. Uropods without curved setae. Uropod 3, peduncle and ramus subequal in length, styliform. Telson slightly longer than wide, narrowing posteriorly, with two long simple setae widely separated.

Description of male. Body, tergites of pleon 1 and 2 with dorsoposterior carina (Fig. 2A, B).

Mean body length of males: 5.7 ± 0.6 mm ($n = 10$).

Mean cephalothorax length: 0.6 ± 0.03 mm ($n = 10$).

Epimeral plates 1–3 acuminate.

Coxae 1–3 (Fig. 2A, B) subequal in shape, subrectangular, longer than wide; coxa 1 shorter than coxae 2 and 3; coxa 4 wider than coxae 1–3 with a deep posterior excavation; coxae 1–4 slightly overlapping anterior coxa, distal margin rounded with small setae widely separated, acumination absent. Coxae 5–7 reduced, shorter than coxae 5 and 6, bilobate except coxa 7; coxa 5 with two subequal lobes, posterior lobe slightly longer than anterior; coxa 6 with two unequal lobes, anterior lobe reduced; coxa 7 with a single lobe, anterior lobe absent.

Head typically gammaridean (Fig. 2A, B), smooth surface, length less than combined length of the first two thoracic segments, reaching the half of the second pereonite, rostrum absent. Eyes pigmented, medium, rounded, located between insertions of antennae 1 and 2.

Antenna 1 (Figs 2A, B, 3A) less than one-half the body length, shorter than antenna 2 (80% length of A2), but longer than peduncle of antenna 2, reaching more than one-half of the third pereonite; peduncle longer than head, reaching beyond half of the length of first pereonite, article 1 and 2 subequal in length, article 1 wider than articles 2 and 3, article 2 longer and wider than article 3, article 3 shorter and thinner than articles 1 and 2, proportions (1.5:1.4:1), article 1 close to the middle length of the

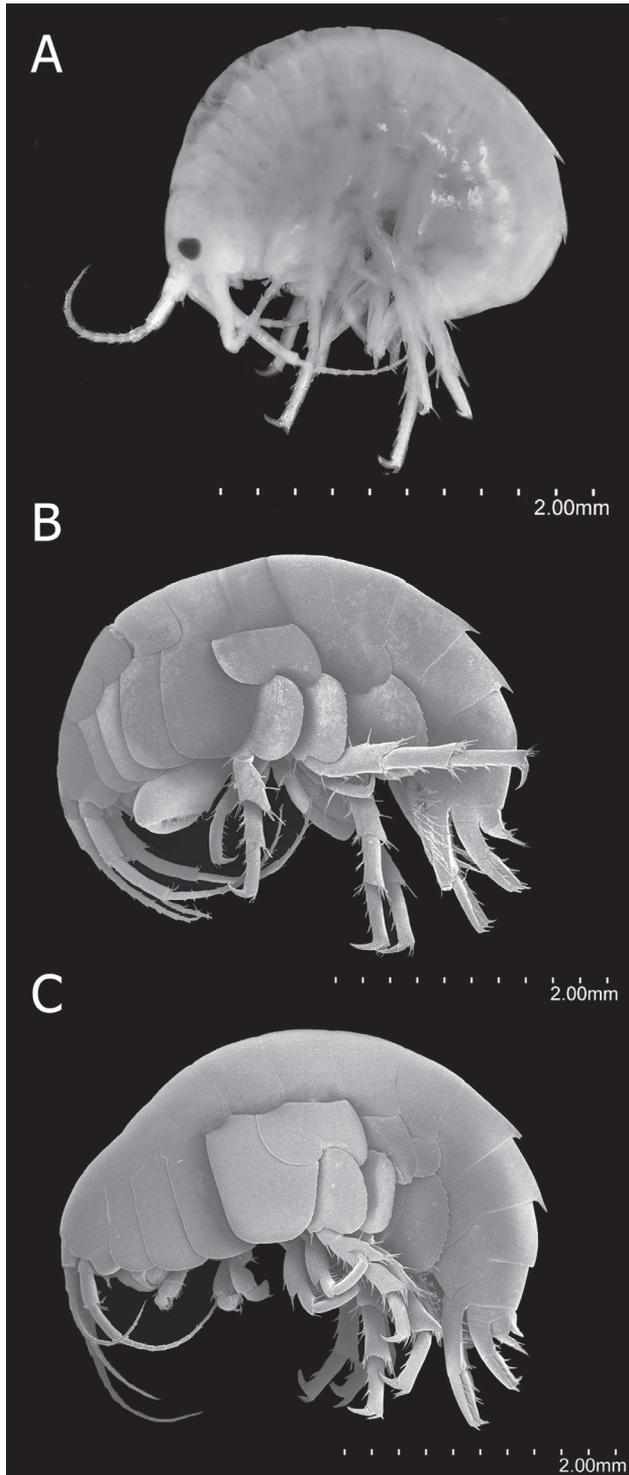


Figure 2. *Hyaella tepehuana* sp. nov. lateral habitus **A** male holotype 5.4mm **B** male paratype 5.6 mm **C** female paratype 5.6 mm. Scale bars: 2 mm.

ventral surface with two short cuspidate setae, one smaller, and one cluster with three cuspidate setae at distal end; flagellum with 9–11 articles reduced gradually toward the distal portion, flagellum longer than peduncle; aesthetasc on flagellum, present on articles 4–11, 4–7 (one pair), 8–11 (one aesthetasc). Accessory flagellum absent.

Antenna 2 (Figs 2A, B, 3B) almost 1.2× longer than antenna 1, slightly longer than one-third body length, reaching half of the fifth pereionite; peduncle reaching the second pereionite, peduncle articles increase gradually in length and decrease in width, article 3 shorter but wider than articles 4 and 5, article 4 a little longer than the length of article 3, article 5 slightly longer than article 4; flagellum with 11–12 articles, slightly longer than peduncle, almost 1.25× the length; without aesthetasc on flagellum.

Buccal parts: upper lip (Fig. 3E) with distal margin rounded and numerous setules present, longer and more distant toward the lateral end, two setae plus accessory setae near the distal margin on both sides (left and right) symmetrically.

Lower lip (Figs 3F, 8G), outer lobes without notches or excavations; mandibular projection of outer lobes rounded; without inner lobes.

Mandibles (Figs 3G, H, 8A, B) without palp, asymmetric. Incisor toothed, six to eight teeth present. Left lacinia mobilis similar to incisor process, with five or six teeth; setal row on left mandible with three or four main pappose setae plus accessory setae. Right mandible with six or seven teeth, lacinia mobilis reduced than the left one, with two pairs of asymmetrical, L-shaped teeth, fused at the base, proximal pair shorter than distal, inner margin denticulate; near the lacinia mobilis base, with one pair of setae; setal row with two main pappose setae plus accessory setae and with setulae near the molar process. Molar process large, cylindrical, and triturative; left mandible lateral view almost rectangular (Fig. 3G) and right mandible with a 60° angle (Fig. 3H); with accessory pappose setae present in both molars.

Maxilla 1 (Figs 3C, 8D, E) with short palp, vestigial, uniaarticulate, longer than wide, distally pointed with one short and stout distal seta (Fig. 8D), palp length almost exceeds half of the distance between base of palp and base of seta on outer plate, but less than half the distance between base of palp and tip of seta on outer plate (Fig. 8E); inner plate slender, shorter than outer plate, with three or four pappose distal setae (two distal and one or two subdistal); outer plate with nine stout serrate setae (Fig. 8E).

Maxilla 2 (Figs 3D, 8C) with plates subequal in length, width, and shape; inner plate shorter and slender, with two or three pappose setae on mid-distal margin, and with seven shorter serrulate setae on distal margin; outer and inner plates with abundant setules.

Maxilliped (Figs 3I, 8F) with inner plate longer than outer plate; distal margin slightly convex, almost flat, in both plates; inner plate distal margin with three cuspidate setae of equal size and with plumose setae; outer plate, inner and distal margins with numerous simple setae. Palp composed of four articles subequal in the maximum length; first article with three simple setae at the inner distal end and one pair at the outer distal end, one on each side; second article with numerous simple setae on the inner margin and three on outer distal end; third article with several setae on distal margin, distal end on the outer margin with three simple setae and comb setae; fourth

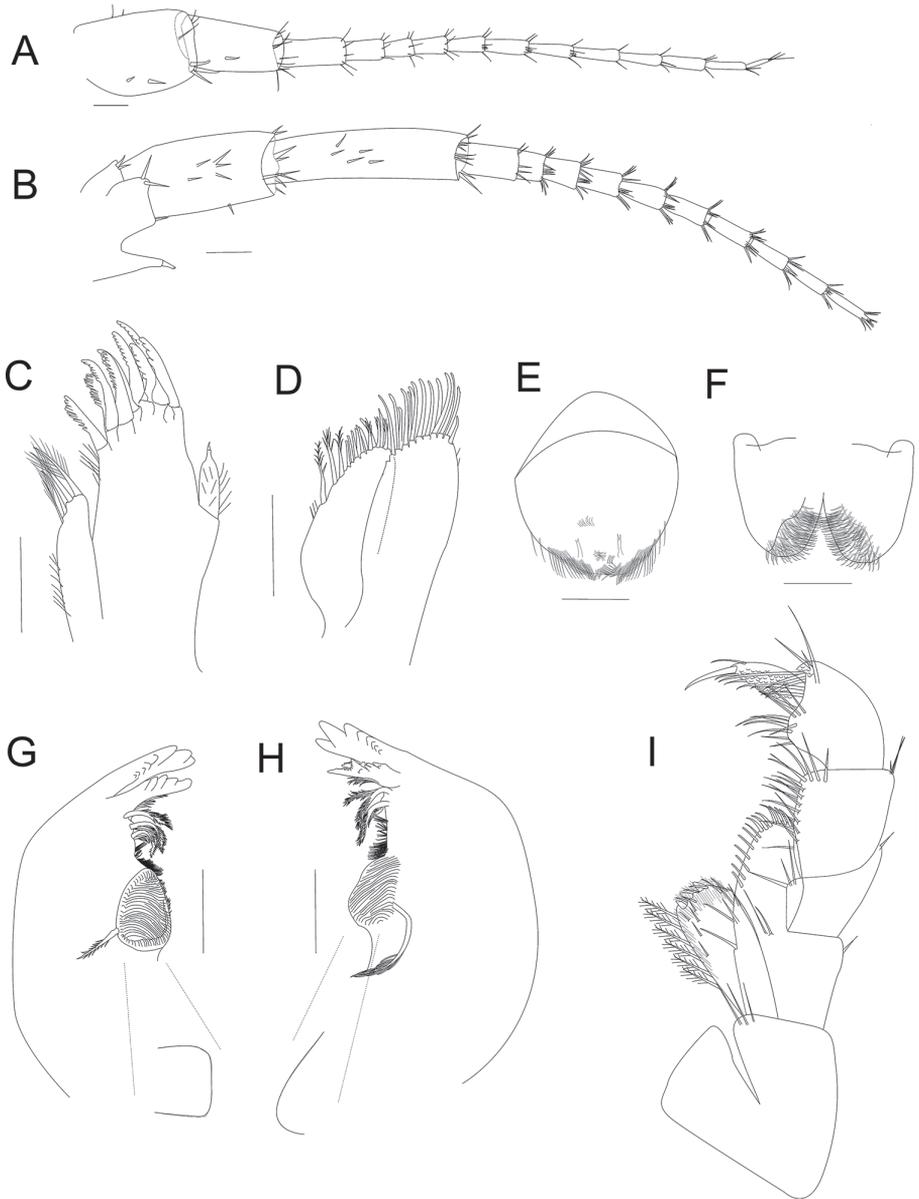


Figure 3. *Hyaella tepehuana* sp. nov., male antennae **A** antenna 1 **B** antenna 2. Buccal parts **C** maxilla 1 **D** maxilla 2 **E** upper lip **F** lower lip **G** left mandible (dotted line shows the form and angle of molar in lateral view) **H** right mandible (dotted line shows the form and angle of molar in lateral view) **I** maxilliped. Scale bars: 100 μ m.

article unguiform, longer than nail, with comb setae, inner margin near the distal half with three setae and one near nail base on outer margin; nail reaching almost two-thirds of the fourth article with serration at distal half.

Gnathopod 1 (Figs 4A, 6A–C) subchelated, hammer shaped, shorter than gnathopod 2. Basis elongated, maximum length close to 3× longer than the maximum width; near to the half of posterior margin with one seta, distal end with two clusters of two setae. Ischium short, almost as long as wide, length almost same as maximum width of basis and maximum length of merus; distal posterior end with two clusters of two setae. Merus longer than wide, almost half of the length of ventral surface with comb scales; distal margin with four setae. Carpus longer than wide, longer and slightly wider than propodus, with strong short and wide posterior lobe forming a scoop-like structure open to the inside; lobe, inner surface with three serrate setae, external surface on the distal half of lobe, near to the margin, with comb scales, anterior distal end with three setae. Propodus 1.5× longer than wide; inner surface near to the distal margin with four serrate setae (three in a row); distal anterior end with two clusters of approximately five setae; distal anterior and posterior surfaces with comb scales; palm transverse, posterior distal end with a robust seta and cup for dactyl. Dactyl claw-like; nail present; anterior surface near to the proximal half with one plumose seta, with comb scales over the anterior surface.

Gnathopod 2 (Figs 4B, 6D, E) subchelated; palm slightly oblique. Basis elongate, more than 3× longer than wide; posterior margin with two long setae. Ischium short, subquadrate, shorter than merus. Merus short; distal end of posterior margin with eight simple setae; distal half of the posterior inner and outer surfaces with comb scales. Carpus shorter than propodus; anterodistal end with two setae; posterior lobe scoop-like, elongate, length similar to the merus maximum length, almost 1.5× the width of merus, with several submarginal pappose setae and comb scales. Propodus robust, almost 1.5× as long as wide, subrectangular; palm slightly shorter than posterior margin, slope slightly irregular, with some long simple setae, several short, and several medium setae; distal margin of palm with one truncated process and presence of one slightly posterior excavation at base, near to the insertion of dactyl; palm posterior distal end with two strong setae, comb scales and cup for dactyl. Dactyl claw-like, congruent with palm, without comb scales; outer margin proximal third with a plumose seta; inner margin crenulate.

Pereopods 3–7 (Figs 4E–I, 7D–I) simple, gradually longer posteriorly. Pereopod 5 shorter than pereopods 4 and 6.

Pereopod 3 (Figs 4E, 7D) with basis elongate; mid-posterior margin with two simple setae; anterodistal and posterodistal ends with simple setae. Ischium subquadrate; posterodistal end with one pair of setae. Merus longer than ischium (more than twice the length); posterior margin with three setae; anterior margin with one seta; anterodistal and posterodistal ends with one cluster of four setae. Carpus shorter and slenderer than merus; posterior margin with four stout setae; posterodistal end with at least five slender setae, longer than the ones from posterior margin; anterodistal end with at least two setae. Propodus almost as large as the posterior margin of merus, slenderer than carpus; posterior margin with eight setae; anterodistal end with three simple setae. Dactyl claw-like; nail present; first proximal third of the anterior margin with one plumose seta; posterior margin with one simple seta close to the nail.

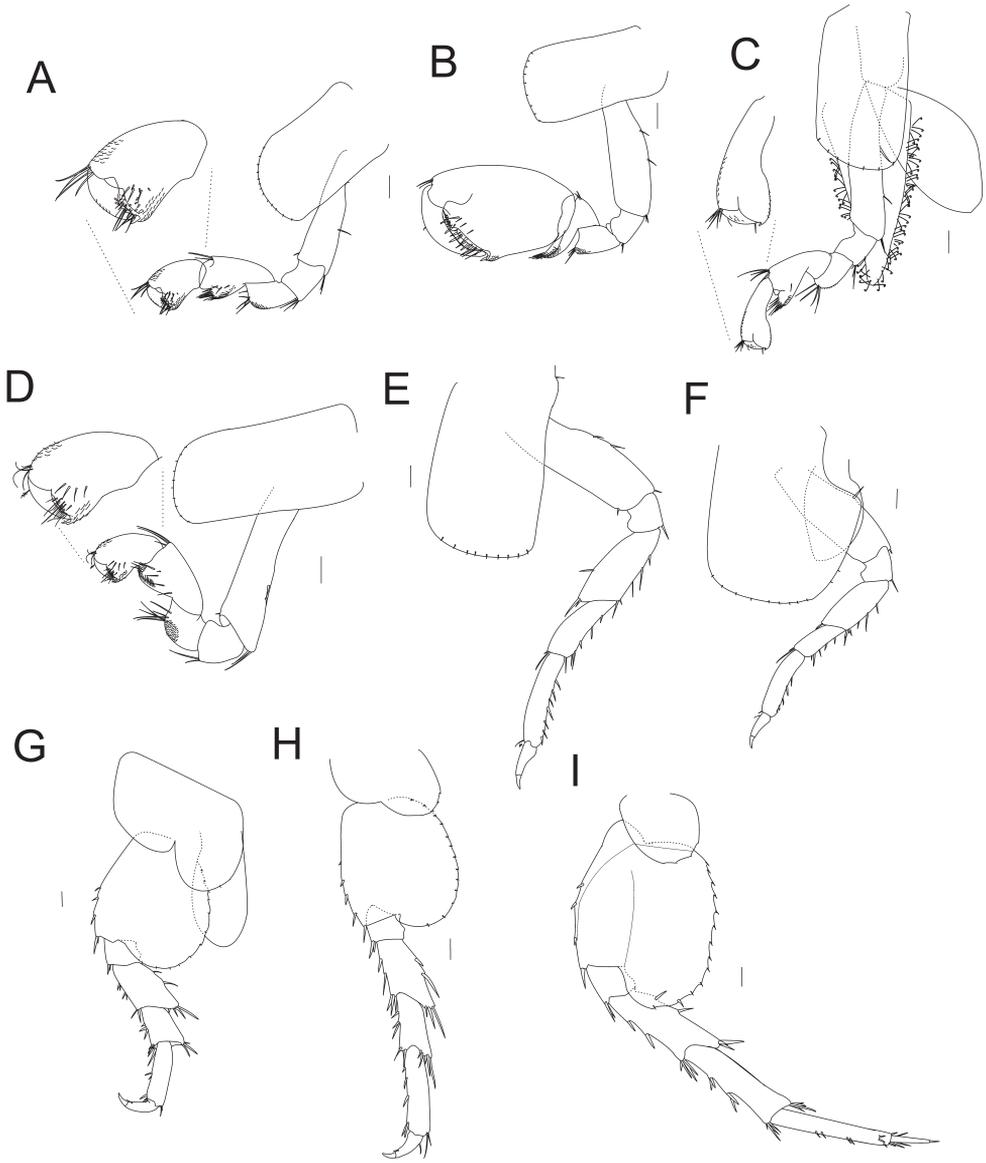


Figure 4. *Hyaella tepehuana* sp. nov. gnathopods **A** male gnathopod 1 **B** male gnathopod 2 **C** female gnathopod 2 **D** female gnathopod 1. Male pereopods **E** pereopod 3 **F** pereopod 4 **G** pereopod 5 **H** pereopod 6 **I** pereopod 7. Scale bars: 100 μ m.

Pereopod 4 (Figs 4F, 7E) similar in shape to pereopod 3 but slightly longer; coxa 4 wider than coxa 3, with a posterior excavation; basis posterior margin with one simple seta.

Pereopods 5–7 (Figs 4G–I, 7F–H) similar in shape; basis posterior lobe rounded and denticulate. Pereopod 7 (Fig. 7I) with basis lobe widely expanded, almost reaching ischium distal margin; wider than lobes of pereopods 5 (Figs 4G, 7F) and 6 (Figs 4H,

7G); width almost 0.75× width of basis (measured at cleft between basis and basis lobe); posterior margin with 14 serrations, each with one setule but one or two serrations with one stout seta in the distal margin; anterior margin with three stout setae and one at distal end.

Pleopods 1–3 (Fig. 5E) not modified, biramous, elongated, rami multi-annulated, with numerous plumose setae; inner margin of peduncle with two short retinacula (coupling hooks) at distal end.

Uropod 1 (Figs 5A, 8H) longer than uropod 2 (Fig. 8H); peduncle longer than rami, proximal half of the dorsal margin with three or four dorsal setae, inner and outer distal end with one seta; rami subequal, inner ramus slightly shorter, with two dorsal setae and four distal setae, outer ramus with three dorsal and three distal setae; male without curved setae on inner ramus.

Uropod 2 (Figs 5B, 8H) longer than peduncle of uropod 1; peduncle as long as rami, with two dorsal setae over the distal half and one at distal end; rami subequal, inner ramus with two dorsal and four distal setae, outer ramus with two or three dorsal and three distal setae.

Uropod 3 (Figs 5C, 8H–I) slightly shorter than peduncle of uropod 2; peduncle rectangular, wider than ramus with four strong distal setae of variable length, inner ramus absent, outer ramus uniarticulate slender, slightly shorter than peduncle, basal width near 3× the apex of ramus, with three or four slender apical setae and one connate seta.

Telson (Figs 5D, 8J) entire, slightly longer than wide, narrowing posteriorly, with two long simple setae widely separated; outer surface bearing two clusters of three plumose setae near the half distal portion, close to the margin, symmetrically distributed.

Coxal gills sac-like, present on segments 2–6 (Fig. 6D). Sternal gills tubular, present on segments 3–7.

Female (Fig. 2C). Similar to male. Gnathopod 1 (Figs 4C, D, 7A–C) with carpus with five setae on the inner face lobe; propodus with four setae in a row over the inner face. Gnathopod 2 smaller than male gnathopod 2, parachelated, palm reverse oblique; basis posterior margin with two setae; propodus slightly longer than twice its maximum width, outer face with three setae in a row and three large setae near the palm, anterior and posterior distal half with comb scales. Pereonite 2 with one anterior excavation or notch for the amplexus. Pereopod 7 lobe with 13 serrations and setules, and two stout setae on the distal margin. Oostegites subtriangular, with setae curled on the margins, reaching almost one-half length of merus (Fig. 4C).

Intraspecific variation: Maxilla 1, inner plate usually with four setae, the smaller adults could have three setae and the young ones two setae. Maxilla 1 palp, length quite variable during the molt process. Maxilla 2 inner plate with two or three setae, even in the same organism.

Habitat. Freshwater, epigean.

Distribution. La Ferrería, Durango, Tunal river (23°57.905'N, 104°39.817'W).

Remarks. *Hyalella tepehuana* sp. nov. is the first species described from northern Mexico. It is easily distinguished from other species of *Hyalella* from the USA, Mexico,

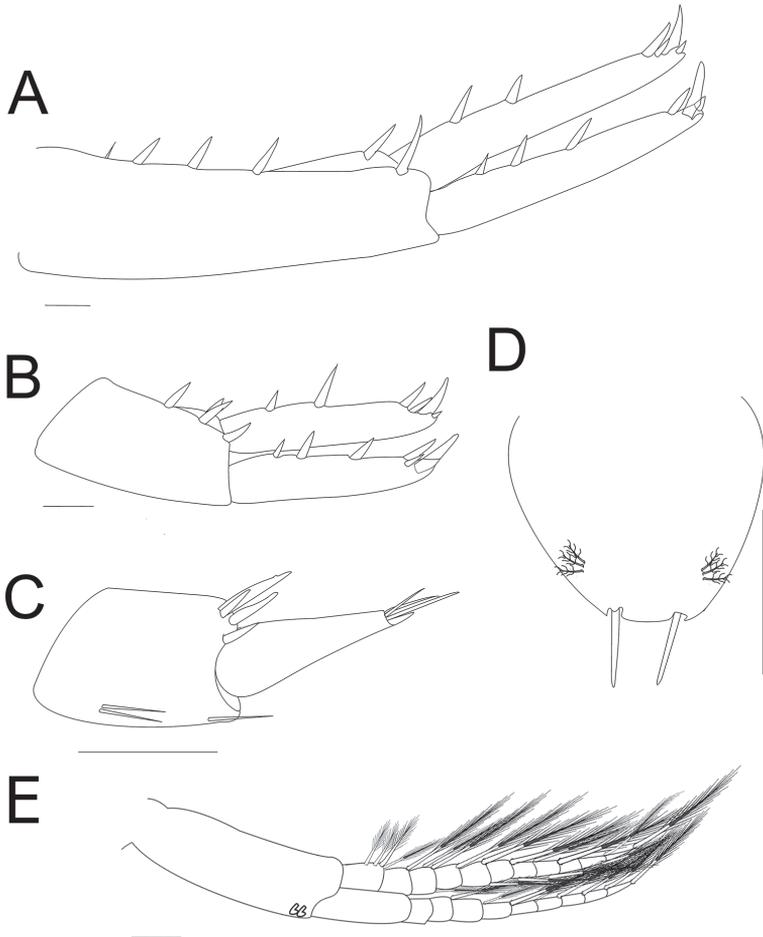


Figure 5. *Hyaella tepehuana* sp. nov. Male uropods **A** uropod 1 **B** uropod 2 **C** uropod 3 **D** telson **E** Pleopod 1. Scale bars: 100 μ m.

and the Caribbean by the atypical number of setae on the inner plate of maxilla 1 (four setae) and maxilla 2 (three setae), and by the shape of the telson. The species morphologically most similar to *H. tepehuana* sp. nov. is *H. faxoni* Stebbing, 1903 from the Volcan Barva in Costa Rica, but *Hyaella tepehuana* sp. nov. differs by the presence of dorsal processes on pereionites 1 and 2, and by the following characters: number of articles on antennae 1 and 2 (9–11 and 11 or 12, respectively, in *H. tepehuana* sp. nov. versus 12 and 15–17, respectively, in *H. faxoni*); number of setae on propodus inner face of gnathopod 1 (four in *H. tepehuana* sp. nov. versus five in *H. faxoni*); number of setae on the posterior basis of male gnathopod 2 (two in *H. tepehuana* sp. nov. versus four in *H. faxoni*); shape of uropod 3 (styliform in *H. tepehuana* sp. nov. versus globose in *H. faxoni*); and telson shape (longer than wide in *H. tepehuana* sp. nov. versus quadrate and wider than long in *H. faxoni*). Furthermore, in the new species, the fourth

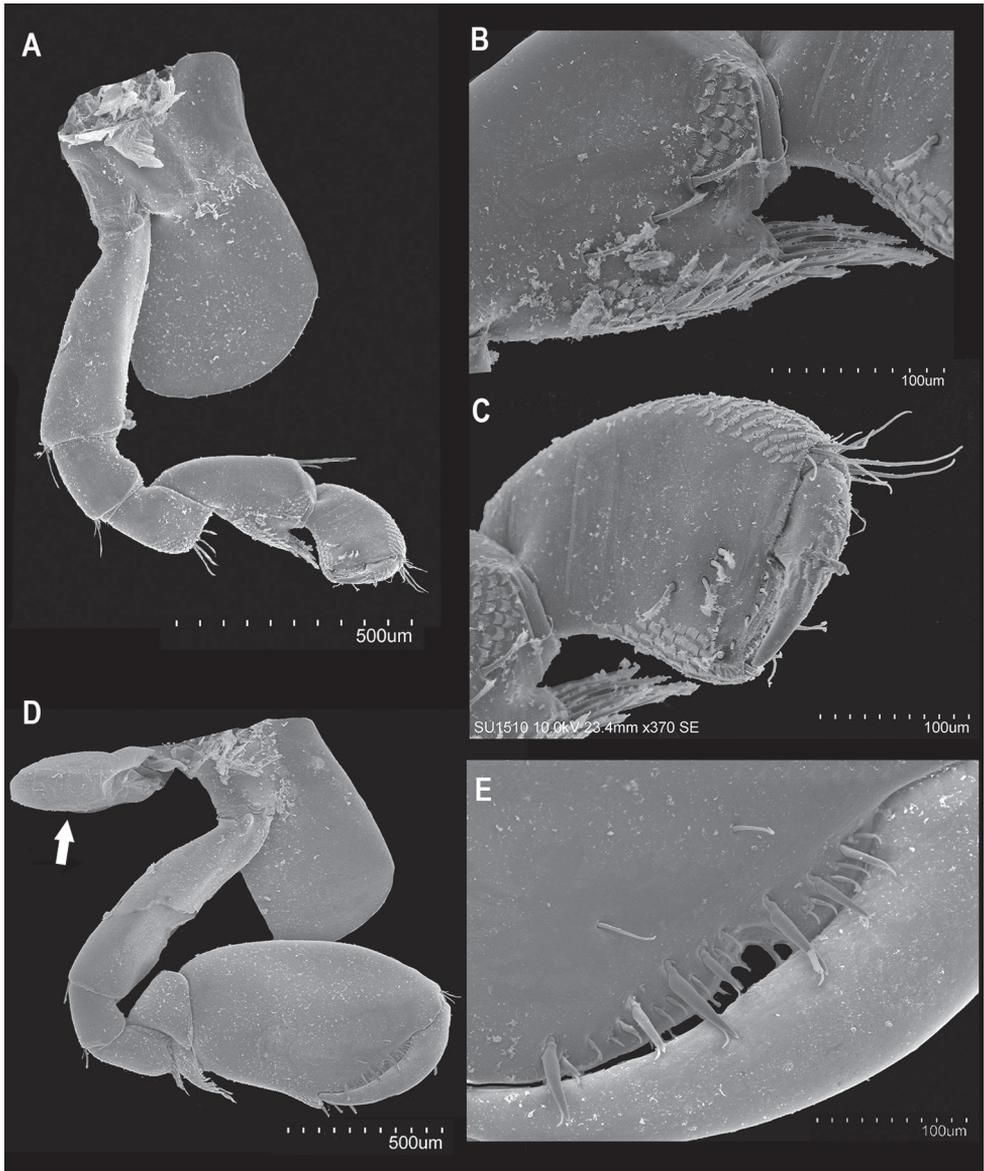


Figure 6. *Hyalella tepehuana* sp. nov. Male gnathopods **A** gnathopod 1 inner face **B** carpus gnathopod 1 inner face **C** propodus and dactyl gnathopod 1 inner face **D** gnathopod 2 inner face (arrow shows coxal gill) **E** palm gnathopod 2 inner face.

article (= dactyl) of maxilliped is more slender than in *H. faxoni*, according to González and Watling (2002b) (Table 1). Unlike *H. faxoni*, *Hyalella tepehuana* sp. nov. and *H. azteca* have a dorsal process on perionites 1 and 2. The differences between these last two species are considerable, based mainly on the morphology of palp of maxilla 1, the number of setae on inner plate of maxilla 1 and maxilla 2, the number of setae on uropods, the shape of the telson and the distance between the distal setae on the telson,

Table 1. Morphological differences of males and females among *Hyaella azteca* (De Saussure, 1858) (based in redescription by Gonzalez and Watling 2002a and the type material), *Hyaella faxoni* Stebbing, 1903 (based in the redescription by Gonzalez and Watling 2002b), and *Hyaella tepehuana* sp. nov.

Character	<i>Hyaella azteca</i> (De Saussure, 1858)	<i>Hyaella faxoni</i> Stebbing, 1903	<i>H. tepehuana</i> sp. nov.
Size (mm)	7.8	8.7	5–6.75
Dorsal process in pereonites 1–2 (mucronations)	yes	no	yes
Maxilla 1, number of pappose setae on the inner plate	3	4	3–4
Maxilla 1, palp apical stout setae	no	yes	yes
Maxilla 2, inner plate pappose setae	2	3	2–3
Mandibles, lacinia mobilis number of teeth	5	5	5–6
Antenna 1, number of flagellum articles	7	12	9–11
Antenna 2, number of flagellum articles	8	15–17	11–12
Uropod 3	styliform	globose	styliform
Male gnathopod 2, hind margin setae	2	4–6	2
Male gnathopod 1, carpus lobe, inner face, number of pappose setae	1–3	1–3	3
Male gnathopod 1, propodus, inner face, number of pappose setae	4	5	3–4
Female gnathopod 1, propodus palm	reverse oblique	slightly reverse oblique	slightly reverse oblique
Uropod 1, outer ramus dorsal setae	2	3	3
Telson	width \approx length, apically pointed with two apposed long simple setae	width > length, quadrate with two short widely apart setae	width < length, apically narrowed (semitriangular) with two long widely apart simple setae

according to the redescription by González and Watling (2002a) (Table 1). These differences seem sufficient to distinguish *Hyaella tepehuana* sp. nov. from *H. azteca* and other species from North America.

Due to the subtle variations within species, and lack of morphological studies and formal descriptions, the identification of species of *Hyaella* in North America is complex. Hence, the new characters proposed by Soucek et al. (2015) to distinguish species are useful: proportion of length of ramus uropod 3 versus the length of stout setae in peduncle, and proportion of gnathopod 2 merus width versus carpus lobe width. The ramus of uropod 3 in *Hyaella tepehuana* sp. nov. is larger than peduncle stout setae. The relative proportions of the merus width and carpus lobe of the gnathopod 2 of *H. tepehuana* sp. nov. are similar as *H. spinicauda* in Michigan and Wisconsin, USA, and some localities in Canada, and different from the proportions found in *H. azteca* (1.5 \times).

Hyaella tepehuana sp. nov. is also similar to the recently described *H. wakulla* Drumm & Knight-Gray, 2019, from Florida, USA. These two species have a similar body length, about 5.5 mm, by which they may be considered to be smaller ecomorphs; however, the main differences between these two species are the number of articles in antennae 1 and 2, and the number of setae on the buccal parts: *Hyaella tepehuana* sp. nov. has more articles in antenna 1 (9–11) and antenna 2 (11–12) while *H. wakulla* has fewer articles in antenna 1 (eight) and antenna 2 (nine). The new species also bears a maximum of four setae on the inner plate of maxilla 1 and bears setules on the palp,

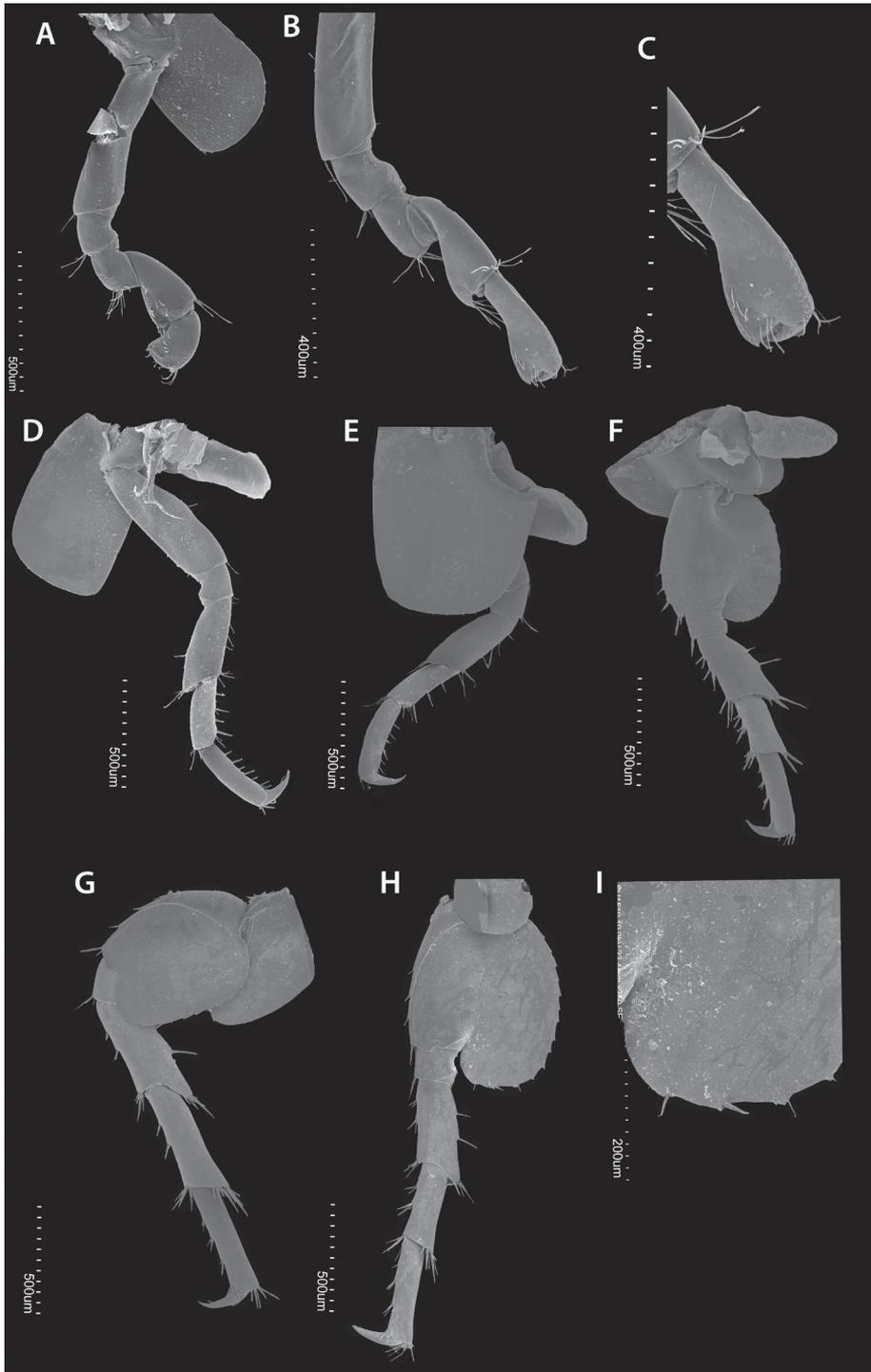


Figure 7. *Hyalella tepahuana* sp. nov. Female gnathopods **A** gnathopod 1 **B** gnathopod 2 **C** propodus gnathopod 2. Male pereiopods **D** pereiopod 3 **E** pereiopod 4 **F** pereiopod 5 **G** pereiopod 6 **H** pereiopod 7 **I** pereiopod 7 basis posterior lobe, distal margin stout setae.

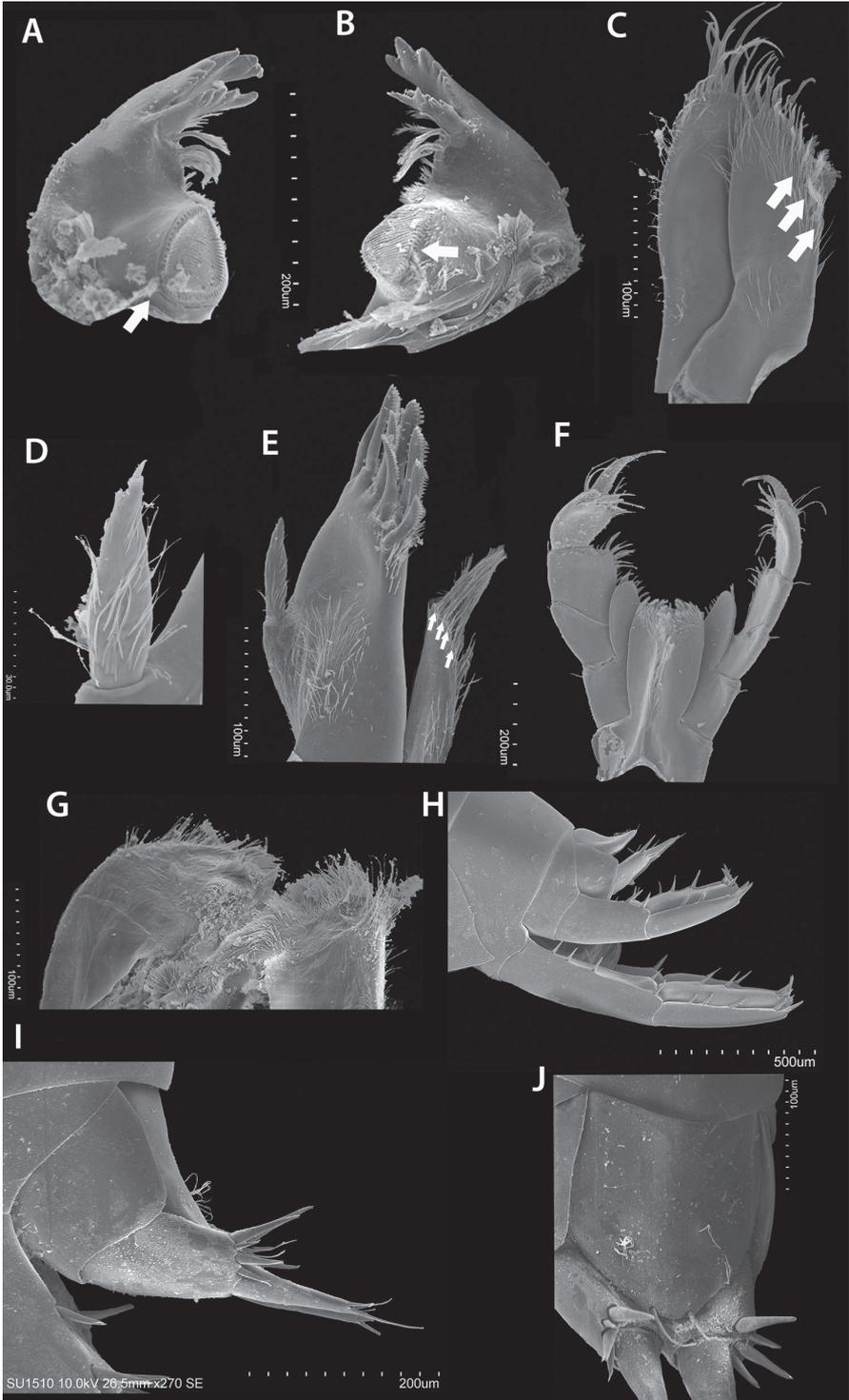


Figure 8. *Hyaella tepehuana* sp. nov., male buccal parts **A** left mandible **B** right mandible **C** maxilla 2 **D** palp maxilla 1 **E** maxilla 1 **F** maxilliped **G** lower lip **H** uropods 1–3 **I** uropod 3 **J** telson. Arrows show setae.

whereas, *H. wakulla* bears three setae on inner plate of maxilla 1, and the palp lacks setules. Also, *H. tepehuana* sp. nov. has a maximum of three pappose setae on the inner plate of maxilla 2, while *H. wakulla* has only two, and the maxilliped fourth article in *H. tepehuana* sp. nov. has fewer than four subterminal setae on the medial margin in contrast to *H. wakulla*, which has a maximum of four subterminal setae in adults. We consider the differences presented here sufficient to consider *Hyaella tepehuana* sp. nov. as a new species.

Key to the species of *Hyaella* (*Hyaella*) in North America, Central America and the Caribbean region*

- 1 Eyes absent 2
- Pigmented eyes present 3
- 2 Antenna 1 is longer than antenna 2; sternal gills on pereonites 3–7; telson with four distal setae *H. muerta*
- Antenna 1 shorter than antenna 2; sternal gills on pereonites 2–7; telson without distal setae *H. cenotensis*
- 3 Body with dorsal mucronations 9
- Body without dorsal mucronations 4
- 4 Ramus of uropod 3 is vestigial or robust, subequal or shorter than peduncle.... 5
- Ramus of uropod 3 slender, subequal or longer than peduncle..... 6
- 5 Ramus of male uropod 3 robust, with seven apical spines *H. sandra*
- Ramus of male uropod 3 vestigial, with two to four spines .. *H. meraspinosa*
- 6 Antenna 1 and 2 are subequal in length (antenna 1 slightly shorter) 7
- Antenna 2 is nearly twice the length of antenna 1 *H. longicornis*
- 7 Hind margin of merus of pereopods 3 and 4 with long setae; telson with two closely apical setae.... *H. caribbeana* (*H. squamosa*, material needs revision and redescription, but the main differences seem to be the length of antennae and the chaetotaxia in the gnathopods (basis, carpus, and propodus))
- Hind margin of article 4 of pereopods 3 and 4 with short setae; telson with two long, broadly-spaced, apical setae 8
- 8 Maxilla 1 inner plate with two setae, pereopod 7 basis lobe ventral margin with three stout setae; pereopod 7 basis anterior margin half distal margin with short stout setae (4); uropod 2 ramus with two dorsal setae; maxilla 2 with serrate setules *H. cheyennis* (*H. inermis*, material needs revision and redescription, but the main difference seems to be the maxilla 2 with serrate setules according to Bueno et al. (2019))
- Maxilla 1 inner plate with more than two setae (4), pereopod 7 basis lobe ventral margin without stout setae; pereopod 7 basis anterior margin half of proximal and distal margin with short stout setae (7); uropod 2 peduncle ramus with three dorsal setae; maxilla 2 probably without serrate setules..... *H. faxoni*

* modified from Baldinger 2004; Marrón-Becerra et al. 2014; Soucek et al. 2015; Marrón-Becerra et al. 2018.

- 9 Inner plate of maxilla 1 narrow, with two to five apical plumose setae..... **10**
- Inner plate of maxilla 1 is broad, subtriangular, with two or three apical plumose setae, followed closely by 22–30 similar medial setae.... *H. montezuma*
- 10 Antenna 1 is longer than half the length of antenna 2, and only first or first two abdominal segments bearing dorsal mucronations..... **11**
- Antenna 1 is less than half the length of antenna 2, with all three abdominal segments bearing dorsal mucronations..... *H. texana*
- 11 Gnathopod 2 of males, carpus posterior lobe is about as long as width of merus; in pereopod 7, the distal/ventral margin of basis posterior lobe, dentate or not, with one or two very small setae if any..... **13**
- Gnathopod 2 of males, carpus posterior lobe approximately 1.5× as long as width of merus, pereopod 7, distal/ventral margin of basis posterior lobe dentate with at least three stout setae **12**
- 12 Pereiopod 3 posterior margin with one setae; pereopod 5 merus and carpus length subequal; pereopod 7 with distal/ventral margin of the basis posterior lobe strongly dentate, with two or more (five) relatively long spines; telson distal margin acute with two apposed setae; female gnathopod 1; carpus inner face with two setae; mandible incisor with six teeth *H. azteca*
- Pereiopod 3 posterior margin with two setae; pereopod 5 merus longer than carpus; pereopod 7 with distal/ventral margin of the basis posterior lobe strongly dentate, and with one or two relatively long spines (fewer than three), distal end of telson narrowing, distal margin rounded or truncated with two widely apart setae; female gnathopod 1, carpus inner face with four setae; mandible incisor with seven teeth..... *H. tepehuana* **sp. nov.**
- 13 Gnathopod 2 propodus in males: palm with a distinct angle step (visible under high power), tip of dactyl approximately aligns vertically with distal end of posterior lobe of carpus; telson distal setae is separated, short, and at least as stout as setae on uropod 3 ramus *H. spinicauda*
- Gnathopod 2 propodus in males: palm without a distinct angle step or notch, tip of dactyl aligning vertically well beyond (posteriorly) distal end of posterior lobe of carpus; telson terminal setae clearly thinner and longer than setae on uropod 3 ramus **14**
- 14 Telson with two long and slender apposed setae; uropod 3 ramus approximately as long as or slightly longer than the longest seta on peduncle; pereopod 7 posterior lobe ventral margin without stout setae; maxilla 1, inner plate with two pappose setae; maxilliped nail short, less than half the length of palp article 4..... *H. wellborni*
- Telson with two long and slender setae widely separated; uropod 3 ramus longer than the longest setae on peduncle; pereopod 7 posterior lobe ventral margin with one stout setae; maxilla 1, inner plate with three pappose setae; maxilliped nail long, more than half length of palp article 4..... *H. maya*

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Checklist of the fresh and brackish water snails (Mollusca, Gastropoda) of Bénin and adjacent West African ecoregions

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Abstract

Currently no comprehensive checklist of fresh and brackish water gastropods from Bénin exists, and those for adjacent West African areas are outdated. Yet, such checklists provide essential biodiversity information and a consistent taxonomic and nomenclatural framework for that biodiversity. Here a first checklist of the fresh and brackish water gastropods from Bénin and adjacent West African ecoregions is presented, based on an extensive literature review and field surveys between September 2014 and June 2019 in six major fresh and brackish water ecosystems in Bénin. This inventory includes information on synonymy, species distribution in West Africa, habitats, and conservation status. The fresh and brackish water gastropod fauna includes 60 species, belonging to 28 genera and 16 families. Pachychilidae, Ampullariidae, Neritidae, and Bulinidae were the most diverse families with 9, 8, 7, and 7 species, respectively. However, literature and field data indicated that 23 species observed in West African basins that extend to Bénin

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do not occur in the territory of Bénin. These species were not detected in our field surveys, most likely because they are rare at collecting sites. Of the 60 species included, five are classified as “Data Deficient”, 43 as “Least Concern”, two as “Nearly Threatened”, one as “Vulnerable”, and six as “Endangered” by the IUCN, whereas the remaining three species were not evaluated. Because the taxonomy of fresh and brackish water gastropods in West Africa is still largely based on morphology, comparative molecular and taxonomic studies may result in substantial revisions of this checklist over the coming years.

Keywords

biodiversity, gastropods, inland water, species inventory, West Africa

Introduction

Mollusca are the second largest animal phylum on Earth, after Arthropoda, and comprise estimated numbers of 50,000–55,000, 25,000–30,000 and 6,000–7,000 of described and valid marine, terrestrial and freshwater species, respectively (Strong et al. 2008; MolluscaBase 2019b). The largest molluscan class, Gastropoda (83% of accepted mollusc species), has repeatedly and successfully colonized continental waters on all continents, except Antarctica (Strong et al. 2008; MolluscaBase 2019b). Despite their economic interest and ecological importance in many aquatic ecosystems (Wanninger and Wollesen 2019), our understanding of their biodiversity is far from complete, especially in developing countries, where expertise, resources and facilities for biodiversity studies are limited (Odountan et al. 2019a). A poor understanding of the biodiversity that underpins ecosystems and their functioning, hampers sustainable management. Indeed, as much legislative work depends on a validated overview of taxonomic biodiversity (Araujo and Jong 2015), biodiversity inventories are essential for the development of monitoring strategies and conservation policies. Moreover, with the growing need to understand natural resources and heritage, biodiversity checklists and databases have become essential tools facilitating communication between taxonomists, naturalist data managers, ecologists, geneticists, museum curators, conservationists, etc. Beyond consolidating taxonomic knowledge, they enable study and management at organismal and ecosystem level, making them essential for national and international conservation (Lydeard et al. 2004; Régnier et al. 2009). As result, there is an increasing demand from policy makers and managers to readily have access to datasets regarding biodiversity (Gofas et al. 2017).

Malacological investigations of fresh and brackish waters are uncommon in West Africa in general and in Bénin in particular. Adanson (1757) and Dautzenberg (1912) investigated the malacological fauna of Senegal and West Africa, respectively, but their works focussed mainly on shells of marine species. The freshwater gastropods of Bénin were studied for the first time by Germain (1917), based on collections by Henry Hubert made around the 1910s. The first identification guide of West African molluscs (from Mauritania to Angola) was published in 1950 (Nicklès 1950), but focused mainly on marine taxa. Towards the end of the 20th century, several malacological stud-

ies have been undertaken on freshwater and brackish taxa in West Africa and in Bénin (Sellin et al. 1980; Danish Bliharziasis Laboratory 1981; Maslin and Bouvet 1986; Zabi and Le Loeuff 1992, 1993; Brown and Kristensen 1993; Le Loeuff and Zabi 1993). These regions were also covered in the first treatise on African freshwater snails (including some considerations on brackish species) on a continental scale (Brown 1980, 1994). These taxonomic papers are now becoming outdated, and in several respects inaccurate. Indeed, since the overviews by Brown (1980, 1994) much taxonomic and faunistic progress has been made (e.g., Jørgensen et al. 2008; Hayes et al. 2015), but the results of these studies have not yet been compiled in an updated overview. Moreover, most research dealing with mollusc diversity in West Africa, including Bénin, focussed on ecology (e.g., Villanueva 2004; Gnohossou 2006; Adandedjan 2012; Odountan 2017; Zinsou 2017; Koudenoukpo 2018) or the transmission of human diseases (e.g., Ibikounlé et al. 2008, 2009, 2013, 2014a, b; Agboho 2018; Onzo-Aboki et al. 2018). As a result, the taxonomic basis of many of these studies was not up to date. This also applies to the List of non-marine molluscs of Benin in Wikipedia (https://en.wikipedia.org/wiki/List_of_non-marine_molluscs_of_Benin), which is incomplete, with outdated nomenclature, it does not include synonyms, and does not provide distributional and conservation information. As such, the Wikipedia list does not inspire much confidence (Kittur et al. 2008). Hence, a new solid and comprehensive synthesis is in order. Therefore, we here provide a comprehensive taxonomic overview of the fresh and brackish water gastropods of Bénin and adjacent West African ecoregions and compile an up to date biodiversity checklist for this fauna. This checklist was developed from literature study and verified through field surveys in six major fresh and brackish ecosystems in Bénin. It provides species synonymy, distribution and habitat data for West Africa, and conservation status. As such we hope that it will act as a reference and research tool for future taxonomic and biomonitoring studies.

Materials and methods

Study region: Bénin and adjacent ecoregions

Bénin is located in West Africa between 6°15' and 12°25'N latitude and between 0°45' and 4°00'E longitude. Its neighbouring countries are Togo in the west, Burkina Faso in the north west, the Republic of Niger in the north (Niger River), and Nigeria in the east. In the south Bénin has a coastline of ~ 125 km along the Atlantic Ocean. Bénin extends from north to the south for ~ 700 km and its width varies between 125 km (along the coast) and 325 km (at the latitude of Tanguiéta). The country has a surface of 112.622 km² (Adam and Boko 1983) and a fairly large network of more or less permanent rivers and standing aquatic ecosystems. Generally, the rivers (e.g., Oueme River, Mono River) are modest in their flow regime and drain into the southern lentic system (e.g., Lake Nokoue, Lake Aheme). This aquatic network is subdivided into four basins, namely the Niger Basin (shared with Mauritania, Guinea, Algeria, Mali, Ivory

Coast, Burkina Faso, Niger, Nigeria, Chad, and Cameroon), the Volta Basin (shared with Mali, Ivory Coast, Burkina Faso, Ghana, and Togo), the Oueme Basin (shared with Togo and Nigeria) and the Mono Basin (shared with Togo). Ecologically these watersheds also contain distinct natural communities, composed of different species with specific ecological dynamics, i.e., they represent distinct freshwater ecoregions (Abell et al. 2008; Graf and Cummings 2011). Sections of the same catchment system are sometimes subdivided into additional ecoregions, and matching freshwater ecoregions that have primarily been established for fish (Abell et al. 2008) with the Transboundary Freshwater dispute Database (<https://tfddmgmt.github.io/tfdd/map.html>). As such, Bénin and its immediate surroundings are covered by ecoregions 505–508 and 513–519 (Fig. 1; Table 1), which form the geographical scope of our study.

Literature study

This checklist is based on a careful literature review to construct an up-to-date biodiversity inventory. These literature sources include peer-reviewed articles, books, reports, manuals, dissertations and other grey literature on the gastropods of Bénin, surrounding countries and their shared drainage basins. Indeed, the development of such a corpus of literature requires the collection of heterogeneous, sometimes contradictory, not to say conflictual, taxonomic opinions across a wide variety of publications.

Field surveys

We supplemented the literature-based biodiversity inventory with field sampling in Bénin. Sampling was conducted in the Sô River, the Oueme River, Lake Nokoue, the Porto-Novo Lagoon, Lake Aheme and the Coastal Lagoon of Ouidah Grand-Popo. In total, 94 field excursions (24, 22, 12, 12, 12, 12, respectively), each of two days per waterbody, were organized between September 2014 and June 2019. Eight to twelve sampling sites were defined within each waterbody to cover a wide range of sub-habitats. These field surveys were performed with an Ekman grab (0.0225 m²) and a

Table 1. Freshwater ecoregions of West Africa investigated and their attributes Ecoregions codes from Abell et al. (2008).

Ecoregions	Covered countries
505: Lower Niger–Benue	Mali, Burkina Faso, Niger, Bénin, Nigeria, Cameroon, Chad
506: Niger Delta	Nigeria,
507: Upper Niger	Guinea, Mali, Ivory Coast, Burkina Faso
508: Inner Niger Delta	Mali, Mauritania
513: Mount Nimba	Guinea, Ivory Coast
514: Eburneo	Ivory Coast, Burkina Faso
515: Ashanti	Ivory Coast, Ghana
516: Volta	Ivory Coast, Mali, Burkina Faso, Ghana, Togo, Bénin
517: Bight Drainage	Ghana, Togo, Bénin, Nigeria,
518: Northern Gulf of Guinea Drainages	Nigeria, Cameroon
519: Western Equatorial Crater Lakes	Cameroon

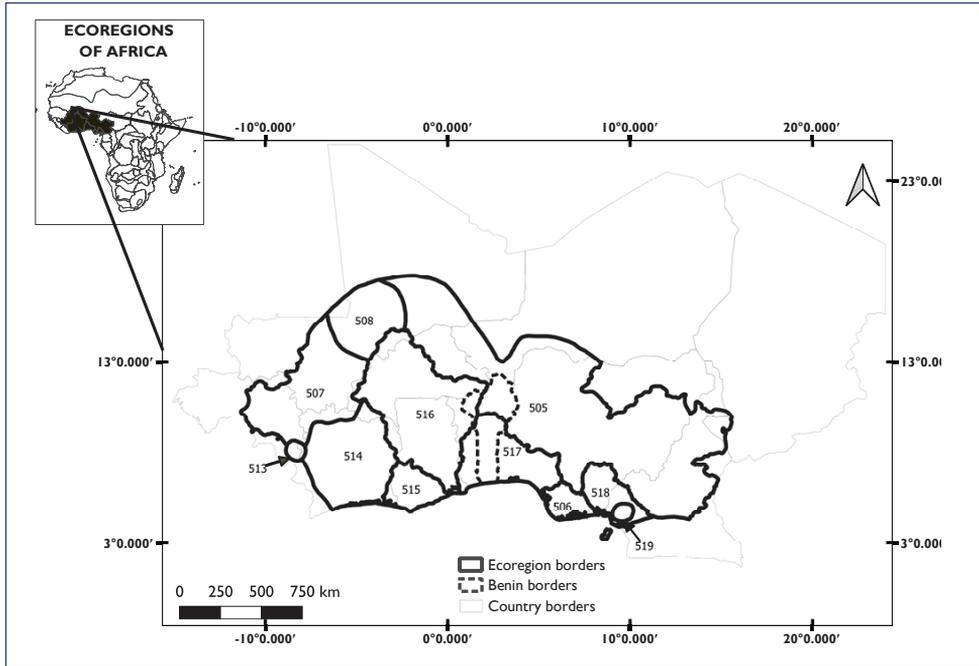


Figure 1. Map of Bénin and surrounding ecoregions covering the major river catchment basins. Ecoregion codes and the countries each ecoregion covers are listed in Table 1.

long-handled kick net (250 μm mesh). Specifically, we inspected the littoral area, the deeper zones, within/under aquatic macrophytes and other environments suitable for molluscs. Snails were put in formalin in prelabelled plastic containers. These containers were subsequently transported to the laboratory, where the snails were washed, and identified using appropriate identification keys (Nicklès 1950; Durand and Lévêque 1981; Brown and Kristensen 1993; Brown 1994) and compared with reference specimens from Dahomey (former name of Bénin) and Bénin (if available) deposited in the collections of the Royal Belgian Institute of Natural Sciences (RBINS) and the Royal Museum for Central Africa (RMCA).

Data compilation

All taxa included in this study were cross-checked for their original name combination, synonymies, type locality data, habitats and dubious records against MolluscaBase (accessed at <http://www.molluscabase.org> during October 2019), and the Worldwide mollusc species Data Base (WMSD accessed at <http://www.bagniliggia.it/> during October 2019) when MolluscaBase did not provide the required data. In addition, the conservation status of each species was determined from the IUCN red list (accessed at <https://www.iucnredlist.org/> during October 2019). The discussion of the occurrence and conservation status of species whose geographical distribution extends beyond the

targeted ecoregions, is mainly limited to the ecoregions covered here. We based our systematic arrangement of subclasses and orders on Bouchet et al. (2017), whereas families, genera and species are listed in alphabetical order.

Results

Our final checklist includes 60 species belonging to 28 genera and 16 families. Information on each of these species is provided below.

Systematic Catalogue

Class GASTROPODA Cuvier, 1795

Subclass Neritimorpha Golikov & Starobogatov, 1975

Order Cycloneritida Frýda, 1998

Family NERITIDAE Rafinesque, 1815

Genus *Clypeolum* Récluz, 1842

Clypeolum owenianum (W. Wood, 1828)

Original combination. *Nerita oweniana* W. Wood, 1828.

Synonyms. *Neritina tiassalensis* Binder, 1956.

Type locality. Africa (Brown 1994).

Habitat. Fresh and Brackish water.

Distribution. Ivory Coast to Cameroon, including Volta River (up to Bator) (Binder 1968; Brown and Kristensen 1993; Le Loeuff and Zabi 1993; Brown 1994; Le Loeuff 1999; Bony 2007; Kouadio et al. 2008, 2011; Edokpayi and Ikhara 2011; Diomandé et al. 2013).

Evidence in Bénin. Along the coast of the Atlantic Ocean (Kristensen and Stensgaard 2010e).

IUCN status. Least Concern.

<https://www.iucnredlist.org/species/40087/10303057>

Remarks. The last whorl encloses earlier whorls almost completely and the lip is commonly expanded in two wing-like projections which appear to be most fully developed in freshwater (Pilsbry and Bequaert 1927). The species is widely distributed and observed beyond our region of interest in countries such as Liberia, the DR Congo and Angola (Brown 1994).

Genus *Nereina* de Cristofori & Jan, 1832

Nereina afra (G. B. Sowerby I, 1836)

Original combination. *Neritina afra* G. B. Sowerby I, 1836.

Synonyms. *Nerita africana* Récluz, 1844; *Neritina aequinoxialis* Morelet, 1848.

Type locality. Fernando Po (= Bioko, Equatorial Guinea).

Habitat. Fresh and Brackish water.

Distribution. From Ivory Coast to Cameroon (Brown and Kristensen 1993; Brown 1994; Bandel and Kowalke 1999; Kouadio et al. 2008, 2011).

Evidence in Bénin. Coastal Lagoon of Bénin (Adandedjan 2012).

IUCN status. Least Concern.

<https://www.iucnredlist.org/species/165778/6119044>

Remarks. Observed in our field data in ecoregion 517.

Genus *Vitta* Adams & Adams, 1854

Vitta adansoniana (Récluz, 1841)

Original combination. *Nerita adansoniana* Récluz, 1841.

Synonyms. *Neritina adansoniana* (Récluz, 1841); *Neritina sangara* Morelet, 1848.

Type locality. Senegal River estuary.

Habitat. Fresh and Brackish water.

Distribution. Ivory Coast to Cameroon (Binder 1968; Brown and Kristensen 1993; Le Loeuff and Zabi 1993; Brown 1994; Guiral et al. 1999; Finlayson et al. 2000).

Evidence in Bénin. Presence uncertain (Kristensen and Stensgaard 2010d) and it was not found in the field surveys.

IUCN status. Least Concern.

<https://www.iucnredlist.org/species/165788/6126163>

Vitta cristata (Morelet, 1864)

Original combination. *Neritina cristata* Morelet, 1864.

Synonyms. -.

Type locality. Como River, Gabon.

Habitat. Fresh and Brackish water.

Distribution. Sierra-Leone, Ivory Coast, Cameroon and Gabon (Binder 1968; Brown and Kristensen 1993; Le Loeuff and Zabi 1993; Brown 1994; Guiral et al. 1999; Le Loeuff 1999; Kouadio et al. 2008, 2011).

Evidence in Bénin. Porto-Novo Lagoon, Coastal lagoon of Ouidah Grand-Popo (Adandedjan 2012; Odountan 2017).

IUCN status. Least Concern.

<https://www.iucnredlist.org/species/14627/4450516>

Remarks. Observed in our field data in ecoregion 517.

Vitta glabrata (G. B. Sowerby II, 1849)

Original combination. *Neritina glabrata* G. B. Sowerby II, 1849.

Synonyms. *Clithon glabrata* (G. B. Sowerby II, 1849); *Clithon glabratum* (G. B. Sowerby II, 1849).

Type locality. Unknown.

Habitat. Fresh and Brackish water.

Distribution. Ivory Coast to Angola (Binder 1968; Sankaré and Etien 1991; Brown and Kristensen 1993; Le Loeuff and Zabi 1993; Guiral et al. 1999; Le Loeuff 1999; Kouadio et al. 2008, 2011; Olomukoro and Azubuike 2009).

Evidence in Bénin. Lake Nokoue, Porto-Novo lagoon, Coastal lagoon (Adandedjan 2012; Odountan 2017; Koudenoukpo 2018).

IUCN status. Least Concern.

<https://www.iucnredlist.org/species/165780/6120407>

Remarks. Observed in our field data in ecoregion 517. Very common in Porto-Novo lagoon.

Vitta kuramoensis (Yoloye & Adegoke, 1977)

Original combination. *Neritina kuramoensis* Yoloye & Adegoke, 1977.

Synonyms. -.

Type locality. Kuramo Water (a branch of Lagos Lagoon), Nigeria.

Habitat. Brackish and marine water.

Distribution. Ivory Coast, Ghana, Bénin, Nigeria and Gabon (Le Loeuff and Zabi 1993; Brown 1994; Guiral et al. 1999; Le Loeuff 1999; Kouadio et al. 2008, 2011; MolluscaBase 2019d).

Evidence in Bénin. Coastal lagoon (Adandedjan 2012).

IUCN status. Not Evaluated.

Remarks. Observed in our field data in ecoregion 517. Sometimes confused with *V. adansoniana* and some specimens identified as *V. adansoniana* may refer to *V. kuramoensis* (Brown 1994).

Vitta rubricata (Morelet, 1858)

Original combination. *Neritina rubricata* Morelet, 1858.

Synonyms. -.

Type locality. Senegambia (= Senegal).

Habitat. Fresh and Brackish water.

Distribution. Ivory Coast to Cameroon and Gabon (Binder 1968; Brown and Kristensen 1993; Le Loeuff and Zabi 1993; Brown 1994; Bandel and Kowalke 1999; Guiral et al. 1999; Le Loeuff 1999; Kouadio et al. 2008).

Evidence in Bénin. Not reported.

IUCN status. Least Concern.

<https://www.iucnredlist.org/species/40090/10304117>

Remarks. According to Brown (1994) syntypes from Morelet's collection (Natural History Museum, London) are labelled from Calabar, Gabon and Congo. The confusion about the type locality and the possible syntypes is well-documented in Breure et al. (2018).

Subclass Caenogastropoda Cox, 1960
 Grade Architaenioglossa Haller, 1890
 Family AMPULLARIIDAE Gray, 1824
 Genus *Afropomus* Pilsbry & Bequaert, 1927

Afropomus balanoideus (Gould, 1850)

Original combination. *Ampullaria balanoidea* Gould, 1850.

Synonyms. *Afropomus balanoidea* (Gould, 1850).

Type locality. Grand Cape Mount, Liberia, Liberia (Cowie 2015).

Habitat. Freshwater.

Distribution. Sierra Leone, Liberia, Ivory Coast, Nigeria (Hubendick 1977; Brown and Kristensen 1993; Brown 1994; Asor et al. 2003; Daget 2003).

Evidence in Bénin. Not reported.

IUCN status. Near Threatened.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165386A6011118.en>

Remarks. Present in neighbouring countries of Bénin (Binder 1963:13), it may occur in Bénin, where its preferred habitats such as ditches, creeks, and small rivers have been surveyed to limited extent only (Brown 1994). As an intermediate host of pulmonary paragonimiasis, the taxon may be of interest to human disease investigators. *Afropomus balanoides* is a misspelling.

Genus *Lanistes* Montfort, 1810

Lanistes chaperi (Kobelt, 1912)

Original combination. *Meladomus libycus chaperi* Kobelt, 1912.

Synonyms. -.

Type locality. Dahomey, Africae occidentalis (=Bénin).

Habitat. Freshwater.

Distribution. Reported from Bénin only (Cowie 2015).

Evidence in Bénin. Original description.

IUCN status. Not Evaluated.

Remarks. Two syntypes of this species exist in the Senckenberg Museum (Frankfurt am Main, Germany): SMF 7451 and 7452. The species has been described as a subspecies of *L. libycus*, and because of that reason it is neither specifically mentioned by Brown and Kristensen (1993), nor by Brown (1994). However, it was considered to be a valid species by Pilsbry and Bequaert (1927), which is maintained by Cowie (2015). Therefore, its ecology should be investigated further.

Lanistes libycus (Morelet, 1848)

Original combination. *Ampullaria libyca* Morelet, 1848.

Synonyms. *Meladomus libycus* (Morelet, 1848); *Meladomus (Lanistes) libycus* var. *albersi* Kobelt, 1912; *Meladomus boettgeri* Kobelt, 1912.

Type locality. Gabon.

Habitat. Freshwater.

Distribution. Coastal countries of West Africa, i.e., Ivory Coast, Ghana, Togo, Bénin, Nigeria, Cameroon, Equatorial Guinea and Gabon (Brown 1994; Diomandé et al. 2009; Jørgensen et al. 2010a; Salawu and Odaibo 2014; Diakitè et al. 2017; Danladi et al. 2019).

Evidence in Bénin. RMCA nos. 37061 and 37066 (Dahomey; ex. coll. Putzeys 1935).

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T175137A7110785.en>

Remarks. This species was not recorded during our sampling in Bénin, although it should occur in the eastern regions of Bénin, around Ketou, Pobè and Sakété. These localities are in close vicinity to Yewa North in Nigeria, where the species is abundant (Salawu and Odaibo 2014).

Lanistes ovum Troschel, 1845

Original combination. *Lanistes (Meladomus) ovum* Troschel, 1845.

Synonyms. *Lanistes (Meladomus) procerus* von Martens, 1866; *Lanistes procerus* von Martens, 1866-; *Lanistes ovum* var. *elator* von Martens, 1866; *Lanistes olivaceus* var. *procerus* von Martens, 1866; *Lanistes ellipticus* var. *luapulensis* Furtado, 1886; *Lanistes affinis* var. *manyarana* Sturany, 1894; *Lanistes ovum* var. *plicosus* von Martens, 1897; *Lanistes ovum* var. *lacoini* Germain, 1907; *Lanistes ovum* var. *major* Germain, 1907; *Lanistes procerus* var. *minor* Germain, 1907; *Lanistes (Meladomus) procerus langi* Pilsbry & Bequaert, 1927; *Lanistes (Meladomus) connollyi* Pain, 1954; *Lanistes (Meladomus) ovum mweruensis* Pain, 1954.

Type locality. Tete, Mozambique, but paralectotypes also come from Sena, Mozambique (Köhler and Glaubrecht 2006).

Habitat. Freshwater.

Distribution. Scattered localities over a large area in Africa including all West African countries (Brown 1994; Albrecht et al. 2018; Ouedraogo et al. 2018).

Evidence in Bénin. Alibori River (Agblonon Houelome et al. 2017).

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165799A6134027.en>

Remarks. Specimens of *L. ovum* have been included in molecular studies (Jørgensen et al. 2008; Schultheiß et al. 2009), which suggested that multiple evolutionary lineages have been lumped into this taxon. Given that the type locality of *L. ovum* is in Mozambique, it is likely that the West African specimens resembling *L. ovum* belong to a distinct taxon. Molecular work is required to resolve the issue.

Lanistes varicus (O. F. Müller, 1774)

Original combination. *Helix varica* O. F. Müller, 1774.

Synonyms. *Ampullaria olivacea* Lamarck, 1816; *Lanistes olivaceus* (Lamarck, 1816); *Ampullaria guinaica* Lamarck, 1822; *Meladomus adansonii* Kobelt, 1911; *Lanistes adansonii* (Kobelt, 1911); *Lanistes millestriatus* Pilsbry & Bequaert, 1927.

Type locality. Unknown.

Habitat. Freshwater (permanent and temporary).

Distribution. Senegal, Gambia, Mali, Ivory Coast, Ghana, Burkina Faso, Niger and Nigeria (Brown 1994).

Evidence in Bénin. Widespread especially at Cotonou garden ASECNA, Toho-Todougba lake, Sèhouè Hlan lake and Acron (Ibikounlé et al. 2009), Cocotomey (Agboho 2018), Oueme River (Zinsou 2017), Sô River (Koudenoukpo 2018), Alibori River (Agblonon Houelome et al. 2017), Porto Novo Lagoon and Coastal lagoon of Ouidah Grand-Popo (Adandedjan 2012).

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T175132A7107425.en>

Remarks. Observed in our field data in ecoregion 517. *Lanistes varicus* is an intermediate host for non-human schistosomes and is often investigated by parasitologists (Ibikounlé et al. 2009). It is an edible species consumed by humans in Bénin (Koudenoukpo 2018). It usually is abundant in natural permanent water bodies. *Lanistes guinaicus* mutation *depressa* Germain, 1917 (513–514) from Région des Tchis, cercle de Mono and Tchaourou (misspelled as Ichaourou)] is an unavailable name because of its infrasubspecific nature (Code, Art. 45.6, Glossary) (Cowie 2015). This taxon seems to be referable to *L. varicus*, but specimens of *L. varicus* from the localities mentioned by Germain (1917) should be further studied to elucidate the status of *L. guinaicus* mutation *depressa*. *L. varicus* as in Adandedjan (2012) is a misspelling.

Genus *Pila* Röding, 1798

Pila africana (von Martens, 1886)

Original combination. *Ampullaria africana* von Martens, 1886.

Synonyms. -

Type locality. Goldküste, Abetifi (= Ghana).

Habitat. Freshwater.

Distribution. Ivory Coast and Ghana (Brown and Kristensen 1993)

Evidence in Bénin. Not reported.

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165375A6007169.en>

Remarks. *Pila africana* is the most common species of *Pila* in countries to the west of Bénin. A lectotype and paralectotypes at the Natural History Museum, Berlin (ZMB) have been assigned by Köhler and Glaubrecht (2006).

Pila ovata (Olivier, 1804)

Original combination. *Ampullaria ovata* Olivier, 1804.

Synonyms. *Lanistes ovatus* (Olivier, 1804); *Ampullaria gradata* Smith, 1881; *Ampullaria erythrostoma* var. *stuhlmanni* von Martens, 1897; *Ampullaria gordonii* var. *bukobae* von Martens, 1897; *Ampullaria ovata* var. *conglobata* von Martens, 1874; *Ampullaria ovata* var. *deckeni* von Martens, 1897; *Ampullaria ovata* var. *emini* von Martens, 1897.

Type locality. Lac Maréotis (Egypt).

Habitat. Freshwater.

Distribution. In West Africa only known from Nigeria and Chad. Common in East Africa from Egypt to northern Mozambique (Brown 1994).

Evidence in Bénin. Not reported.

IUCN status. Least Concern.

<https://www.iucnredlist.org/species/165765/6110071>

Remarks. The Nigerian specimens identified as *Pila ampullacea* (Linnaeus, 1758) by (Gadzama 2012) seem to belong to *Pila ovata* (Olivier, 1804). Molecular work is required to examine whether *P. ovata* indeed has a very wide geographical distribution, or whether it consists of several cryptic species that have been lumped together.

Pila wernei (Philippi, 1851)

Original combination. *Ampullaria wernei* Philippi, 1851.

Synonyms. -.

Type locality. White Nile.

Habitat. Freshwater.

Distribution. In West Africa, present in Mali, Nigeria, Cameroon, Chad and doubtfully in Guinea, Ivory Coast, Burkina Faso, Ghana, Togo, Bénin and Niger (Jørgensen et al. 2010b).

Evidence in Bénin. Presence uncertain (Jørgensen et al. 2010b).

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T175127A7104032.en>

Remarks. This species is common in the Niger River from Mali to Nigeria and therefore could be present in Niger tributaries in Bénin, namely the Mékrou, Alibori and Sota. It seems that reports from coastal environments in West Africa are misidentifications. Köhler and Glaubrecht (2006) designated a lectotype (Museum für Naturkunde, Berlin: ZMB 1335), a paralectotype exists apparently at the Museo Nacional de Historia Natural, Santiago, Chile (MNHNCL). This species has, like *P. ovata*, a wide geographical distribution, which, however, needs to be examined with molecular data.

Family VIVIPARIDAE Gray, 1847

Genus *Bellamyia* Jousseau, 1886

Bellamyia unicolor (Olivier, 1804)

Original combination. *Cyclostoma unicolor* Olivier, 1804.

Synonyms. *Vivipara duponti* De Rochebrune, 1881; *Bellamyia bellamyia* Jousseau, 1886; *Viviparus unicolor* (Olivier, 1904).

Type locality. Alexandria, Egypt.

Habitat. Freshwater.

Distribution. The species is widely distributed in the northern hemisphere part of sub-Saharan Africa, and along the Nile (Brown 1994). In West Africa it occurs in Burkina Faso and Nigeria (Gadzama 2012; Gadzama et al. 2015; Ouedraogo et al. 2015, 2018).

Evidence in Bénin. Not reported.

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T98275044A84313812.en>

Remarks. This species has been considered to be a bucket taxon that requires taxonomic revision (Schultheiß et al. 2014). The type of the genus is *B. bellamyia* Jousseaume, 1886, by original designation, which is considered a synonym of *Vivipara duponti* De Rochebrune, 1881, which represents a West African form of *Bellamyia unicolor* (Olivier, 1804). The type locality of *B. bellamyia* is Kora, Haut-Senegal, and of *V. duponti* the Bakoy River [= Bakoye River] at Pangalla. It is possible that one or both species would prove to be genetically distinct from *B. unicolor* upon molecular examination.

Order Littorinimorpha Golikov & Starobogatov, 1975

Family ASSIMINEIDAE H.Adams & A.Adams, 1856

Genus *Assimineia* Fleming, 1828

Assimineia hessei Boettger, 1887

Original combination. *Assimineia hessei* Boettger, 1887.

Synonyms. -.

Type locality. swamp behind the English trade house at Banana, West Zaire (= Democratic Republic of Congo).

Habitat. Brackish water.

Distribution. Nigeria, DR Congo (Brown 1994).

Evidence in Bénin. Not reported.

IUCN status. Endangered.

<https://www.iucnredlist.org/species/175138/7111055>

Remarks. This salt-tolerant species is associated with mangrove habitats, and easily overlooked because of its small size (length of ~3 mm). As such, it may potentially occur elsewhere, including in mangroves in Bénin.

Family BITHYNIIDAE Gray, 1857

Genus *Gabbiella* Mandahl-Barth, 1968

Gabbiella africana (Frauenfeld, 1862)

Original combination. *Bithynia africana* Frauenfeld, 1862.

Synonyms. *Bithynia tournieri* Binder, 1955.

Type locality. West Africa (without further detail).

Habitat. Fresh and brackish water.

Distribution. Mali, Ivory Coast, Togo, and doubtfully in Ghana (Kristensen and Stensgaard 2010b; Camara et al. 2012; Bony et al. 2013).

Evidence in Bénin. Coastal lagoon of Ouidah Grand-Popo at many sites including Alongo, and Agonnékanmè (Adandedjan 2012).

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165403A6017400.en>

Remarks. Observed in our field data in ecoregion 517. Previous records of this species were all in freshwater habitats, whereas the specimens reported in Bénin occurred in brackish water. Brown (1994) suggested that *Bithynia tournieri* Binder, 1955 may be conspecific, which is followed here, but in the absence of molecular studies the systematics of *Gabbiella* are very poorly known. The contrast in habitat between previously recorded specimens and those from Bénin could be indicative for specific differences, but until compelling evidence indicates otherwise, we consider the Bénin specimens conspecific.

***Gabbiella tchadiensis* Mandahl-Barth, 1968**

Original combination. *Gabbiella tchadiensis* Mandahl-Barth, 1968.

Synonyms. -.

Type locality. South East shore at Bol in Lake Chad, Chad.

Habitat. Freshwater.

Distribution. Tchad and Nigeria (Brown 1994).

Evidence in Bénin. Not reported.

IUCN status. Endangered.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165387A6011471.en>

Remarks. This species occurs in the catchment of Lake Chad, including the Komadugu Yobe River. This catchment falls beyond the ecoregions under study here, but the taxon is considered to have had a more extensive Late Pleistocene-Holocene distribution in the Chad Basin (Van Damme 1984). Additionally, recent specimens have been reported also from Lake Léré on the border between Cameroon and Chad (Brown 1994), which is part of the Niger Basin and the reason for inclusion here.

Family HYDROBIIDAE Stimpson, 1865

Genus *Hydrobia* Hartmann, 1821

***Hydrobia accrensis* Connolly, 1929**

Original combination. *Hydrobia accrensis* Connolly, 1929.

Synonyms. -.

Type locality. Quarry near Accra, Ghana.

Habitat. Freshwater.

Distribution. Ghana and Togo (Kristensen and Stensgaard 2010c).

Evidence in Bénin. Not reported.

IUCN status. Near Threatened.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165400A6016291.en>

Remarks. As for Bithyniidae, the lack of knowledge on the anatomy of hydrobiid species combined with a lack of molecular studies currently hampers confident systematic placement of African Hydrobiidae (see e.g., Seddon et al. 2011).

Hydrobia guyenoti Binder, 1955

Original combination. *Hydrobia guyenoti* Binder, 1955.

Synonyms. -.

Type locality. Toupah Bay in Lagune Ebrié, Ivory Coast.

Habitat. Fresh and brackish water.

Distribution. Ivory Coast in Lagune Ebrié (Brown 1994).

Evidence in Bénin. Coastal lagoon (Adandedjan 2012).

IUCN status. Endangered.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165381A6009233.en>

Remarks. This species endemic to West Africa is not mentioned by MolluscaBase but is included in WMSDB and regional reports (Smith et al. 2009; Adandedjan 2012). It may be more widespread than previously assumed. It is one the smallest species of the genus: 2.7×1.8 mm. The whorls are strongly convex with a deep suture. The central radular tooth has a single basal denticle on each side and long lateral lobes (Brown 1994).

Hydrobia lineata Jekelius, 1944

Original combination. *Hydrobia lineata* Jekelius, 1944.

Synonyms. -.

Type locality. Bingerville Bay, in fresh water, Ivory Coast.

Habitat. Freshwater.

Distribution. Ivory Coast, Togo and Bénin (Brown 1994).

Evidence in Bénin. Reported in Lac Toho Todougba (Brown 1994).

IUCN status. Data Deficient.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165380A6008870.en>

Remarks. Observed in our field data in ecoregion 517. Only one specimen was observed and that was empty shells, not a living specimen. The species is known from fossils only according to MolluscaBase (2019c), but some authors reported extant specimens (Smith et al. 2009; Badahoui et al. 2010). The species requires taxonomical study (Seddon et al. 2011).

Family LITTORINIDAE Children, 1834

Genus *Littoraria* Gray, 1833

Littoraria angulifera (Lamarck, 1822)

Original combination. *Phasianella angulifera* Lamarck, 1822.

Synonyms. *Littorina angulifera* (Lamarck, 1822).

Type locality. Unknown.

Habitat. Brackish and marine and water.

Distribution. Senegal, Sierra Leone, Liberia, Ghana and Nigeria (Rosewater 1981).

Evidence in Bénin. Cotonou (Rosewater 1981).

IUCN status. Not Evaluated.

Remarks. Observed in our field data in ecoregion 517. Specimens from mangroves of the Coastal lagoon of Ouidah, Grand-Popo that have been assigned to *Littoraria scabra* (Linnaeus, 1758) by Adandedjan et al. (2012) seem to be referable to *Littoraria angulifera* (Lamarck, 1822). *Littoraria scabra* is very polymorphic, but endemic to the Indo-West Pacific region (Reid et al. 2010).

Subcohort Cerithiimorpha Golikov & Starobogatov, 1975

Remarks. A temporary order named Caenogastropoda has been established (MolluscaBase 2019a) to group a number of superfamilies that belong to the Subclass Caenogastropoda but not to the Order Littorinimorpha. This group has previously been referred to as the subcohort Cerithiimorpha (Bouchet et al. 2017), which is followed here. We do not use the temporary Order [unassigned] Caenogastropoda to avoid confusion with the Subclass Caenogastropoda.

Family PACHYCHILIDAE Fischer & Crosse, 1892

Genus *Potadoma* Swainson, 1840

Potadoma angulata Thiele, 1928

Original combination. *Potadoma angulata* Thiele, 1928.

Synonyms. -

Type locality. Samanga (known as Sanaga River), Cameroon.

Habitat. Freshwater.

Distribution. Currently, this species has only been recorded from Cameroon (Brown 1994).

Evidence in Bénin. Not reported.

IUCN status. Endangered.

<https://www.iucnredlist.org/species/184556/8292306>

Remarks. This endemic species of Cameroon is known only from the southernmost parts of ecoregion 505.

Potadoma bicarinata Mandahl-Barth, 1967

Original combination. *Potadoma bicarinata* Mandahl-Barth, 1967.

Synonyms. -

Type locality. Volta River at Asikoko village near Frankadua, Ghana.

Habitat. Freshwater.

Distribution. Currently, this species has only been recorded from Ghana (Mandahl-Barth 1967).

Evidence in Bénin. Unconfirmed, may be reported by Adandedjan (2012) under *Potadoma* sp.

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165383A6009945.en>

Remarks. The distribution of *Potadoma* is disjunct, most taxa occur in West Africa, whereas some others in Central Africa (Brown 1994). The phylogenetic affinities of taxa from both regions are currently unknown. Especially the West African taxa, several of which occur in the ecoregions under study here, display high morphological disparity. Many of the endemic *Potadoma* species from Cameroon fall just beyond the boundaries of the ecoregions considered here, such as *P. zenkeri* (von Martens, 1901). Late Cenozoic fossils suggest that the genus was more widespread before, including in the Albertine Rift (e.g., Van Damme and Pickford 2003; Salzburger et al. 2014), the Turkana Basin (Van Bocxlaer et al. 2008) and even in Botswana (Riedel et al. 2009).

Potadoma freethi (Gray, 1831)

Original combination. *Melania freethi* Gray, 1831.

Synonyms. *Melania foenaria* Reeve, 1860; *Melania guineensis* Reeve, 1860; *Potadoma freethi dykei* Spence, 1925; *Melania nigrita* Morelet, 1851; *Melania nigritina* Morelet, 1848; *Potadoma freethii guineensis* Pilsbry & Bequaert, 1927.

Type locality. Fernando Po (= Bioko, Equatorial Guinea).

Habitat. Freshwater.

Distribution. From Ivory Coast to Nigeria (Brown 1994; Owojori et al. 2006; Kouadio et al. 2008).

Evidence in Bénin. Reported by Kristensen and Stensgaard (2010e).

IUCN status. Least Concern.

<https://www.iucnredlist.org/species/175120/7099504>

Remarks. *P. freethi* is the type species of the genus, by original designation (Gray 1847). Observed in our field data in ecoregion 517. Several subspecies, such as *P. f. dykei* Spence, 1925 and *P. f. guineensis* Reeve, 1860 have been described and these are included here. Two other subspecies have been described from Central Africa (DR Congo), i.e., *P. f. tigrina* Connolly, 1938 and *P. f. graptoconus* Pilsbry & Bequaert, 1927, but it seems doubtful these would belong to *P. freethi* given the disjunct distribution of the genus *Potadoma*. *Melania conulus* Lea & Lea, 1851, is another species described from Fernando Po of which the original description is similar to *P. freethi*, but more research is required before we can confirm it to be a synonym. Therefore, *M. conulus* is considered to be a “taxon inquirendum” (MolluscaBase 2019a: taxon 1115355).

Potadoma liberiensis (Schepman, 1888)

Original combination. *Melania liberiensis* Schepman, 1888.

Synonyms. *Melania sancti-pauli* Schepman, 1888; *Potadoma bequaerti* Binder, 1963.

Type locality. St Paul's River near Bavia, Liberia.

Habitat. Freshwater.

Distribution. Guinea, Liberia and Ivory Coast (Mandahl-Barth 1967; Diomandé et al. 2009).

Evidence in Bénin. Perhaps included in *Potadoma* sp. reported by Adandedjan (2012).

IUCN status. Data Deficient.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165385A6010770.en>

Remarks. The synonyms concern variants in which spiral ridges are developed to variable extent.

Potadoma moerchi (Reeve, 1859)

Original combination. *Melania moerchi* Reeve, 1859.

Synonyms. -.

Type locality. 'Guinea danica' according to Brot (1874), confirmed as Ghana by Pilsbry and Bequaert 1927.

Habitat. Freshwater.

Distribution. Ghana, Togo, Bénin and South-West Nigeria (Mandahl-Barth 1967; Salawu and Odaibo 2014).

Evidence in Bénin. Reported by Brown (1994).

IUCN status. Least concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165382A6009591.en>

Remarks. Observed in our field data in ecoregion 517.

Potadoma nyongensis Spence, 1928

Original combination. *Potadoma nyongensis* Spence, 1928.

Synonyms. -.

Type locality. Nyong River at 3°35'N, 10°10'E, Cameroon.

Habitat. Freshwater.

Distribution. Currently, the species is only recorded from its type locality and the Man River at Sakbayeme Cameroon (Brown 1994).

Evidence in Bénin. Not reported.

IUCN status. Endangered.

<https://www.iucnredlist.org/species/184695/8315726>

Remarks. *Potadoma nyongoensis*, as in MolluscaBase, is probably a misspelling. The type locality of this endemic species of Cameroon falls beyond the boundaries of the ecoregions considered here, but Man River at Sakbayeme is part of our study area.

Potadoma togoensis Thiele, 1928

Original combination. *Potadoma togoensis* Thiele, 1928.

Synonyms. -.

Type locality. White Volta River at Apaso, Ghana.

Habitat. Freshwater.

Distribution. Ghana and Togo (Brown 1994).

Evidence in Bénin. Perhaps included in *Potadoma* sp. reported by Adandedjan (2012).

IUCN status. Data Deficient.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165394A6014033.en>

Remarks. Observed in our field data in ecoregion 517.

Potadoma trochiformis (Clench, 1929)

Original combination. *Goodrichia trochiformis* Clench, 1929.

Synonyms. -.

Type locality. Man River near Sakbayeme (NE of Edea), Cameroon.

Habitat. Freshwater.

Distribution. Currently, the species is reported only from its type locality (Brown 1994).

Evidence in Bénin. Not reported.

IUCN status. Endangered.

<https://www.iucnredlist.org/species/184704/8318057>

Remarks. Possibly synonymous with *P. nyongensis* (see Mandahl-Barth 1967; Brown 1994).

Potadoma vogeli Binder, 1955

Original combination. *Potadoma vogeli* Binder, 1955.

Synonyms. -.

Type locality. Agnéby (river or stream) at Abgenville, Ivory Coast.

Habitat. Freshwater.

Distribution. Ivory Coast (Brown 1994).

Evidence in Bénin. Not reported.

IUCN status. Vulnerable.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165393A6013707.en>

Family PALUDOMIDAE Stoliczka, 1868

Genus *Cleopatra* Troschel, 1856

Cleopatra bulimoides (Olivier, 1804)

Original combination. *Cyclostoma bulimoides* Olivier, 1804.

Synonyms. *Paludina senegalensis* Morelet, 1860; *Cleopatra pirothi* Jickeli, 1881; *Cleopatra bulimoides* var. *richardi* Germain, 1911; *Cleopatra bulimoides* var. *welwitschi* von Martens, 1897.

Type locality. Kalidje Canal near Alexandria, Egypt.

Habitat. Freshwater.

Distribution. In West Africa this species occurs in Senegal, Guinea, Mali, Ivory Coast, Burkina Faso, Ghana, Togo, Bénin, Niger, Nigeria, and Chad (Brown 1994; Kristensen and Stensgaard 2010a), it also occurs in Northeast Africa, including the northern part of the East African Rift.

Evidence in Bénin. Observed during our field data in ecoregion 517.

IUCN status. Least Concern.

<https://www.iucnredlist.org/species/175131/7106773>

Remarks. Observed in our field data in ecoregion 517. *Cleopatra bulimoides* is conchologically a highly polytypic species, with many nominal species in possible synonymy, such as *C. cyclostomoides* (Küster, 1852) and *C. congener* Preston, 1913. The species boundaries of *C. bulimoides* need to be explored by molecular methods.

Genus *Pseudocleopatra* Thiele, 1928

Pseudocleopatra togoensis Thiele, 1928

Original combination. *Pseudocleopatra togoensis* Thiele, 1928.

Synonyms. -.

Type locality. Volta River near Apaso, Ghana (in Togo according to Thiele, but apparently in SE Ghana near Akwamu).

Habitat. Freshwater.

Distribution. Ghana (Brown 1994).

Evidence in Bénin. Not reported.

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165404A6017727.en>

Remarks. *Pseudocleopatra togoensis* is the type species of the genus.

Pseudocleopatra voltana Mandahl-Barth, 1973

Original combination. *Pseudocleopatra voltana* Mandahl-Barth, 1973.

Synonyms. -.

Type locality. Volta River at Daboya, Ghana.

Habitat. Freshwater.

Distribution. Ghana (Brown 1994).

Evidence in Bénin. Not reported.

IUCN status. Data Deficient.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165376A6007457.en>

Family POTAMIDIDAE H.Adams & A.Adams, 1854

Genus *Tympanotonos* Schumacher, 1817

Tympanotonos fuscatus (Linnaeus, 1758)

Original combination. *Murex fuscatus* Linnaeus, 1758.

Synonyms. *Murex radula* Linnaeus, 1758; *Murex fuscatus radula* Linnaeus, 1758 ; *Nerita aculeata* O. F. Müller, 1774; *Tympanotonos radula* (Linnaeus, 1758); *Murex terbelli* Gmelin, 1791; *Potamides granulatus* (Lamarck, 1816).

Type locality. ‘M. Mediterraneo’, an incorrect reference to the Mediterranean Sea (Brown 1994).

Habitat. Brackish water.

Distribution. Senegal to Angola (Brown and Kristensen 1993; Brown 1994)

Evidence in Bénin. Sô River, Coastal lagoon of Ouidah Grand-Popo, Lake Aheme, Lake Nokoue, Porto-Novo Lagoon (Gnohossou 2006; Adandedjan 2012; Odountan 2017; Koudenoukpo 2018).

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165803A6137267.en>

Remarks. Observed in our field data in ecoregion 517. *Tympanotonus* and *Tympanotomus* are very common misspellings and ill-founded emendations of the genus name *Tympanotonos* (Pilsbry & Bequaert, 1927).

Family THIARIDAE Gill, 1871 (1823)

Genus *Melanoïdes* Olivier, 1804

Melanoïdes manguensis (Thiele, 1928)

Original combination. *Melania manguensis* Thiele, 1928.

Synonyms. -.

Type locality. Oti River at Mangu, East Ghana (located by Thiele in Togo).

Habitat. Freshwater.

Distribution. Ghana and Ivory Coast (Brown 1994)

Evidence in Bénin. Not reported.

IUCN status. Data Deficient.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165379A6008515.en>

Remarks. Presence of this species in Togo is uncertain (Brown 1994).

Melanoïdes tuberculata (O. F. Müller, 1774)

Original combination. *Nerita tuberculata* O. F. Müller, 1774.

Synonyms. *Melania* (*Melanoïdes*) *tuberculata* (O. F. Müller, 1774); *Melania tuberculata* (O. F. Müller, 1774); *Melanoïdes tuberculata tuberculata* (O. F. Müller, 1774); *Melanoïdes* (*Melanoïdes*) *tuberculata* (O. F. Müller, 1774); *Melanoïdes* (*Melanoïdes*) *tuberculata tuberculata* (O. F. Müller, 1774); *Melanoïdes tuberculatus* (O. F. Müller, 1774); *Striatella tuberculata* (O. F. Müller, 1774); *Melanoïdes fasciolata* Olivier, 1804; *Turritella tuberculata* Link, 1807; *Turritella turricula* Link, 1807; *Melania cancellata* Say, 1829; *Melania mauriciae* Lesson, 1831; *Melania terebra* Lesson, 1831; *Melania trunculata* Lamarck, 1822; *Melania virgulata* Quoy & Gaimard, 1834; *Melania ornata* von dem Busch, 1842; *Melania flammigera* Dunker, 1844; *Melania rivularis* Philippi, 1847; *Melania suturalis* Philippi, 1847; *Melania rustica* Mousson, 1857; *Melania commersoni*

Morelet, 1860; *Melania inhambanica* von Martens, 1860; *Melania zengana* Morelet, 1860; *Melania dominula* Tapparone Canefri, 1883; *Melania flyensis* Tapparone Canefri, 1883; *Melania pellicens* Tapparone Canefri, 1883; *Melania singularis* Tapparone Canefri, 1883; *Melania baldwini* Ancey, 1899; *Thiara baldwini* (Ancey, 1899); *Melania tuberculata* var. *victoriae* Dautzenberg, 1908; *Melania carica* Oppenheim, 1919; *Melania dadiana* Oppenheim, 1919; *Melanoides (Melanoides) carica* (Oppenheim, 1919); *Melanoides (Melanoides) dadiana* (Oppenheim, 1919); *Melanoides tuberculata dadiana* (Oppenheim, 1919); *Melanoides tuberculata* var. *dautzenbergi* Pilsbry & Bequaert, 1927.

Type locality. Coromandel coast, India.

Habitat. Freshwater.

Distribution. Widespread in West Africa including Ivory Coast, Burkina Faso, Ghana, Niger, Bénin and Nigeria (Brown 1994; Gadzama 2012; Gadzama et al. 2015; Diakité et al. 2017).

Evidence in Bénin. Widespread in Bénin at freshwater sites including in Coastal lagoon of Ouidah Grand-Popo around Aho Channel (Adandedjan 2012), Nokoue lake and Porto-Novo Lagoon around Totchè Channel (Gnohossou 2006; Adandedjan 2012; Odountan et al. 2019b), Acron and Djidja (Agboho 2018), Pehunco town (Ibikounlé et al. 2014b), Alibori River (Agblonon Houelome et al. 2017), Oueme River (Zinsou 2017), Lake Ahémé around Tohonou (Odountan 2017).

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T155675A120117210.en>

Remarks. Observed in our field data in ecoregion 517. The taxonomic status of the parthenogenetic *M. tuberculata* is problematic, first because it contains African and Oriental strains, and the species has invaded many tropical freshwater habitats around the globe. Native and invasive strains both occur in West Africa (Van Bocxlaer et al. 2015).

Melanoides voltae (Thiele, 1928)

Original combination. *Melania voltae* Thiele, 1928.

Synonyms. -.

Type locality. Volta River at Apaso, Ghana.

Habitat. Freshwater.

Distribution. Ghana and Nigeria (Brown 1994; Mafiana and Beyioku 1998).

Evidence in Bénin. Not reported.

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165406A6018396.en>

Genus *Pachymelania* E.A. Smith, 1893

Pachymelania aurita (O. F. Müller, 1774)

Original combination. *Nerita aurita* O. F. Müller, 1774

Synonyms. *Strombus tympanorum africanus* Chemnitz, 1786; *Melania zonata* Philippi, 1848; *Melania balteata* Philippi, 1851; *Melania aurita* Reeve, 1860;

Melania histrionica Reeve, 1860; *Io rota* Reeve, 1860; *Melania subaurita* Brot, 1868; *Melania soriculata* Morelet, 1864; *Claviger auritus* Brot, 1874; *Clavigerina aurita* von Martens, 1903.

Type locality. Unknown.

Habitat. Brackish water.

Distribution. Senegal to Angola including Ivory Coast, Togo, and Nigeria (Binder 1968; Brown 1994; Imoobe 2008; Tampo 2015).

Evidence in Bénin. At coastal area of the Coastal lagoon of Ouidah Grand-Popo (Adandedjan 2012); Oueme River (Zinsou 2017); Sô River (Koudenoukpo 2018); Lake Nokoue and Lake Aheme (Odountan 2017).

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165769A6112482.en>

Remarks. Observed in our field data in ecoregion 517. The species is morphologically variable with respect to the number of spiral cords, threads and tubercles. A molecular systematic study of *Pachymelania* is required to assess species boundaries and morphological variation.

Pachymelania byronensis (W. Wood, 1828)

Original combination. *Strombus byronensis* W. Wood, 1828.

Synonyms. *Melania owenii* Gray, 1831; *Melania tuberculosa* Rang, 1832; *Melania rangii* Deshayes, 1838; *Pachymelania bryoni* Smith, 1893.

Type locality. Coast of Upper Guinea.

Habitat. Freshwater.

Distribution. Ivory Coast to Nigeria (Brown and Kristensen 1993; Brown 1994).

Evidence in Bénin. Coastal lagoon of Ouidah Grand-Popo (Adandedjan 2012), Oueme River (Zinsou 2017), Sô River (Koudenoukpo 2018), Lake Nokoue and Lake Aheme (Odountan 2017).

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T175140A7112397.en>

Remarks. Observed in our field data in ecoregion 517.

Pachymelania fusca (Gmelin, 1791)

Original combination. *Murex fuscus* Gmelin, 1791.

Synonyms. *Murex fuscatus* Maton, 1804; *Pirena granulosa* Lamarck, 1822; *Melania quadriseriata* Gray, 1831; *Melania matoni* Gray 1831; *Melania mutans* Gould, 1843; *Melania tessellata* Lea, 1850; *Melania fuscaia* Hanley, 1854–1858; *Melania fusca* Reeve, 1860; *Melania loricata* Reeve, 1860; *Melania matoni* var. *loricata* Boettger, 1885; *Melania quadriseriata* var. *carinata* Brot, 1868; *Claviger matoni* Brot, 1874; *Clavigerina fusca quadriseriata* von Martens, 1903.

Type locality. Unknown.

Habitat. Fresh and brackish water.

Distribution. Senegal to Angola (Brown and Kristensen 1993; Brown 1994).

Evidence in Bénin. Mainly at sites close to the Atlantic Ocean in Lake Nokoue and Lake Aheme (Oudountan 2017), Coastal lagoon of Ouidah Grand-Popo (Adandedjan 2012), Oueme River (Zinsou 2017), Sò River (Koudenoukpo 2018).

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165779A6119724.en>

Remarks. Observed in our field data in ecoregion 517. Like *P. aurita* this species has a very variable morphology.

Subclass Heterobranchia Burmeister, 1837

Order Ellobiida Van Mol, 1867 [see Bouchet et al. (2017) for this emendation]

Family ELLOBIIDAE L. Pfeiffer, 1854(1822)

Remarks. The family name was first introduced in synonymy, but is now available under art. 11.6 with the authorship determined by art. 50.7 (see Bouchet et al. 2017)

Genus *Melampus* Monfort, 1810

Melampus liberianus H. Adams & A. Adams, 1854

Original combination. *Melampus liberianus* H. Adams & A. Adams, 1854.

Synonyms. *Melampus obovatus* H. Adams & A. Adams, 1854.

Type locality. Liberia.

Habitat. Brackish (mangrove) and marine water.

Distribution. River estuaries from Liberia to DR Congo, including in Ghana, Cameroon and São Thomé (Pilsbry and Bequaert 1927; Brown 1994).

Evidence in Bénin. Not reported.

IUCN status. Least Concern.

<https://www.iucnredlist.org/species/175139/7111601>

Remarks. *Melampus obovatus* represents a subadult stage of *M. liberianus* (Dohrn 1878).

Superorder Hygrophila Férussac, 1822

Remarks. Hygrophila was originally spelled as “hygrophiles” (vernacular), subsequently latinized by Herrmannsen (1847 [in 1846–1852]: 547) and established as a suborder. Later, it was treated by Thiele (1926 [in 1925–1926]: 136) as a “Sippe” [= superfamily] but it is now considered a- Superorder (see Bouchet et al. 2017)

Family BULINIDAE Fischer & Crosse, 1880

Genus *Bulinus* O. F. Müller, 1781

Bulinus globosus (Morelet, 1866)

Original combination. *Physa globosa* Morelet, 1866.

Synonyms. *Bulinus (Physopsis) globosus* (Morelet, 1866); *Isidora (Physopsis) globosa* (Morelet, 1866); *Physa masakaensis* Preston, 1913; *Physopsis choziensis* Preston, 1913.

Type locality. Dande River (Luanda Province), Angola.

Habitat. Freshwater.

Distribution. Widespread in West Africa including Mali, Ivory Coast, Burkina Faso, Ghana, Togo, Bénin, Niger, Nigeria, Cameroon, Chad, Equatorial Guinea and Gabon (Ntonifor and Ajayi 2007; Okafor and Ngang 2008; Salawu and Odaibo 2014; Diakité et al. 2017; Abe et al. 2018; Ouedraogo et al. 2018)

Evidence in Bénin. Widespread, especially at Djèffa and Ganhatin (Assogba and Youssao 2002); Acron, Cotonou garden ASECNA, Djidja, Nikki, Péhunco and Pèrèrè towns, Sô Ava, Pahou, Sand quarries, and Sô Tchanhoué (Ibikounlé et al. 2009, 2013, 2014a; Agboho 2018); Alibori River (Agblonon Houelome et al. 2017); Sô River (Koudenoukpo 2018).

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T99504682A120114163.en>

Remarks. Observed in our field data in ecoregion 517. *Bulinus globosus* and *Bulinus* spp. in general are important intermediate hosts for trematode parasites. Especially parasites of the genus *Schistosoma* cause debilitating tropical diseases in humans and livestock. *Bulinus globosus* is part of the *B. africanus* species complex (Jørgensen et al. 2011). Beyond the recognition of several species complexes, our general understanding of taxonomic diversity and species relationships within *Bulinus* is still limited (see Jelnes 1986), especially within the *B. truncatus/tropicus* complex where several polyploidisation events have taken place (Jørgensen et al. 2011). *Bulinus globosus* is diploid ($2n = 36$) (see Jelnes 1986).

***Bulinus forskalii* (Ehrenberg, 1831)**

Original combination. *Isidora forskalii* Ehrenberg, 1831.

Synonyms. *Bulinus (Pyrgophysa) forskalii* (Ehrenberg, 1831); *Bulinus (Pyrgophysa) mariei* (Crosse, 1879); *Physa apiculata* Morelet, 1867; *Physa capillacea* Morelet, 1867; *Physa clavulata* Morelet, 1867; *Physa gradata* Melvill & Ponsonby, 1898; *Physa simplicata* Morelet, 1867; *Physa turriculata* Morelet, 1867; *Physa wahlbergi* Krauss, 1848; *Pyrgophysa mariei* Crosse, 1879.

Type locality. Damietta, Egypt.

Habitat. Freshwater.

Distribution. Widespread in West Africa including Mali, Ivory Coast, Burkina Faso, Ghana, Togo, Bénin, Niger, Nigeria, Cameroon, Chad, Equatorial Guinea and Gabon (Ntonifor and Ajayi 2007; Okafor and Ngang 2008; Salawu and Odaibo 2014; Diakité et al. 2017; Abe et al. 2018; Ouedraogo et al. 2018).

Evidence in Bénin. Widespread especially at Djèffa and Ganhatin (Assogba and Youssao 2002); Cotonou garden ASECNA, Nikki, Péhunco and Pèrèrè towns, Sô Ava, Pahou's sand quarries, Cocotomey, Djidja, and Sô Tchanhoué (Ibikounlé et al. 2009, 2013, 2014a; Agboho 2018), Alibori River (Agblonon Houelome et al. 2017), Sô River (Koudenoukpo 2018).

IUCN status. Least Concern.

<https://www.iucnredlist.org/species/165794/6130451>

Remarks. Observed in our field data in ecoregion 517. *Bulinus forskali* as in Ag-boho (2018), is a misspelling. The *B. forskalii* species complex appears to be the most deeply split *Bulinus* species complex (Jørgensen et al. 2011). Species of this complex have a much higher spire than species of other *Bulinus* complexes (Brown 1994). Further investigation did not clarify the issue: study of its shell morphology suggests *B. jousseaumei* to be distinct (Kristensen and Christensen 1991), whereas enzyme analyses support synonymization (Jelnes 1986).

***Bulinus jousseaumei* (Dautzenberg, 1890)**

Original combination. *Isidora jousseaumei* Dautzenberg, 1890.

Synonyms. -.

Type locality. Senegal River near Medine, Mali.

Habitat. Freshwater.

Distribution. Widespread in West Africa including Mali, Burkina Faso, Togo, Niger, and Nigeria (Brown 1994, Salawu and Odaibo 2014).

Evidence in Bénin. Reported (Salawu and Odaibo 2014)

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165388A6011857.en>

Remarks. Two specimens observed in our field data in ecoregion 517 seem to be referable to *B. jousseaumei* (Dautzenberg, 1890). The species is not native in Bénin (Salawu and Odaibo 2012), but seems to be introduced. *Bulinus jousseaumei* belongs to the *B. africana* species complex, and is either a distinct species (Mandahl-Barth 1965) or a form of *B. globosus* (Wright 1961).

***Bulinus senegalensis* O. F. Müller, 1781**

Original combination. *Bulinus senegalensis* O. F. Müller, 1781.

Synonyms. -.

Type locality. Podor, Senegal.

Habitat. Freshwater.

Distribution. Mainly Sahelian, from Guinea through the middle Niger Basin to Nigeria (Brown 1994).

Evidence in Bénin. Not reported.

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165398A6015514.en>

Remarks. Being first introduced as ‘*Le Bulin*’ by Adanson (1757), *Bulinus senegalensis* is the type species of the genus. The species belongs to the *B. forskalii* species complex (Brown 1994), is diploid ($2n = 36$) and enzyme analyses indicated that it is distinct from *B. forskalii* (Jelnes 1986). The species occurs mainly in seasonal rain pools and aestivates during drought (Brown 1994). It was not observed in our survey

of perennial waterbodies in Bénin. The species is an important host of *Schistosoma haematobium* in various regions of West Africa.

***Bulinus truncatus* (Audouin, 1827)**

Original combination. *Physa truncata* Audouin, 1827.

Synonyms. *Bulinus (Bulinus) truncatus* (Audouin, 1827); *Bulinus (Bulinus) truncatus truncatus* (Audouin, 1827); *Bulinus (Isidora) truncatus* (Audouin, 1827); *Bulinus (Isidora) truncatus truncatus* (Audouin, 1827); *Physa rohlfsi* Clessin, 1886; *Bulinus (Bulinus) truncatus rohlfsi* (Clessin, 1886); *Bulinus rohlfsi* (Clessin, 1886); *Physa mutandaensis* Preston, 1913.

Type locality. Egypt.

Habitat. Freshwater.

Distribution. Widespread in West Africa including Mali, Ivory Coast, Burkina Faso, Ghana, Togo, Bénin, Niger, Nigeria, Cameroon, Chad, Equatorial Guinea, and Gabon (Ntonifor and Ajayi 2007; Okafor and Ngang 2008; Salawu and Odaibo 2014; Diakit   et al. 2017; Abe et al. 2018; Ouedraogo et al. 2018).

Evidence in B  nin. Widespread especially at Dj  ffa and Ganhatin (Assogba and Youssao 2002), Accron, Cotonou garden ASECNA, Djidja, Nikki, P  hunco and P  r  r   towns, S   Ava, Pahou, Sand quarries, and S   Tchanhou   (Ibikounl   et al. 2009, 2013, 2014a; Agboho 2018), Alibori River (Agblonon Houelome et al. 2017), S   River (Koudenoukpo 2018).

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T99507883A120114540.en>

Remarks. Observed in our field data in ecoregion 517. *Bulinus truncatus* is tetraploid ($2n = 72$) (Jelnes 1986) and morphologically very variable (Brown 1994). Detailed studies with high-throughput sequencing are required to address many of the outstanding questions related to the biology of this species, for example, on heterozygosity and potential interspecies molecular variation across the wide geographic range of *B. truncatus*. Species such as *B. guernei* (Dautzenberg, 1890), *B. contortus* Michaud, 1829, *B. coulboisi* (Bourguignat, 1888), *B. mutandaensis* (Preston, 1913), and *B. sericinus* (Jickeli, 1874) are regularly considered to be synonyms of *B. truncatus* (Brown, 1994).

***Bulinus umbilicatus* Mandahl-Barth, 1973**

Original combination. *Bulinus umbilicatus* Mandahl-Barth, 1973.

Synonyms. -.

Type locality. Zalingei in Darfur Province, West Sudan.

Habitat. Freshwater.

Distribution. Widespread in West Africa mainly in Mali, Niger, Nigeria, and Chad (Brown 1994).

Evidence in B  nin. Not reported.

IUCN status. Least Concern.

<https://www.iucnredlist.org/species/175134/7109049>

Remarks. *Bulinus umbilicatus* is diploid ($2n = 36$), belongs to the *B. africanus* species complex (Jelnes 1986; Brown 1994), and displays intergradation with *B. globosus* both at the level of allozymes and shell morphology (Jelnes 1986; Kristensen and Christensen 1991). Like *B. senegalensis*, the taxon frequently occurs in seasonal aquatic habitats and aestivates during dry periods (Brown 1994).

Genus *Indoplanorbis* Annandale & Prashad, 1921

Indoplanorbis exustus (Deshayes, 1833)

Original combination. *Planorbis exustus* Deshayes, 1833.

Synonyms. *Planorbis indicus* Benson, 1836; *Planorbis coromandelicus* Dunker, 1856; *Planorbis zebrinus* Dunker, 1856; *Planorbis hindu* Clessin, 1886; *Planorbis indicus* var. *zonatus* Clessin, 1886.

Type locality. marshes on the coast of Malabar, South West India.

Habitat. Freshwater.

Distribution. Ivory Coast and Nigeria (Brown 1994; Koné et al. 2013).

Evidence in Bénin. Freshwater habitats of Parakou city, Pahou, Sand quarry, Acron, Djassin, Djéffa, Tchivié, Cotonou, ASECNA garden, and Sô Ava (Ibikounlé et al. 2009, 2013; Agboho 2018).

IUCN status. Least Concern.

<https://www.iucnredlist.org/species/165594/17211568>

Remarks. Observed in our field data in ecoregion 517. Some authors have erroneously used “1834” as the year of publication. The species is native to Asia, and has been introduced into West Africa by man (Kristensen and Ogunnowof 1987). Originally, these introductions were to Ivory Coast and Nigeria, but our data suggest that the taxon is spreading in West Africa. Although *Indoplanorbis* hosts *Schistosoma* species that parasitise domestic livestock in Asia, no evidence exists to our knowledge that it transmits schistosomes in Africa.

Family LYMNAEIDAE Rafinesque, 1815

Genus *Radix* Montfort, 1810

Radix natalensis (Krauss, 1848)

Original combination. *Limnaeus natalensis* Krauss, 1848.

Synonyms. *Limnaea (Radix) natalensis* Krauss, 1848; *Limnaea natalensis* Krauss, 1848; *Radix (Exsertiana) natalensis* (Krauss, 1848); *Radix hovarum* (Tristram, 1863); *Limnaea hovarum* Tristram, 1863; *Limnaeus natalensis* var. *exsertus* von Martens, 1866; *Limnaea orophila* Morelet, 1867; *Limnaea electa* Smith, 1882; *Limnaea caillaudi* Bourguignat, 1883; *Limnaea acroxa* Bourguignat, 1883; *Limnaea caillaudi* (Bourguignat, 1883); *Limnaea gravieri* Bourguignat, 1885; *Limnaea nyansae* von Martens, 1892; *Limnaea arabica* Smith, 1894; *Limnaea arabica* Smith, 1894; *Limnaea arabica* Smith,

1894; *Limnaea elmeteitensis*. Smith, 1894; *Limnaea humerosa* von Martens, 1897; *Limnaea undussumae* von Martens, 1897; *Limnaeus dakaensis* Sturany, 1898.

Type locality. Natal, South Africa.

Habitat. Freshwater.

Distribution. Widespread in West Africa including Senegal, Burkina Faso, Ivory Coast, Nigeria (Gadzama 2012; Koné et al. 2013; Salawu and Odaibo 2014; Diakité et al. 2017).

Evidence in Bénin. Djèffa and Ganhatin (Assogba and Youssao 2002), Acron, Baaka, Cotonou ASECNA garden, Cotonou beach temporary ponds, Lake Nokoue, Lake Toho-Todougba, and Sèhouè bridge (Ibikounlé et al. 2009; Agboho 2018), Nikki, Pehunco and Pèrèrè towns (Ibikounlé et al. 2014a, b)

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T165761A120112796.en>.

Remarks. Observed in our field data in ecoregion 517. Some authors have erroneously used “1948” (Agboho 2018) as year of the publication of the name. The species occurs throughout most of Africa, including Madagascar, several islands in the Indian Ocean and Arabia. It usually lives in permanent waters and it is rare in seasonal habitats, unless they are directly connected to permanent waters. Molecular work is required to examine whether *R. natalensis* indeed has a very wide geographical distribution or whether it consists of several cryptic species that have been lumped together.

Family PLANORBIDAE Rafinesque, 1815

Genus *Biomphalaria* Preston, 1910

Biomphalaria pfeifferi (Krauss, 1848)

Original combination. *Planorbis pfeifferi* Krauss, 1848.

Synonyms. *Planorbis (Coretus) pfeifferi* Krauss, 1848; *Planorbis (Planorbula) pfeifferi* Krauss, 1848; *Biomphalaria madagascariensis* (Smith, 1882); *Planorbis hildebrandti* von Martens, 1882; *Planorbis madagascariensis* Smith, 1882; *Planorbis bowkeri* Melvill & Ponsonby, 1893; *Planorbis nairobiensis* Dautzenberg, 1908; *Planorbis hermanni* Boettger, 1910.

Type locality. Natal in Umgeni Valley, South Africa.

Habitat. Freshwater.

Distribution. Widespread especially in Ivory Coast, Burkina Faso Niger, Nigeria (Okafor and Ngang 2008; Salawu and Odaibo 2014; Diakité et al. 2017; Abe et al. 2018; Ouedraogo et al. 2018).

Evidence in Bénin. Widespread especially at Djèffa and Ganhatin (Assogba and Youssao 2002), Toho Todougba Lake, Kpinnou Lake, Sonon, Nikki, Pehunco and Pèrèrè towns, Sô Ava (Ibikounlé et al. 2009, 2013, 2014a; Agboho 2018).

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2015.RLTS.T165782A85689765.en>

Remarks. Observed in our field data in ecoregion 517. Enzyme studies on populations of *B. pfeifferi* from Cameroon and Senegal have found consistent biological dif-

ferences (Mimpfoundi and Greer 1990), suggesting that multiple West African species of *Biomphalaria* may have been lumped in *B. pfeifferi*.

***Biomphalaria camerunensis* (Boettger, 1941)**

Original combination. *Australorbis camerunensis* Boettger, 1941.

Synonyms. *Biomphalaria alexandrina wansoni* Mandahl-Barth, 1957.

Type locality. Mongongo, NW of Mount Cameroon, Cameroon.

Habitat. Freshwater.

Distribution. From Ghana eastwards to Central African Republic (Brown 1994).

Evidence in Bénin. Not reported.

IUCN status. Least Concern.

<https://www.iucnredlist.org/species/175130/7105918>

Remarks. In Cameroon *B. camerunensis* is confined to the southern equatorial climatic zone (Greer et al. 1990), and it was never found in the same site as *B. pfeifferi* (Brown, 1994).

Genus *Gyraulus* Charpentier, 1837

***Gyraulus costulatus* (Krauss, 1848)**

Original combination. *Planorbis costulatus* Krauss, 1848.

Synonyms. *Planorbis (Gyraulus) costulatus* Krauss, 1848; *Caillaudia angulata* Bourguignat, 1883.

Type locality. Natal in Umgeni Valley, South Africa.

Habitat. Freshwater.

Distribution. From Senegal to Angola including Ivory Coast, Bénin and Nigeria (Brown 1994; Salawu and Odaibo 2014).

Evidence in Bénin. Alibori River (Agblon Houelome et al. 2017).

IUCN status. Least Concern.

<https://www.iucnredlist.org/species/165767/6111409>

Remarks. Observed in our field data in ecoregion 517. The taxonomy of African *Gyraulus* is poorly known, but Meier-Brook (1983) found the African species *G. costulatus* and *G. connollyi* to have distinct anatomical characteristics that warranted him to place them in the subgenus *Caillaudia* Bourguignat, 1883. So far, this alternate representation has not been formally accepted.

Genus *Hovorbis* Brown & Mandahl-Barth, 1973

The genus was formerly known as *Afrogyrus* Brown and Mandahl-Barth, 1973, which however is an invalid junior homonym of the coleopteran genus *Afrogyrus* Brinck, 1955. Özdikmen and Darilmaz (2007) altered the name to *Africanogyrus*, however, the available name *Hovorbis* Brown and Mandahl-Barth, 1973 has priority.

***Hovorbis coretus* (de Blainville, 1826)**

Original combination. *Planorbis coretus* de Blainville, 1826.

Synonyms. *Planorbis coretus* de Blainville, 1826; *Africanogyrys coretus* (de Blainville, 1826); *Afrogyrys coretus* (de Blainville, 1826); *Planorbis misellus* Morelet, 1867; *Planorbis* (*Spiralina*) *anderssoni* Ancey, 1890; *Planorbis anderssoni* Ancey, 1890.

Type locality. Podor, Senegal.

Habitat. Freshwater.

Distribution. Bénin, Burkina Faso, Cameroon, Chad, Ivory Coast, Equatorial Guinea, Ghana, Guinea, Guinea-Bissau, Niger, Nigeria, Sierra Leone, Togo. (see www.iucnredlist.org/species/165775/120113348).

Evidence in Bénin. Not reported.

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T165775A120113348.en>

Remarks. Two specimens observed in our field data in ecoregion 517 seem to be referable to *H. coretus*. This species was first introduced as ‘*Le Coret*’ by Adanson (1757). Several potential synonyms are mentioned in Brown (1994), but more study of these taxa is required to verify their status.

Genus *Segmentorbis* Mandahl-Barth, 1954***Segmentorbis angustus* (Jickeli, 1874)**

Original combination. *Segmentina angusta* Jickeli, 1874.

Synonyms. *Planorbis* (*Segmentina*) *emicans* Melvill & Ponsonby, 1892; *Segmentina* (*Hippeutis*) *emicans* (Melvill & Ponsonby, 1892); *Segmentina kempfi* Preston, 1912.

Type locality. Toquor River at Mekerka (west of Asmara) in Hamasen Province, Ethiopia.

Habitat. Freshwater.

Distribution. Ivory Coast (Diakité et al. 2017); Nigeria and Cameroon (Kristensen and Stensgaard 2010; Salawu and Odaibo 2012; Salawu and Odaibo 2014).

Evidence in Bénin. Not reported.

IUCN status. Least Concern.

<https://www.iucnredlist.org/species/165771/6114438>

Remarks. *Segmentorbis angustus* is the type species of the genus. The small body size of *Segmentorbis* species (<6 mm) implies that it may be sometimes be overlooked in freshwater snail surveys. *Segmentorbis angustus* occurs in permanent waterbodies, often within the vegetation.

***Segmentorbis kanisaensis* (Preston, 1914)**

Original combination. *Segmentina kanisaensis* Preston, 1914.

Synonyms. *Segmentorbis formosa* Connolly, 1928.

Type locality. Nile at Kanisa, South Sudan.

Habitat. Freshwater.

Distribution. Widely distributed in West Africa from Gambia to Chad (Albrecht et al. 2008)

Evidence in Bénin. Not Reported.

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T165763A6107847.en>

Remarks. This species can be readily distinguished from *S. angustus* by its depressed shell with strongly carinated periphery. It is sometimes found together with *S. angustus*, but also occurs in temporary waters (Brown 1994).

Family PHYSIDAE Fitzinger, 1833

Genus *Afrophysa* Starobogatov, 1967

Afrophysa brasiliensis (Küster, 1844)

Original combination. *Physa brasiliensis* Küster, 1844.

Synonyms. *Physa mosambiquensis* Clessin, 1886; *Physa (Aplecta) waterloti* Germain, 1911; *Aplexa waterloti* Brown, 1994.

Type locality. “Brasil” but Taylor (2003) restricted it to Porto Alegre, Rio Grande do Sul.

Habitat. Freshwater.

Distribution. Ghana, Togo and Nigeria (MolluscaBase 2018).

Evidence in Bénin. Porto-Novo (Germain 1911), Sô River (Odountan 2017; Koudenoukpo 2018).

IUCN status. Least Concern (evaluated under *Aplexa waterloti*).

<https://www.iucnredlist.org/species/165396/6014756>

Remarks. Observed in our field data in ecoregion 517. *Physa (Aplecta) waterloti* Germain, 1911 was established by Taylor (2003) as junior synonym of *Afrophysa brasiliensis* based on type specimens (from Bénin) which were morphologically degraded and very bad. Molecular work on specimens from the type locality in Brazil and West Africa is required to resolve relationships within *Afrophysa*.

Genus *Physella* Haldeman, 1842

Physella acuta (Draparnaud, 1805)

Original combination. *Physa acuta* Draparnaud, 1805.

Synonyms. *Haitia acuta* (Draparnaud, 1805); *Lymnaea heterostropha* Say, 1817; *Physa fontana* Haldeman, 1841; *Physa inflata* Lea, 1841; *Physa charpentieri* Küster, 1850; *Physa heterostropha nigricans* var. *callosa* Rigacci, 1866; *Physa heterostropha* var. *gibbosa* Rigacci, 1866; *Physa heterostropha* var. *minor* Rigacci, 1866; *Physa lata* Tryon, 1865; *Physa plicata* De Kay, 1843; *Physa philippii* Küster, 1844; *Physa primeana* Tryon, 1865; *Physa say de Blainville*, 1826; *Physa striata* Menke, 1828; *Physa tenuissima* Lea, 1864.

Type locality. River Garonne, France.

Habitat. Freshwater.

Distribution. Widely distributed in West Africa from Senegal to Angola (Van Damme et al. 2017)

Evidence in Bénin. Acron, Cocotomey, Djèffa, and Djidja (Agboho 2018).

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T155538A91354457.en>

Remarks. Observed in our field data in ecoregion 517. The taxon is native to America and has been introduced in many other regions around the world including Europe, Asia and Africa. The African *Physella* fauna likely consists of a composite from multiple introductions. Nominal species such as *Physa borbonica* Férussac, 1827, *P. cubensis* Pfeiffer, 1839, *P. canariensis* Bourguignat, 1856, *P. tenerifae* Mousson, 1872, *P. mamoi* Benoit, 1875, and *Aplecta orbigny* Mazé, 1883, considered as synonyms of *P. acuta* (e.g Brown 1994), are not mentioned in MolluscaBase.

Genus *Stenophysa* von Martens, 1898

Stenophysa marmorata (Guilding, 1828)

Original combination. *Physa marmorata* Guilding, 1828.

Synonyms. *Limnea (Physa) rivalis* Sowerby, 1822; *Aplexa marmorata* (Guilding, 1828); *Physa acuminata* Villa & Villa, 1841; *Aplecta sowerbyana* d'Orbigny, 1841.

Type locality. St. Vincent, Lesser Antilles.

Habitat. Freshwater.

Distribution. Ivory Coast (Bony et al. 2008), Nigeria (Oloyede et al. 2017).

Evidence in Bénin. Djèffa and Ganhatin (Assogba and Youssao 2002), Sô Ava (Ibikounlé et al. 2013).

IUCN status. Least Concern.

<http://dx.doi.org/10.2305/IUCN.UK.2011-2.RLTS.T189786A8768994.en>

Remarks. Observed in our field data in ecoregion 517.

Discussion

This study provides the first checklist of fresh and brackish water gastropods in Bénin and adjacent ecoregions, i.e., ecoregions 505–508 and 513–519 of Abell et al. (2008). It comprises a total of 60 species, classified in 28 genera. More specifically, Architaenioglossa, Cerithiimorpha, Cycloneritida, Ellobiida, Hygrophila, and Littorinimorpha comprise 9, 19, 7, 1, 17, and 7 species, respectively. From the 16 families listed, Pachychilidae, Ampullariidae, Neritidae, Bulinidae, and Thiaridae were the most diverse with 9, 8, 7, 7, and 6 species, respectively. Of the 60 species listed, 37 are recorded (sometimes uncertain) in Bénin (~ 62 %), indicating a considerable species richness. The high richness in Pachychilidae relates to the diversity within the genus *Potadoma*, whereas the high richness in Ampullariidae relates to the diversity within the genera

Pila and *Lanistes* throughout (sub-)tropical Africa (Cowie 2015). However, almost half of the Pachychilidae and one third of the ampullariids that are recorded in this study have not been recorded directly from Bénin. The fact that only a small part of Bénin's aquatic environments, especially around the Niger River, the largest river in West Africa, have been sampled might explain why some species that are broadly distributed in West Africa such as *Pila wernei* (endemic to the Niger River basin) and *Bulinus senegalensis* O. F. Müller, 1781 have not been detected in our sampling.

Our findings with literature-based data also provoked some taxonomic concerns, because several papers on the fresh and brackish water malacofauna of Bénin or West-Africa, contained several (nomenclatural) errors. A case in point is the erroneous listing of *Codakia orbicularis* (Linnaeus, 1758), *Cardita calyculata* (Linnaeus, 1758), *Thais coronata califera* (Lamarck, 1822), *Thais nodosa* (Linnaeus, 1758), *Turritella* Lamarck, 1799, *Polinices* Montfort, 1810, *Patella* Linnaeus, 1758 as non-marine gastropod species in Bénin (e.g., Adandedjan 2012). These taxa were excluded in this study. In addition, we were unable to find information on *Melanooides anomala* (Smith, 1877) reported from Bénin (Adandedjan et al. 2012), and this taxon was consequently omitted. This identification seems to refer to *Melanooides anomala* (Dautzenberg & Germain, 1914), which has its type locality in the DR Congo and is endemic to the Congo Basin (Brown 1994). The difference in authorship and the report of the species outside its known region is suspect and calls for verification. Similarly, records that cannot be checked, e.g., *Lanistes ovum* (Agblonon Houelome et al. 2017) because specimens have not been illustrated and nor deposited in publicly accessible institutions, should be treated with caution. Hence, until compelling evidence indicates otherwise, we regard such doubtful species records in the literature as misidentifications.

Although only four species are threatened (Endangered/Vulnerable), a significant number of species has been assessed as Data Deficient, Not assessed or Not applicable. One of the main reasons for Data Deficiency in molluscs is taxonomic uncertainty and poor geographic knowledge (Seddon et al. 2011). Moreover, in West Africa, there are only few, reliable, recent survey data available, so that more species were marked as Data Deficient (Seddon et al. 2011). Therefore, a large field inventory is required that should focus on diverse habitats of fresh and brackish water from North to South with the possibility of molecular analyses. Moreover, species such as *Afrophysa brasiliensis* (Küster, 1844), *Lanistes guinaicus* mutation *depressa* Germain, 1917, *Lanistes chaperi* (Kobelt, 1912), *Lanistes ovum* Troschel, 1845, *Physa* (*Aplecta*) *waterloti* Germain, 1911, *Pila ovata* (Olivier, 1804), and *Radix natalensis* (Krauss, 1848) need further taxonomic study.

Bénin and its transboundary basins present a diversified fresh and brackish water gastropod fauna. The current checklist contains information on 60 species. However, many of these species require more detailed taxonomic and phylogenetic scrutiny, our current knowledge remains in its infancy. This checklist is hence an updated baseline for further taxonomic and ecological studies of the fresh and brackish water gastropods of Bénin and adjacent West African ecoregions.

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Variability of the key features and revision of a group of closely related species of grassflies (Diptera, Chloropidae, *Meromyza*)

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Abstract

The following external morphological features of members of the genus *Meromyza* Mg. have been analyzed: the ratio of the height of frontal triangle to the length of the head; the presence of black setae on the lower surface of genae; the degree of manifestation of ocellus spot; the ratio of the length of mesonotum to scutellum; the length and color of the mid strip of the mesonotum and its degree of manifestation on the scutellum; and the thickness of hind femurs. Additionally, the size and shape of anterior and posterior processes of the postgonite, and the type of attachment of the posterior process have been investigated. The authors have determined the range of variability of key features applicable to the reliable identification of closely related species of grass flies in the “meigeni” species group of the genus *Meromyza*, as well as the usefulness of CO1 mtDNA sequences for this purpose. The authors propose to re-instate the name *M. laeta* Meigen, 1838 (instead of using the name *M. meigeni* Nartshuk, 2006), to include seven species into the cluster “meigeni”, and to substitute the name of the cluster “meigeni” with the name “variegata”.

Keywords

CO1 mtDNA, meigeni group, morphological features, postgonite, variegata group

Introduction

The high variability of key external morphological features of *Meromyza* flies (Diptera, Chloropidae, *Meromyza* Meigen, 1830) has been noted by many authors (Nartshuk and Fedoseeva 2010). High variability in the color of palpi and mid stripe of the mesonotum, and shape of ocellus and occipital spots, etc. make it difficult to precisely identify specimens. Before features of the genital apparatus became key to the identification of *Meromyza* flies, only six species of this genus had been described, and species synonymy was highly disputed (Fedoseeva 1960). To date, *Meromyza* includes more than 90 species, and the identification key is based on a combination of external morphological features, and specific features of male genital apparatus (Nartshuk and Fedoseeva 2010). Recently, it was shown that the precision of species identification can be verified using molecular-genetic analysis of CO1 mtDNA gene (Safonkin et al. 2016). The results of genetic analysis combined with the shape of the anterior process of the postgonite made it possible to divide the genus into eight clusters, which were named after the species closest to the hypothetical ancestor (Safonkin et al. 2016). The flies of the “meigeni” cluster are populous throughout Europe. Excessive variability of external features in females of the “meigeni” cluster makes species identification based on female features very difficult. Despite the similarity of male anterior and posterior processes of the postgonite, the degree of their variability and its usefulness for species identification has not been previously studied. Upon our findings, the larvae of *Meromyza variegata* Meigen, 1830 from this cluster damage oat shoots, which necessitates the correct identification of adults.

The aim of this study was to determine the variability of key features of “meigeni” cluster flies, to select features unique for valid identification of the species, and to re-examine the naming of this cluster.

Material and methods

Collection sites of the material. *Meromyza* species were acquired from the collection of the Severtsov Institute of Ecology and Evolution (Moscow, Russia), and comprised material collected in different years in the Moscow, Tver and Tula regions of the Russian Federation, as well as the Brest region of the Republic of Belarus. In 2018, we also collected flies from the Czech Republic in the Pilsen Region (June 20, 49°75'82"N, 13°15'61"E), Jihlava (June 23, 49°39'66"N, 15°59'96"E), Brno (June 24, 49°23'01"N, 16°53'33"E) and Olomouc (June 25, 49°63'40"N, 17°34'35"E). We used *M. bohémica* Fed. as a model for the analysis of population variability of morphological features in the “meigeni” cluster.

External key features. Based on our own and published data, we analyzed face profile, the shape of the 3rd antennal segment, the length of the head, the color of arista and palpi, the height and width of the frontal triangle and their ratio, the ratio of the length of the head to the height of frontal triangle, the ratio of the genae height to the height of the 3rd antennal segment, the wrinkledness of the apex of frontal triangle, the shape and size of the ocellus spot, the presence of black setae on the lower surface

of genae, the parafacial angle; the pattern of the occiput; the length of mesonotum and scutellum and their ratio, the length of the mid stripe of mesonotum, the color and length of mesonotal stripes, the presence of a stripe on scutellum, the color of the abdomen, the thickness of hind femurs, and body length.

Postgonites. The shape of the postgonite was studied using images acquired with a CamScan MV 2300 scanning electron microscope (Czech Republic). Measurements of the lateral surface of the postgonites for 80 *Meromyza* specimens were carried out using images acquired by a Keyence VHV-1000 light microscope (Japan), with an integrated data analysis program and with standard settings used throughout the study. We investigated a shape and an area of the anterior process of the postgonite, and the type of attachment of the posterior process. Measurements were recorded in micrometers. To prepare the samples, we extracted the postgonites from the abdomen of flies and affixed them onto paper.

We performed molecular-genetic analysis based on the nucleotide sequences of CO1 mtDNA locus previously obtained and deposited by us in GenBank (Safonkin et al. 2016). We deposited new nucleotide sequences of CO1 mtDNA from *Meromyza bohemica* Fedoseeva, 1962, *M. femorata* Macquart, 1835 and *M. rufa* Fedoseeva, 1962 in GenBank with accession numbers MN 037808–MN 037814. The construction of the phylogenetic tree with new nucleotide sequences and analysis of phylogenetic relations were performed using the MEGA5 (Tamura et al. 2011) software package. Statistical data analysis was performed using Statistica 10.

Results

Species differences of the cluster “meigeni”. Based on the analyzed features (see methodology), most features either do not differ, or their dimensional boundaries overlap. We determined that the following features are the most applicable for species identification (Table 1): 1) The head length and the height of the frontal triangle are maximal in *M. femorata* and minimal in *Meromyza mosquensis* Fedoseeva, 1960; 2) Black setae on the lower surface of genae are found in *M. bohemica* and *Meromyza elbergi* Fedoseeva, 1979, and sometimes in *M. femorata* and *M. variegata*; 3) A dark occipital spot is observed in *M. mosquensis* and *M. elbergi*, and there are light lateral occipital stripes in some species (only *M. bohemica* has brown stripes); 4) The length of the mesonotum and scutellum and their ratio are maximal in *M. meigeni* Nartshuk, 2006, and minimal in *M. femorata*; 5) The stripe of the mesonotum reaches the scutellum in *M. meigeni* and passes through the scutellum in *M. mosquensis* and *M. elbergi*; 6) The darkened part of the mesonotal mid stripe varies slightly in all species and differs significantly in color (*M. femorata* and *M. rufa* have lighter stripes); 7) *M. mosquensis* and *M. elbergi*, and occasionally *M. femorata*, have a marked stripe on the mesonotum; 8) The thickness of hind femurs significantly varies among species.

Males differ in the structure and size of the postgonites. The difference in the area of the anterior process of the postgonite is statistically significant in most species (Table 2). The species also differ in shape of the anterior and posterior processes of the postgonite, and by the type of attachment of the posterior process to the anterior one, and

Table 1. Characteristics of key features of the species in the proposed “variegata” cluster of grassflies *Mero-myza* according to present and literature data; *N* - number of specimens measured, * only literature data.

Characteristics	<i>M. bohémica</i> <i>N</i> = 9	<i>M. elbergi</i> *	<i>M. femorata</i> <i>N</i> = 11	<i>M. laeta</i> (<i>M. meigeni</i>) <i>N</i> = 18	<i>M. mosquensis</i> <i>N</i> = 13	<i>M. rufa</i> <i>N</i> = 3	<i>M. variegata</i> <i>N</i> = 11
Length of the head, mm	0.587±0.019	–	0.607±0.017	0.487±0.016	0.467±0.010	0.524±0.004	0.570±0.023
Height of frontal triangle, mm	0.376±0.013	–	0.419±0.012	0.329±0.112	0.313±0.009	0.320±0.008	0.380±0.024
Ratio of the genae height to the height of the 3rd antennal segment	0.83±0.02	1.5	1.03±0.05	0.77±0.06	0.75±0.04	0.64±0.03	0.74±0.04
Setae and the bristles on the lower surface of genae	light with some black setae	many black setae	black or white	light	light	light	light sometimes black
Color of the palpi	light	black in distal part	black in distal part	black	light	light	light
Occiput pattern (spot/lateral strips)	no/brown	brown/brown, sometimes the occiput is dark	no/no	not intensive/not bright	dark /dark, sometimes the occiput is dark	no or not intensive/ yes	not intensive/not bright
Length of mesonotum, mm	0.916±0.018	–	0.947±0.037	0.769±0.017	0.762±0.020	0.742±0.029	0.898±0.039
Length of the scutellum, mm	0.305±0.008	–	0.323±0.012	0.218±0.006	0.225±0.007	0.222±0.012	0.288±0.008
Ratio of mesonotum to scutellum	3.01±0.06	–	2.93±0.06	3.54±0.11	3.39±0.06	3.37±0.32	3.11±0.07
Stripe of the mesonotum passes through the scutellum	no	yes, broad	no	sometimes	yes, broad	no	no
Proportion of colored part of the mid stripe of the mesonotum (%)	72.9±2.1	–	73.3±2.5	69.4±1.3	100	72.1±2.7	73.1±2.3
Color of the mid and lateral stripes	brown, light brown, black outer margins of lateral stripes	black, sometimes brown	reddish, rich red, yellow, sometimes brown	brown dominates over black, lateral stripes are often black, sometimes all stripes are brown or yellow	brown to black	rust-colored, brown, yellow-brown, black outer margins of lateral stripes	brown, sometimes black, lateral stripes are darker with black margins
Ratio of hind femurs to hind tibia	3.27±0.11	almost three times over	4.13±0.23	3.19±0.26	3.03±0.12	3.83±0.20	3.80±0.15
Length of the body, mm	3–3.5	3.5–4.5	4.5–5	3–3.5	3–3.5	3.5–4	4–5

by the line of attachment of the posterior process relative to the line of attachment of the anterior process of the postgonite to the hypandria.

The population variability of key features was analyzed in *M. bohémica* Fed. as the model species. The first feature was the black setae on the lower surface of genae; a 10% and 15% variability in number of individuals with more than five setae was observed in the same population, and among studied populations, respectively.

Table 2. Characteristics of the postgonite of the proposed “variegata” species of grassflies *Meromyza*.

Characteristics	<i>M. bohemia</i>	<i>M. femorata</i>	<i>M. laeta</i> (<i>M. meigeni</i>)	<i>M. mosquensis</i>	<i>M. rufa</i>	<i>M. variegata</i>
Attachment of posterior process of the postgonite to the anterior one	laterally	posterior	posterior	posterior	posterior	posterior
Line of attachment of posterior process of the postgonite to the anterior one	above	down	almost down	above	above	almost down
Shape of posterior process of the postgonite	acuminate, slightly curved forward	acuminate, slightly curved forward	acuminate, curved forward	not acuminate, curved forward	round-ended, getting broader downward	acuminate, curved forward
Tip of the anterior process of the postgonite	sharply stubbed, acuminate	acuminate, Stubbed, transverse folds	oval	slightly stubbed, oval	obtusely stubbed	diagonally stubbed
Area of anterior process of the postgonite, μm^2 (n specimens)	4365.0 \pm 139.4(9)	7228.7 \pm 93.1 (23)	4512.6 \pm 91.6 (4)	5507.9 \pm 87.0 (22)	3053.8 \pm 296.3 (3)	9010.2 \pm 134.3 (20)

Table 3. The number of setae and the proportion of specimens (%) with a large number of setae (more than five) on the lower surface of the genae in males and females in four populations of *M. bohemia*: number of specimens (*N*), number of setae <5 or >5 (fewer or more than five).

Collection sites, percent of flies with more than 5 setae (%>5)	Side of the genae	Males				Females				Population Average % >5
		<i>N</i>	<5	<i>N</i>	>5	<i>N</i>	<5	<i>N</i>	>5	
Plzen	right	70	2.2 \pm 0.2	24	6.4 \pm 0.4	42	1.7 \pm 0.2	23	6.0 \pm 0.4	
	left		2.1 \pm 0.2		6.6 \pm 0.5		2.2 \pm 0.3		6.3 \pm 0.4	
% >5					25.5				35.4	30.5
Jihlava	right	38	2.1 \pm 0.2	38	6.1 \pm 0.6	40	2.3 \pm 0.2	10	6.7 \pm 0.4	
	left		2.0 \pm 0.2		6.2 \pm 0.6		2.4 \pm 0.3		5.9 \pm 0.6	
% >5					19.1		20.0			19.6
Brno	right	91	2.0 \pm 0.2	20	5.5 \pm 0.4	67	1.9 \pm 0.2	11	5.5 \pm 0.3	
	left		2.0 \pm 0.1		5.4 \pm 0.4		2.1 \pm 0.2		5.7 \pm 0.4	
% >5					18.0				14.1	16.1
Olomouc	right	30	2.1 \pm 0.3	11	5.5 \pm 0.4	21	1.6 \pm 0.2	10	5.6 \pm 0.4	
	left		1.8 \pm 0.3		5.5 \pm 0.4		1.5 \pm 0.3		5.8 \pm 0.3	
% >5					26.8				32.3	29.5
Average	right	229	2.1 \pm 0.1	64	5.9 \pm 0.2	170	1.9 \pm 0.1	54	6.0 \pm 0.2	
	left		2.0 \pm 0.1		6.0 \pm 0.3		2.1 \pm 0.1		6.0 \pm 0.2	
% >5					21.8				24.1	23.9

The second feature was the length of the mid stripe of the mesonotum. In most adult flies, the length of the stripe was $\frac{3}{4}$ the length of the mesonotum. The largest proportion of males and females with extreme length ratios ($\frac{2}{3}$ and 1) were found in the eastern population.

The third feature was the color of the mid stripe of the mesonotum. Red is the most common color, with greater number of specimens with dark red or brown stripes found in the central and western populations.

The phylogenetic tree based on the CO1 mtDNA of previously obtained nucleotide sequences (Safonkin et al. 2016) and new ones from *M. bohemia*, *M. femorata*

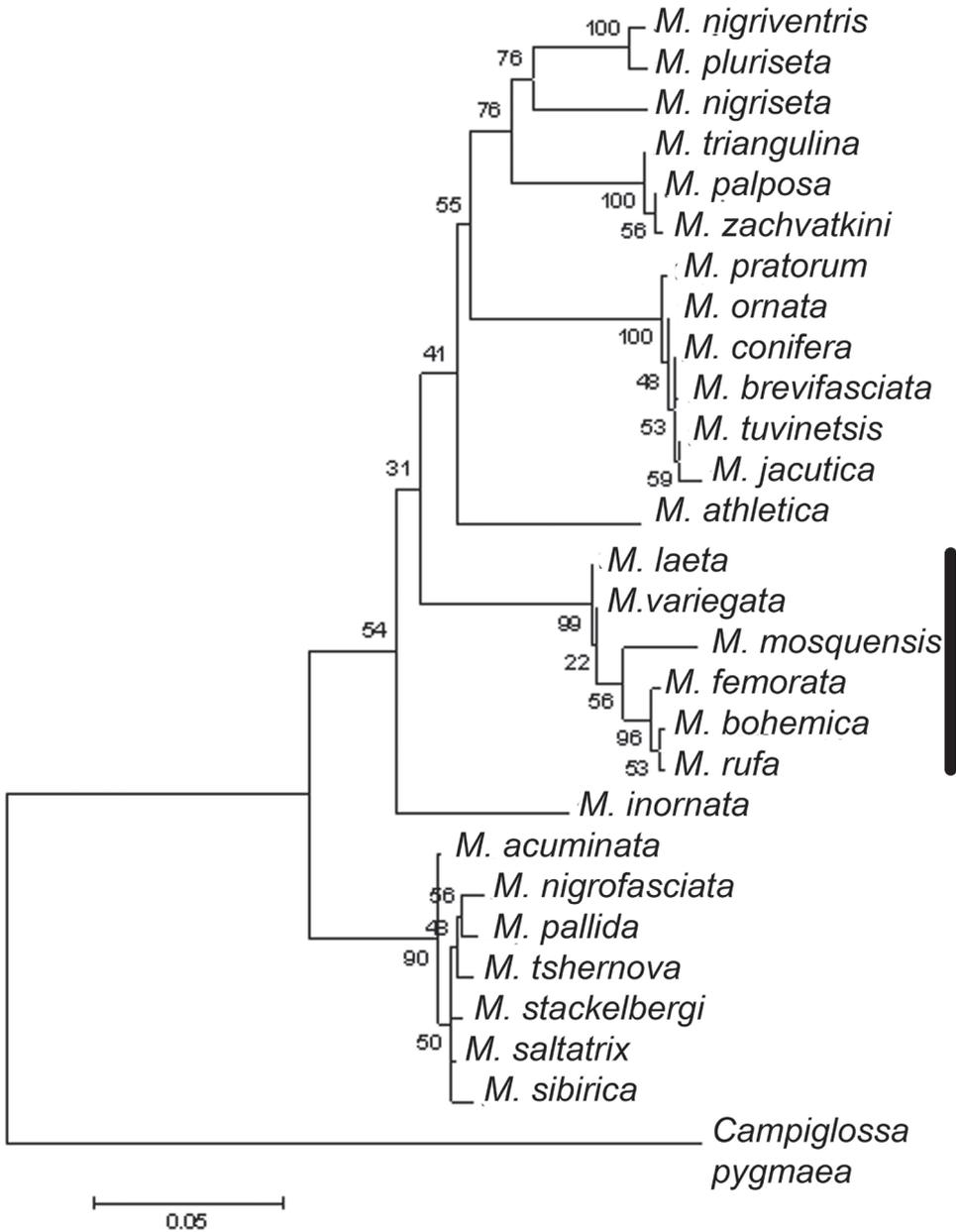


Figure 1. Maximum-likelihood phylogenetic tree showing relationships between the species of the “variegata” cluster and other species of *Meromyza* flies, based on CO1 mtDNA locus. The bootstrap values are given at the nodes. Vertical line – cluster “variegata”. *M. laeta* Fedoseeva, 1960 = *M. meigeni* sensu Nartshuk, 2006. The scale bar shows the genetic distances between the haplotypes. The outgroup was *Campiglossa pygmaea* Novak, 1974 (Diptera, Tephritidae) (GenBank: HM062547.1).

and *M. rufa* puts the sequences from these species into the cluster composed of *M. meigeni*, *M. mosquensis*, *M. variegata*; *M. meigeni* and *M. variegata* are the most close to a hypothetical haplotype of the cluster, also we can easily see that other species of this cluster divide from *M. variegata* (Fig. 1).

Discussion

The first attempt to divide 28 new species of *Meromyza* into four distinct groups based on the male's postgonite morphology was made by Hubicka (1970). Eight species, *M. rufa*, *M. bohémica*, *M. lolli* Hubicka 1967, *M. eduardi* Hubicka, 1966, *M. femorata*, *M. laeta* Meigen, 1838, *M. variegata*, and *M. stackelbergi* Fedoseeva, 1967, were placed by this author into a separate group ("variegata"). Our comparative analysis of key features described by Hubicka (1970) for species of this group revealed that postgonite morphology of *M. eduardi* and *M. stackelbergi* differed sharply from the other six species in this group which cannot be reliably distinguished by external morphological features. Three of the species from the "variegata" group, *M. bohémica*, *M. lolli*, *M. femorata*, had black setae on the lower surface of genae, but three others, *M. laeta*, *M. mosquensis*, *M. rufa* and *M. variegata*, did not have this feature. Based on the analysis of population variability of *M. bohémica*, we concluded that this feature is not unique. Also, our comparative analysis of population variability in occipital stripes and the color range of the stripes of the mesonotum in *M. bohémica* and *M. lolli*, considered by Hubicka (1970) as key features of species in the "variegata" group, showed that these morphological features are also non-unique. This fact is confirmed by the most complete key feature tables (Fedoseeva 2003, Nartshuk and Fedoseeva 2010), in which *M. lolli* is absent. In our opinion, five species from the "variegata" group described by Hubicka (1970) can be included in a separate cluster, previously designated by us as "meigeni" (Safonkin et al. 2016). The difficulty of identification of species of the considered group is confirmed in the case of *M. lidiae* Nartshuk, 1992. This species, according to Nartshuk (1992), is close to *M. laeta*. However, Nartshuk and Fedoseeva (2011) pointed out that *M. lidiae* is a junior synonym for *M. variegata*. Nartshuk (2006) described the new species *M. meigeni* based on the absence of the holotype *M. laeta* though pointing out, that *M. meigeni* may be identical to the previously described *M. laeta* (Nartshuk 2006, Nartshuk and Fedoseeva 2011). In the key to species of the genus (Nartshuk and Fedoseeva 2010), *M. laeta* is replaced by *M. meigeni*. However, we think that the species-specific description presented by the aforementioned authors (shape of the aedeagus and anterior process of the postgonite) is not sufficiently reliable to consider *M. meigeni* as a new species. Despite the description of *M. meigeni* by Nartshuk as a species unique to Slovenia, the shape of the aedeagus and anterior process of the postgonite, the key features of this species, are similar to those in *M. laeta* which was described as a species by different authors (Meigen 1830, 1838, Fedoseeva 1960, Hubicka 1970) from numerous regions of Europe. In our opinion, it suggests possible regional variability of *M. laeta* for a number of key features. We propose to go back to the traditional name of the species previously defined as *M. laeta* (Meigen 1830, 1838).

Molecular-genetic analysis of the CO1 mtDNA gene revealed a concordance between the haplotype and size of the postgonite anterior process (Safonkin et al. 2016); the species closer to the hypothetical ancestors of the cluster demonstrate the largest size of the anterior process of the postgonite. As stated in the above paper, the cluster in question was named as “meigeni”, since the Network (phylogenetic program) places *M. meigeni* closer to a hypothetical haplotype than *M. variegata*. The dendrogram displays *M. meigeni* and *M. variegata* as practically equidistant from the hypothetical haplotype (Fig. 1). Also, the area of the anterior process of the postgonite in *M. variegata* is significantly larger than in *M. meigeni* (Table 2). Based on the concordance between molecular phylogenetic results (Fig. 1) and the size of the postgonite anterior process, we propose to name the cluster in question as “variegata” instead of “meigeni”. The cluster’s name is taken from the name of the species closer to the ancestor haplotype and with the largest size of the anterior process of the postgonite.

Thus, it is possible to identify seven species in the “variegata” cluster according to a combination of external key features and the postgonite structure: *M. rufa*, *M. bohémica*, *M. femorata*, *M. laeta*, *M. variegata*, *M. mosquensis*, and *M. elbergi*. Based on the original description (Nartshuk 1992), *M. zimzerla* Nartshuk, 1992 can be also placed into the “variegata” cluster. Molecular analysis of the second part of the CO1 mtDNA gene confirms the identification of six selected species of the “variegata” cluster. Currently, there is no molecular analysis data for *M. elbergi* and *M. zimzerla*, though the key features of *M. elbergi* are close to those in species of the “variegata” cluster, but the original description of *M. zimzerla* puts this species close to *M. variegata*, one of the species with large inter-population variability. Nartshuk and Fedoseeva (2011) pointed out that *M. variegata* sensu Fedoseeva, 1960 = *M. zimzerla* Nartshuk, 1992.

Our comparative analysis showed a high degree of variability of external key features among species of the “variegata” cluster. The structure of the postgonite and, especially, the size of its anterior process is species specific in males, whereas the females cannot be reliably identified as particular species only by external morphology. For example, in the keys of Fedoseeva (2003) and Nartshuk and Fedoseeva (2010), the presence of a ‘large number’ of black setae on the lower surface of the genae is the main criterion in identification of *M. bohémica*. Based on our analysis of the populations of this species, only one-fifth to one-third of female specimens can be identified by this character as *M. bohémica*.

The same is true of *M. meigeni*; the main diagnostic feature in the identification of this species is the mid stripe of the mesonotum which reaches the scutellum but does not pass through it. However, in 1.4–29% of individuals of *M. bohémica* populations the mid stripe reaches the shield, instead. Also, the color of the stripes of the mesonotum varies from light to dark among specimens of *M. bohémica* populations. Such an important feature for species identification of the “variegata” cluster as palp color, can vary considerably from light to dark. In addition, the species are divided into two groups based on the shape of the palpi. However, the analysis of the shape of the palp requires examination of the object from the same angle, which is not always feasible when using the dry specimens.

Conclusions

We suggest, that approaches to the identification of the “variegata” cluster species should include external morphological and dimensional features of both males and females. Additionally, the analysis of males by peculiarities of the postgonites (structure and size of the area of the anterior process of the postgonite) and the analysis of females by the CO1 mtDNA should be used. This will allow the reliable species identification. A similar approach should be used in the identification of adults in other clusters of the genus *Meromyza*.

Key to species included in the proposed cluster “variegata”

- 1 Palpi strongly darkened.....2
- Palpi light, slightly darkened on the top (up to 1/2 height).....4
- 2 Black setae on the lower surface of genae (postgonite; Fig. 2B)*M. elbergi*
- Without black setae on the lower surface of genae3
- 3 Hind femurs heavily thickened, postgonite area, body size, height of frontal triangle, height of genae relative to the 3rd segment of the pedicel is large, the end of the projecting part of anterior process of postgonite with transverse folds, posterior process of the postgonite is adjacent to the anterior one from the back (Fig. 2C).....*M. femorata*
- Hind femur thinner, postgonite area, body size, height of frontal triangle, height of genae relative to the 3rd segment of the pedicel is less, the end of projecting part of anterior process of postgonite is round, posterior and anterior processes of postgonite are fused (Fig. 2D)..... *M. laeta*
- 4 Sampling of specimens with black setae on the lower surface of genae. The length of mesonotum is 3-fold over the scutellum length (postgonite; Fig. 2A)*M. bohémica*
- Without black setae on the lower surface of genae. Mesonotum is 3.1–3.4-fold over the scutellum..... 5
- 5 Dark occiput spot, mid strip of mesonotum passes through scutellum, small height of frontal triangle (0.31 mm) (postgonite; Fig. 2E)
..... *M. mosquensis*
- without dark occiput spot and the strip on the scutellum, large height of frontal triangle (0.32–0.38 mm)6
- 6 Color of the strips of mesonotum rusty-red, yellow-brown, sometimes brownish, small genae height relative to the 3rd segment of the pedicel and small length of the mesonotum. Wide posterior process of the postgonite (Fig. 2F) *M. rufa*
- Color of the stripes from brown to black, large height of genae relative to the 3rd segment of the pedicel and the length of the mesonotum. Acuminate curved posterior process of the postgonite (Fig. 2G) *M. variegata*

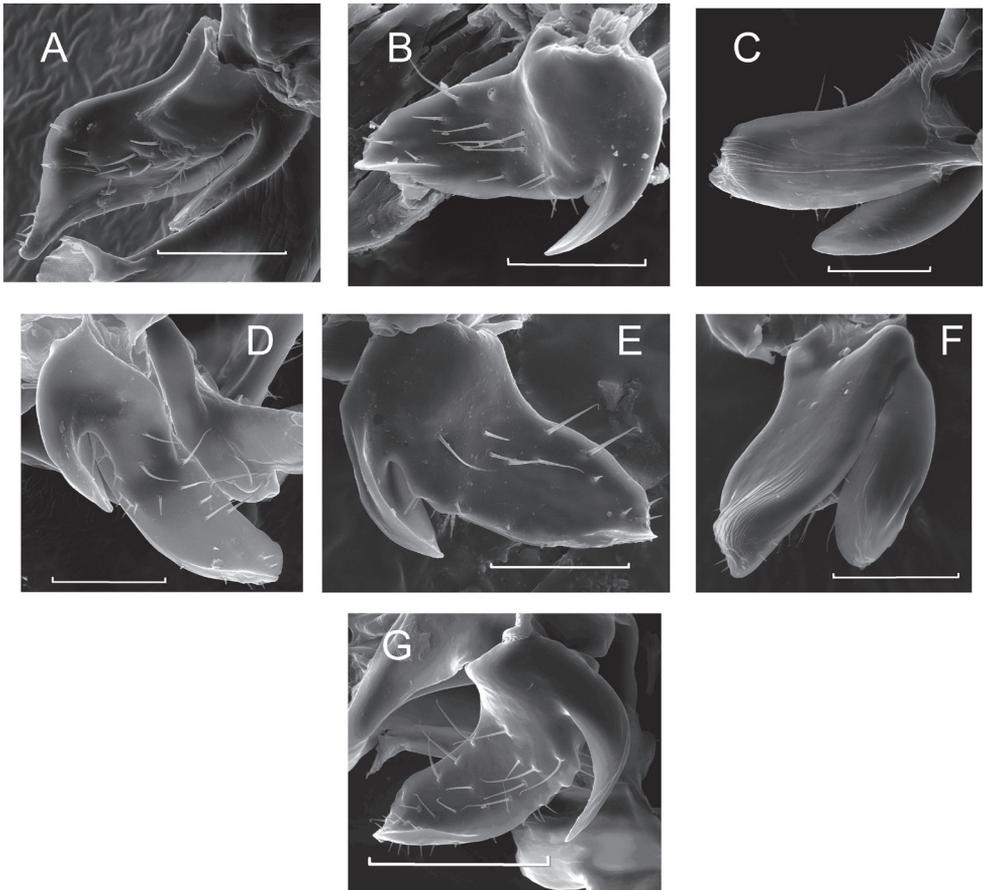


Figure 2. The postgonites of *M. bohemica* (A), *M. elbergi* (B), *M. femorata* (C), *M. laeta* (D), *M. mosquensis* (E), *M. rufa* (F), *M. variegata* (G). Scale bar: 50 μ m.

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A new species of *Dendropsophus* (Anura, Hylidae) from southwestern Amazonia with a green bilobate vocal sac

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Abstract

Recent studies have shown that species diversity of the South American frog genus *Dendropsophus* is significantly underestimated, especially in Amazonia. Herein, through integrative taxonomy a new species of *Dendropsophus* from the east bank of the upper Madeira River, Brazil is described. Based on molecular phylogenetic and morphological analyses, the new species is referred to the *D. microcephalus* species group, where it is differentiated from its congeners mainly by having a green bilobate vocal sac and an advertisement call comprising 1–4 monophasic notes emitted with a dominant frequency of 8,979–9,606 Hz. Based on intensive sampling conducted in the study area over the last ten years, the new species is restricted to the east bank of the upper Madeira River, although its geographic range is expected to include Bolivian forests close to the type locality.

Keywords

Amphibia, advertisement call, Amazonian biodiversity, *Dendropsophus microcephalus* species group, *Dendropsophus bilobatus* sp. nov., integrative taxonomy, morphology, upper Madeira River

Introduction

The genus *Dendropsophus* Fitzinger, 1843 is a taxonomically difficult group of small and, for the most part, morphologically similar species. The group exhibits high species diversity – 108 species are currently recognized, of which 66 occur in Brazilian Amazonia (Segalla et al. 2016; Frost 2019) – as well as cryptic diversity due to a high degree of both phenotypic similarity among species and intraspecific polymorphism (Gehara et al. 2014; Caminer et al. 2017). Whereas traditional morphological methods have often failed to reveal cryptic species and accurately delimit species boundaries, non-morphological methods (e.g., molecular phylogenetics and bioacoustics) have proven to be very useful for reliably documenting the full extent of species diversity in the genus (e.g., Fouquet et al. 2015; Rivanadeira et al. 2018).

The advertisement call is the most common mate-recognition signal among anurans; it has a direct impact on sexual selection and speciation (e.g., Sullivan et al. 1995; Boul et al. 2006). Consequently, advertisement call characteristics are widely used to identify anuran species both in field-based faunal inventories and in taxonomic studies (Schneider and Sinsch 2007; Köhler et al. 2017). During herpetological surveys of the amphibian and reptile fauna in the vicinity of the upper Madeira River (southwestern Amazonia, Rondônia, Brazil) in 2011–2013, we recorded several anuran advertisement calls that were markedly different from calls of all described species of *Dendropsophus* known from Brazilian Amazonia. The frogs emitting these calls morphologically resemble members of the *D. microcephalus* species group (sensu Faivovich et al. 2005), and preliminary bioacoustic analyses revealed that their calls are monophasic, i.e., they consist of only one call type (e.g., Orrico et al. 2014), and have a remarkably high dominant frequency (above 8 kHz).

In the *Dendropsophus microcephalus* species group, a similarly high dominant frequency has been reported only for two “monophasic” species: *D. meridianus* (Lutz, 1954) and *D. ozzyi* Orrico, Peloso, Sturaro, Silva, Neckel-Oliveira, Gordo, Faivovich & Haddad, 2014 (Pombal and Bastos 1998; Orrico et al. 2014). A high dominant frequency (~ 9 kHz) was also reported for *D. minusculus* (Rivero, 1971) from Belem, Brazil, by Duellman and Pyles (1983), but a low dominant frequency (~ 3 kHz) was recorded by Tarano (2011) from a population of the same species closer to the type locality in Venezuela. We suspect that the report by Duellman and Pyles (1983) may represent a species misidentification, and that the population referred to *D. minusculus* instead likely corresponds to *D. ozzyi*.

We believe that the unknown *Dendropsophus* with high dominant frequency calls represent at least two new species, which differ markedly in body shape, coloration and molecular characters. Herein, we provide formal description of the most strikingly distinct of these species, which to date is known only from the east bank of the upper Madeira River. In addition to its distinctive advertisement call, the species is characterized by a green bilobate vocal sac. Our description combines morphological, bioacoustic and molecular data.

Materials and methods

Specimens examined

We examined adult specimens of three forms of *Dendropsophus* collected in nine long-term ecological research (hereafter RAPELD) sampling sites (Magnusson et al. 2013) on the east and west banks of the upper Madeira River (Fig. 1, Table 1). Collected individuals were killed by topical application of a 2% benzocaine solution. Tissue samples were then taken from all specimens and stored in 100% ethanol. Finally, all specimens were fixed in 10% neutral-buffered formalin and stored in 70% ethanol. Voucher specimens are deposited in the herpetological collection of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA-H). *Dendropsophus* species used for comparisons are listed in Appendix 1.

Morphological characters

The format for the description follows Moravec et al. (2008). Specimens were sexed based on the presence or absence of secondary sexual characters (e.g., vocal sac and vocal slits) in males. Morphometric measurements were taken to the nearest 0.1 mm using a dissecting microscope and digital calipers. Thirteen morphometric measurements follow Duellman (1970) and Heyer et al. (1990): SVL, snout–vent length; HL, head length; HW, head width; EN, eye–nostril distance; ED, horizontal eye diameter; TD, horizontal tympanum diameter; HAL, hand length; 3FD, third finger disk diameter; 4TD, fourth toe disk diameter; TL, tibia length; THL, thigh length; FL, foot length; TAL, tarsus length. Webbing formulae of toes follow Savage and Heyer (1967) as adapted by Myers and Duellman (1982). Field notes and photographs taken by A. P. Lima were used to describe coloration in life.

Molecular analysis

We included samples of three forms of small-sized *Dendropsophus* collected in the area of the upper Madeira River during surveys in 2011–2013. The aim of those surveys was to detect phylogenetic diversity of *Dendropsophus* species distributed in this region. For the final dataset, we retrieved additional sequences of *Dendropsophus* from GenBank to locate phylogenetic positions of our new material in relation to DNA sequences published earlier, most importantly by Faivovich et al. (2005) and Jansen et al. (2011, 2019). We included species representing all *Dendropsophus* species groups (sensu Faivovich et al. 2005). Primary attention was paid to Amazonian species of the *D. microcephalus* species group. In concordance with earlier published phylogenies, we used *Xenohyla truncata* (Izecksohn, 1959) as an outgroup. The final dataset comprised 63 samples representing 34 nominal taxa, three new forms and the outgroup. All sequences acquired from GenBank are identified by GenBank accession numbers (Appendix 2).

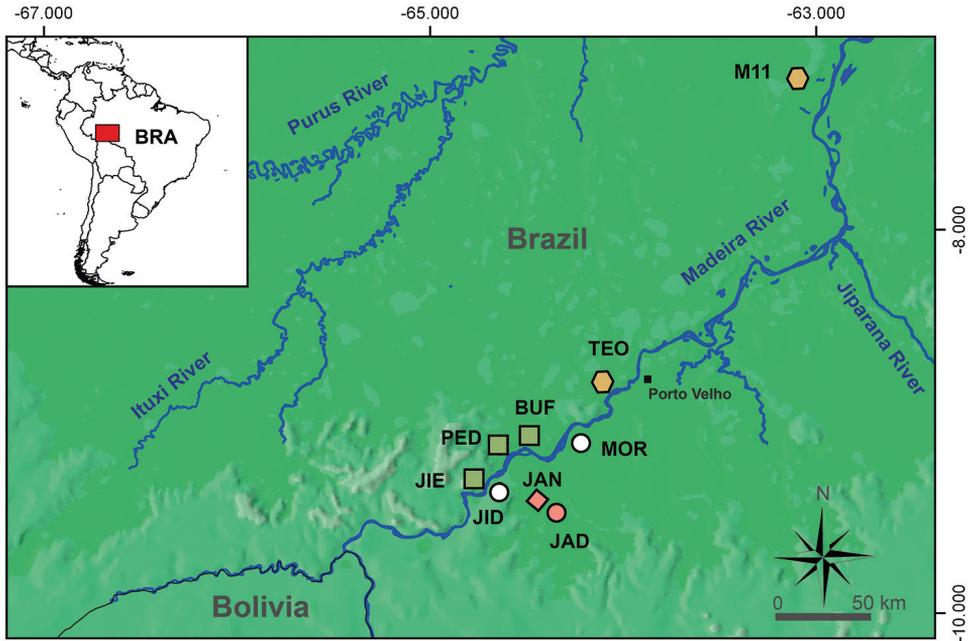


Figure 1. Schematic map showing RAPELD sampling sites in the upper Madeira River, Brazilian Amazonia. Symbols: green squares, *Dendropsophus* sp. A; orange pentagons, *Dendropsophus* sp. B; red diamond and circle, type and paratype localities of *Dendropsophus* sp. nov., respectively; white circles, referred specimens of *Dendropsophus* sp. nov. West bank: M11, Module 11; TEO, Teotônio; BUF, Bufalo; PED, Pedras; JIE, Jirau-Esquerdo. East bank: MOR, Morrinhos; JAD, Jaci-Direito; JAN, Jaci-Novo; JID, Jirau-Direito.

Table 1. RAPELD sampling sites in the upper Madeira River, Brazilian Amazonia.

Sampling site	Acronym	Geographic coordinates	Madeira River bank
Module 11	M11	07°13'06"S, 63°05'31"W	West
Teotônio	TEO	08°48'26"S, 64°05'56"W	West
Bufalo	BUF	09°09'32"S, 64°37'59"W	West
Pedras	PED	09°06'28"S, 64°30'46"W	West
Jirau-Esquerdo	JIE	09°17'52"S, 64°46'10"W	West
Jaci-Novo	JAN	09°24'45"S, 64°26'33"W	East
Jaci-Direito	JAD	09°27'44"S, 64°23'32"W	East
Jirau-Direito	JID	09°21'43"S, 64°41'31"W	East
Morrinhos	MOR	09°04'34"S, 64°14'46"W	East

Genomic DNA was extracted from muscle tissue of 16 specimens of the three new forms. DNA extractions were obtained using the Wizard Genomic DNA Purification Kit (Promega Corporation, USA) following the manufacturer’s protocols. We used the 16sbr (GCCGTCTGAACTCAGATCGCAT) and 16sar (CGCCTGTTTAT-CAAAAACAT) primers (Palumbi et al. 1991) to amplify a fragment of the 16S rRNA containing 495 base pairs (bp). The reaction conditions had a pre-heating step at 73 °C

for 60 s, 35 cycles of denaturation at 92 °C for 10 s, primer annealing at 50 °C for 35 s, and primer extension at 72 °C for 90 s, followed by a final extension step of five minutes at 72 °C. PCR products were purified through Exonuclease I and Thermosensitive Alkaline Phosphatase (Thermo Fisher Scientific, USA) and followed ABI BigDye Terminator Cycle Sequencing Kit protocols (Life Technologies, USA) as recommended by the manufacturer. Amplicons were sequenced using the forward primer in MacroGen (MacroGen Inc., Seoul, Korea).

Sequences were visually checked and edited with GENEIOUS 7.1.7 (GeneMatters Corp, Minneapolis, MN, USA). The final 16S rRNA matrix was composed of 63 terminals and 495 bp. BIOEDIT (Hall 1999) was used to align the final matrix through the ClustalW algorithm (Thompson et al. 1994). The most probable evolutionary model explaining sequence divergence was estimated using the Akaike Information Criterion (AIC) in JMODELTEST 2.1.7 (Darriba et al. 2012), which recovered the GTR+G+I as the most probable evolutionary model.

Phylogenetic trees were inferred through Maximum Likelihood (ML). The ML phylogenetic tree was calculated under the GTRGAMMA model with IQTREE webserver (Trifinopoulos et al. 2016). Clade support was estimated through 5,000 ultrafast bootstrap approximation replicates. MEGA 6.06 (Tamura et al. 2013) was used in order to estimate the uncorrected-pairwise genetic distance (p-distance) and Kimura-2-Parameters genetic distance (K2P; Kimura 1980) between the new *Dendropsophus* forms and other members of the *D. microcephalus* species group included in the phylogenetic analyses.

Bioacoustics

Advertisement calls of three males of the new *Dendropsophus* species (INPA-H 41302, 41303, 41304) were recorded in the sampling site Jaci-Novo during the rainy season on 15 February 2013. Calls were recorded with a Marantz PMD660 digital professional recorder (Marantz, Japan) and a Sennheiser K6/ME66 directional microphone (Sennheiser, Germany). The microphone was positioned approximately 1 m from each male. Recordings were made in wave format at a sampling rate of 44.1 kHz with 16-bits resolution. Air temperature taken with a digital thermometer during the recording was 25–26 °C ($N = 3$). Recordings are housed in the bioacoustic repository of the Amazonian Biodiversity Studies Centre at INPA (CENBAM 706, 707, 708).

Seven advertisement calls were analyzed for each recorded male. Advertisement call parameters were measured in RAVEN 1.5 (Bioacoustics Research Program 2015). Raven parameters were set as follows: window type = Blackman window, 3 dB filter bandwidth = 82 Hz, FFT window size = 2048 samples; FFT overlap = 80%, hop size = 4 ms. The following temporal and spectral parameters were inferred: call duration, inter-call interval, call period, number of notes, note duration, number of pulses per note, pulse duration, inter-pulse interval, dominant frequency (measured trough the function *Peak Frequency*), and bandwidth. The bandwidth was measured 20 dB below the peak frequency to avoid the overlap with background noise. Terminology of call measurements follows Köhler et al. (2017) while terminology of call structure follows

Littlejohn and Harrison (1985). Graphic representation of the advertisement calls was produced in the R environment (R Core Team 2016) through the package seewave v.2.1 (Sueur et al. 2008). Seewave was set as follows: window = Hanning, FFT size = 150 samples, FFT overlap = 85%.

Results

Molecular analysis

The Maximum Likelihood (ML) analysis based on 16S rRNA recovers several well-supported clades within *Dendropsophus* (Fig. 2). Samples collected in the area of the upper Madeira River form three monophyletic lineages nested within a major clade (ML support = 96), which includes species of the *D. microcephalus* species group sensu Faivovich et al. (2005).

Specimens from the east bank of the river, which are characterized by high-pitched calls and a bilobate vocal sac of green color when deflated (hereafter referred as *Dendropsophus bilobatus* sp. nov.), are grouped in a well-supported clade (ML support = 96) consisting of *D. mathiassoni* (Cochran & Goin, 1970), *D. juliani* Moravec, Aparicio & Köhler, 2006, *D. juliani* A (sensu Jansen et al. 2011), *D. minusculus*, *D. rozenmani* Jansen, Santana, Teixeira & Köhler, 2019, *D. sanborni* (Schmidt, 1944), *D. elianeae* (Napoli & Caramaschi, 2000) and *D. cachimbo* A (sensu Jansen et al. 2011). Within this clade, *Dendropsophus bilobatus* sp. nov. is placed with low support (ML support = 60) as sister to the group formed by the last five of the above-mentioned species (Fig. 2). Interspecific pairwise genetic distances between *Dendropsophus bilobatus* sp. nov. and its close relatives range from 3.4 to 5.8% (p-distance) and 3.4 to 6.1% (K2P). The average intraspecific genetic distance is 0.3% (K2P and p-distance; Table 2).

Dendropsophus specimens from the west bank of the upper Madeira River cluster in sister position to *D. reichlei* Moravec, Aparicio, Guerrero-Reinhard, Calderon & Köhler, 2008 from Bolivia (ML support = 98). These frogs sort into two well-supported sister lineages (ML support = 98; Fig. 2). The first lineage (hereafter referred to as *Dendropsophus* sp. A) comprises specimens collected in the RAPELD Teotonio and M11 sampling sites (distance apart ~ 250 km). The second lineage (*Dendropsophus* sp. B) comprises specimens from the RAPELD Pedras and Bufalos sampling sites (distance apart ~ 20 km). Genetic distances between *D. sp. A* and *D. sp. B* range from 1.6% (p-distance) to 1.7% (K2P). Genetic distances between *D. reichlei* and *D. sp. A* (K2P and p-distance = 3.1%) are slightly higher than those between *D. reichlei* and *D. sp. B* (K2P and p-distance = 2.8%). The average intraspecific genetic distance is higher in *Dendropsophus* sp. A (K2P and p-distance = 0.4%) than in *D. sp. B* (K2P and p-distance = 0.1%).

Because *Dendropsophus bilobatus* sp. nov. also differs from other congeneric species by its remarkably distinct morphology, we here describe it as a new species. Resolution of the taxonomic status of *D. sp. A* and *D. sp. B* is pending the results of additional species delimitation tests, which will be treated in a future study.

Taxonomy

Dendropsophus bilobatus sp. nov.

<http://zoobank.org/18906B0C-5EEA-416B-A672-FF8AD98DA448>

Figures 2–6, Table 2, 3

Material. Holotype. INPA-H 41300 (field number APL 19703; GenBank accession number MN977837), an adult male from the RAPELD Jaci-Novo sampling site (09°24'45"S, 64°26'33"W; 117 m a.s.l.), flooded forest at the west bank of the Jaci-Parana River (east tributary of the upper Madeira River), municipality of Porto Velho, district of Jaci-Parana, state of Rondônia, Brazil, collected on 26 March 2013 by Albertina P. Lima.

Paratopotypes. Five males: INPA-H 41302 (field number APL 19442), 41303 (field number APL 19443; GenBank accession number MN977835), 41304 (field number APL 19444), 41305 (field number APL 19445; GenBank accession number MN977836), and 41306 (field number APL 19446), collected on 15 February 2013 by Albertina P. Lima.

Paratypes. Two males: INPA-H 41301 (field number APL 19419; GenBank accession number MN977834) and 41307 (field number APL 19448), from the RAPELD Jaci-Direito sampling site (09°27'44"S, 64°23'32"W; 121 m a.s.l.), east bank of the Jaci-Parana River (an east tributary of the upper Madeira River), municipality of Porto Velho, district of Jaci-Parana, state of Rondônia, Brazil, collected on 14 and 15 February 2013, respectively, by Albertina P. Lima.

Referred material. Three males: INPA-H 41308 (field number APL 16652) and 41309 (field number APL 16653), from the RAPELD Jirau-Direito sampling site (09°21'43"S, 64°41'31"W; 131 m a.s.l.), east bank of the upper Madeira River, municipality of Porto Velho, state of Rondônia, Brazil, collected on 20 January 2011 by Albertina P. Lima; and INPA-H 41310 (field number APL 16428), from the RAPELD Morrinhos sampling site (09°04'34"S, 64°14'46"W; 95 m a.s.l.), municipality of Porto Velho, state of Rondônia, Brazil, collected on 13 January 2011 by Albertina P. Lima.

Generic placement. We assign this species to *Dendropsophus* based on our molecular phylogenetic analysis (Fig. 2) and on its general morphological similarity to other members of the genus.

Diagnosis. A species of the *Dendropsophus microcephalus* species group, distinguished from other species of *Dendropsophus* by the following combination of characters: (1) small size, SVL 18.8–20.8 mm ($N = 8$) in males (females unknown), head slightly wider than body; (2) snout short, truncate in dorsal and lateral views; (3) tympanum evident, round, about one third of eye diameter, tympanic annulus distinct anteriorly, ventrally and partly posteriorly; supratympanic fold barely evident; (4) dentigerous processes of vomers small, barely prominent, and separated medially between posterior halves of choanae; (5) skin on dorsal surfaces smooth; (6) tarsal fold and tubercles on outer edge of tarsus absent; ulnar folds and tubercles absent; (7) axillary membrane extensively developed; (8) fingers about half webbed; toes about three-fourths webbed; (9) bifid distal subarticular tubercle under fourth finger; (10) pectoral glands absent;

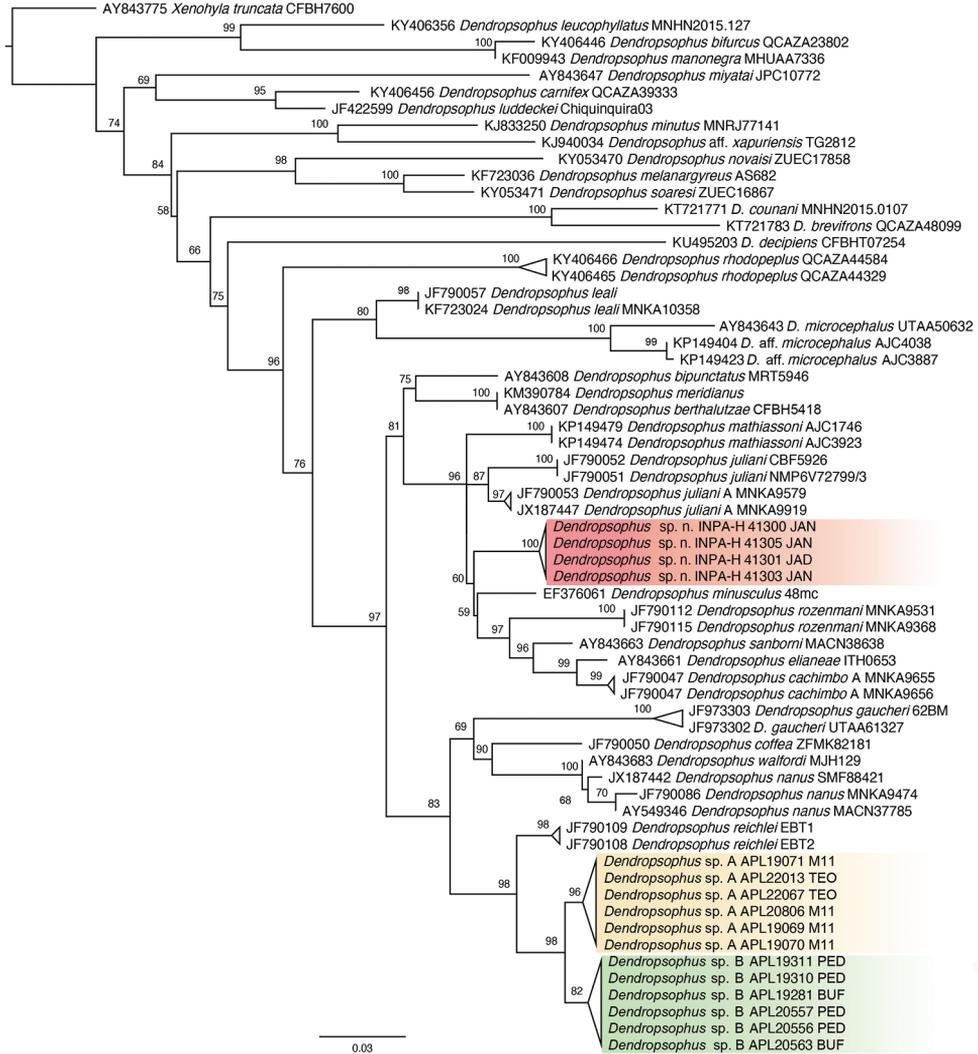


Figure 2. Maximum Likelihood phylogenetic tree of *Dendropsophus* inferred from the 16S rRNA mitochondrial gene (495 bp). Bootstrap values are shown close to nodes. Highlighted clades represent taxa from the upper Madeira River. Red horizontal bar denotes specimens from the east bank of the river; green and orange bars indicate specimens from the west bank.

(11) generally darker coloration of the loreal-tympanic region contrasts sharply with the lighter dorsal head coloration, one or two white spots below the eye; (12) in life, ground coloration of dorsum light brown; head greenish brown laterally; flanks ventrally and posteriorly a translucent pinkish white without chromatophores; hidden surfaces of thighs yellow without melanophores; (13) in life, throat green in males; belly yellowish-white in pectoral and central parts, translucent pinkish-white in posterior and lateral parts; ventral surfaces of thighs translucent pinkish white; (14) in life, iris pale to dark

brown with barely visible tiny brown veins, iris periphery dark brown to black; bones white; (15) advertisement call consisting of 1–4 notes (usually 1–2 notes), emitted regularly in series of 7–35 calls; high-pitched, monophasic, pulsed notes (3–8 pulses) with a duration of 12–24 ms and a dominant frequency of 8,979–9,606 Hz.

Comparisons. *Dendropsophus bilobatus* sp. nov. is readily distinguished from all congeners by having a green bilobate subgular vocal sac (some members of the *D. marmoratus* species group have a bilobate vocal sac, but not green) and a monophasic advertisement call with a remarkably high dominant frequency (8,979–9,606 Hz). Below we describe additional important differences between the new species and other members of the *D. microcephalus* species group (sensu Faivovich et al. 2005) that occur in Brazilian Amazonia and surrounding areas of Bolivia, Colombia, Peru and Ecuador. Characters of *D. bilobatus* are set in parentheses if not otherwise stated.

Three species of the *Dendropsophus microcephalus* species group have advertisement calls with a high dominant frequency: *D. meridianus* (Lutz, 1954), *D. minusculus*, and *D. ozzyi*. However, *D. meridianus* differs from *D. bilobatus* in having a snout slightly acuminate in lateral view (truncate), a single subgular yellow vocal sac (bilobate, green), dark dorsal lines or stripes on the dorsum (absent), absence of white subocular spots (present; Lutz 1973), and the dominant frequency of the advertisement call reaches 8,000 Hz (it reaches 9,606 Hz in *D. bilobatus*; Lutz 1973, Pombal and Bastos 1998); *D. minusculus* can be distinguished by its yellow single subgular vocal sac (bilobate, green) and by the absence of white subocular spots (present; Zina et al. 2014); and *D. ozzyi* differs in its single subgular transparent vocal sac (bilobate, green), absence of white subocular spots (present), vivid orange palmar end plantar surfaces (palmar surface greenish yellow, plantar surface orange), webbing formula of feet I 2–2+ II 1+–3– III 1+–2+ IV 2+–1+ V (I 1+–2– II 1+–1^{1/2} III 1^{1/2}–2– IV 2–1+ V), presence of glandular structures restricted on toes III and IV (glandular structures present also on toes II and V), and in single notes of the advertisement call (pulsed notes; Orrico et al. 2014).

The dark-greenish-brown coloration of the loreal-tympanic region of *Dendropsophus bilobatus*, which sharply contrasts with the light brown dorsal head coloration, resembles the head color pattern of *D. coffea* (Köhler, Jungfer & Reichle, 2005), *D. cruzi* (Pombal & Bastos, 1998), *D. studerae* (Carvalho-e-Silva, Carvalho-e-Silva & Izecksohn, 2003), *D. juliani*, *D. meridianus*, *D. microcephalus* (Cope, 1886), *D. minusculus*, *D. shiwiarum* Ortega-Andrade & Ron, 2013, *D. tintinnabulum* (Melin, 1941), and *D. reichlei*, but the new species is easily distinguished from each named species as follows (species already distinguished above are not listed here): *D. coffea* lacks white subocular spots (present) and has dark brown dorsal stripes (absent; Köhler et al. 2005); in *D. cruzi*, the thigh is longer than the tibia (tibia longer than thigh; Pombal and Bastos 1998); *D. studerae* has tuberculate dorsal skin (smooth; Carvalho-e-Silva et al. 2003); *D. juliani* has an acutely rounded snout in dorsal view (truncate), absence of white subocular spots (present), and greenish yellow plantar surfaces (orange; Moravec et al. 2006); *D. microcephalus* has maximum male SVL 24.5 mm (20.8 mm), an acutely rounded snout in dorsal view (truncate), an ovoid tongue (cordiform), and a weak tarsal fold (absent; Duellman 1970); *D. shiwiarum* has the discs of finger III

and toe IV with pointed tips (pointed tips absent), a prominent conical tubercle on the dorsal surface of fingers III and IV (tubercle absent), both palmar and plantar surfaces unpigmented (palmar surface greenish yellow, plantar surface orange), and a lower dominant frequency of the advertisement call (3,984–5,254 Hz in *D. shiwiarum* vs. 8,979–9,606 Hz in *D. bilobatus*; Ortega-Andrade and Ron 2013); *D. tintinnabulum* has a triangular-to-rounded snout in dorsal view (truncate) and orange palmar surfaces (greenish; Teixeira and Giaretta 2017), and lacks white subocular spots (present); and *D. reichlei* has a rounded snout in dorsal view (truncate) and a distinct canthus rostralis (absent), and lacks a glandular nuptial pad (present; Moravec et al. 2008).

In our phylogenetic analysis, the clade that contains *Dendropsophus bilobatus* is closely related to *D. bipunctatus* (Spix, 1824), *D. meridianus* and *D. berthallutzae* (Bokermann, 1962) from the southern and southeastern Brazilian coast (Fig. 2). In addition to differences in shape and color of the vocal sac and in advertisement call, these three species can be distinguished from *D. bilobatus* as follows: *D. bipunctatus* has a granulate dorsum (smooth), maximum SVL in males 25 mm (maximum male SVL 20.8 mm) and several small spots surrounded by a dark network that are distributed across the subocular area and lateral snout (spots only on subocular area and are not surrounded by dark network; Lutz 1973); *D. berthallutzae* has a snout that is slightly mucronate in dorsal view (truncate) and longer than eye diameter (snout shorter than eye diameter), and a minute outer metatarsal tubercle (absent; Lutz 1973).

Nine other small Amazonian species have been associated with the *Dendropsophus microcephalus* species group. These species differ from *D. bilobatus* in having the following combinations of characters: *D. joannae* (Köhler & Lötters, 2001) has tuberculate dorsal skin (smooth), a red inner iris in life (iris light to dark brown), and uniform head coloration without white subocular spots (coloration of loreal-tympanic region sharply outlined, subocular spots present; Köhler and Lötters 2001); *D. leali* (Bokermann, 1964) has a uniform ground head coloration without white subocular spots (coloration of loreal-tympanic region sharply outlined, subocular spots present; Köhler and Lötters 2001) and a biphasic call (monophasic; A. P. Lima personal data); *D. haraldschultzi* (Bokermann, 1962), *D. nanus* (Boulenger, 1889), *D. sanborni* (Schmidt, 1944) and *D. walfordi* (Bokermann, 1962) have a more pointed snout (snout short, truncate in dorsal and lateral views), a more or less conspicuous pattern of numerous thin brown lines on a yellowish dorsum (lines absent) and a biphasic call (monophasic; Hödl 1977; Teixeira et al. 2016; Missassi et al. 2017); *D. mathiasoni* (Cochran & Goin, 1970) has dorsolateral lymphatic sacs (absent; Cochran and Goin 1970); *D. rhodopeplus* (Günther, 1858) has a yellow dorsum with bright purple or red marks (purple or red marks absent; Duellman 2005); and *D. riveroi* (Cochran & Goin, 1970) has a canthus rostralis (absent) but lacks glandular nuptial pads in males (present; Ortega-Andrade and Ron 2013).

Ten other small Amazonian species belong to the *Dendropsophus rubicundulus* clade of the *D. microcephalus* species group (sensu Faivovich 2005). These species can be distinguished from *D. bilobatus* as follows: *D. anataliasiasi* (Bokermann, 1972), *D. araguaya* (Napoli & Caramaschi, 1998), *D. cerradensis* (Napoli & Caramaschi, 1998), *D. jimi*

Table 2. Interspecific pairwise genetic distances (expressed as a percentage and based on the 16S rRNA mitochondrial gene) between species of *Dendropsophus*, especially those in the *D. microcephalus* species group. Lower diagonal: uncorrected p-distance. Upper diagonal: Kimura-2-parameters (K2P).

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1 <i>D. rhodopeplus</i>	8.0																				
2 <i>D. leali</i>	7.5	8.5																			
3 <i>D. gaucheri</i>	10.6	8.0	14.9																		
4 <i>D. microcephalus</i>	9.5	7.9	13.3	3.7																	
5 <i>D. microcephalus</i> COL	9.6	6.6	12	3.6	11.1																
6 <i>D. sp. A</i>	10.4	7.8	9.2	11	10.2	1.7															
7 <i>D. sp. B</i>	9.5	7.2	8.7	10.1	9.7	1.6	2.8														
8 <i>D. reichlei</i>	8.6	6.3	8.1	9.6	8.4	3.1	2.8	5.0													
9 <i>D. coffea</i>	8.6	6.6	8.3	8.9	8.8	6.4	6.3	4.8	4.7												
10 <i>D. nanus/walfordi</i>	8.7	7.7	8.1	9.2	9.7	6.4	5.9	5.5	4.5	6.0											
11 <i>D. meridianus</i>	8.6	6.0	8.7	10.7	10.1	7.2	6.6	5.6	4.7	5.7	4.6										
12 <i>D. bipunctatus</i>	9.2	6.3	9.8	9.8	8.4	7.1	6.4	6.2	6.8	6.7	4.4	4.8									
13 <i>D. berthaltzae</i>	8.5	5.9	8.5	10.6	9.9	7.2	6.6	5.4	4.6	5.6	2.0	4.6	6.6								
14 <i>D. cachimbo</i> A	8.8	8.0	8.7	11.1	10.5	8.2	7.3	6.6	7.4	6.9	6.4	6.1	6.3	5.4							
15 <i>D. rozenmani</i>	8.7	8.2	9.5	11	9.9	8.2	7.1	6.3	7.6	7.4	5.2	7.1	5.1	5.1	5.9						
16 <i>D. mathiassoni</i>	7.0	7.0	8.5	9.8	9.5	8.5	8.1	6.7	6.6	6.4	4.9	5.5	4.8	5.0	5.6	4.0					
17 <i>D. minusculus</i>	9.9	8.5	7.1	11.8	11.4	9.7	9.6	7.8	8.5	7.0	5.7	6.8	5.4	5.7	5.6	3.9	4.9				
18 <i>D. sanborni</i>	8.5	7.9	8.5	10.6	9.7	8.5	7.7	6.9	8.1	7.0	6.2	5.9	6.1	3.3	4.5	4.6	4.7	4.6			
19 <i>D. juliani</i>	8.5	7.0	8.7	10.2	9.7	7.7	7.1	6.0	6.7	5.9	5.1	5.5	5.0	4.6	4.7	4.4	4.7	4.4	4.6		
20 <i>D. juliani</i> A	7.9	7.5	8.6	9.8	9.6	7.7	7.2	6.3	6.2	5.3	4.7	4.7	4.5	4.5	4.9	3.2	3.9	3.2	2.3	2.4	
21 <i>D. bilobatus</i> sp. nov.	8.4	7.3	7.8	9.6	9.9	7.4	7.1	6.7	6.8	6.2	5.8	5.7	5.7	5.0	5.8	4.5	4.6	3.9	4.1	3.4	3.4

(Napoli & Caramaschi, 1999), *D. rhea* (Napoli & Caramaschi, 1999), *D. rozenmani*, *D. rubicundulus* (Reinhardt & Lütken, 1862) and *D. tritaeniatus* (Bokermann, 1965) lack white subocular spots (present) and have conspicuous dark brown stripes or small dark brown spots arranged in longitudinal lines on the dorsum (dorsum with irregular pattern of irregular yellow spots or small dark brown dots; Martins and Jim 2004, Teixeira et al. 2013, Teixeira and Giarretta 2015, Jansen et al. 2019); and *D. cachimbo* (Napoli & Caramaschi, 1999) and *D. elianeae* have a uniformly green or yellowish green dorsum (dorsum light brown with irregular pattern of yellow spots or small dark brown dots) and lack white subocular spots (spots present; Jansen et al. 2019).

The two unnamed forms of *Dendropsophus* in the *D. microcephalus* species group from the west bank of the upper Madeira River (*D. sp. A* and *D. sp. B*) differ from *D. bilobatus* in having a single yellow subocular spot (bilobate, green) and pointed discs on toes and fingers (rounded).

Five *Dendropsophus* species distantly related to the *D. microcephalus* species group are reported from the area of the upper Madeira River (A. P. Lima personal data): *D. kamagarini* Rivadeneira, Venegas & Ron, 2018, *D. koehlini* (Duellman & Trueb, 1989), *D. leucophyllatus* (Beireis, 1783), *D. minutus* (Peters, 1872) and *D. sarayacuensis* (Shreve, 1935). These species differ clearly in their larger size and coloration (Rodríguez and Duellman 1994, Peloso et al. 2016, Caminer et al. 2017, Rivadeneira et al. 2018).

Currently, *Dendropsophus amicum* (Mijares-Urrutia, 1998), *D. battersbyi* (Rivero, 1961), *D. bromeliaceus* Ferreira, Faivovich, Beard, & Pombal, 2015 and *D. yaracuyanus* (Mijares-Urrutia & Rivero, 2000) are not assigned with certainty to any species group. However, *Dendropsophus bilobatus* differs from *D. amicum*, *D. battersbyi* and *D. yaracuyanus* by the SVL in males of 18.8–20.8 mm in males (SVL 22.8 mm in the male holotype of *D. amicum*, SVL 33 mm in the male holotype of *D. battersbyi*, SVL 28.5–30.4 mm in males of *D. yaracuyanus*; Rivero 1961, Mijares-Urrutia 1998, Mijares-Urrutia and Rivero 2000); from *D. bromeliaceus* by the presence of subocular spots and webbing formula of fingers I 2⁺–2 II 1^{1/2}–2^{2/3} III 2⁻–2 IV (subocular spots absent, I trace II 2⁻–3⁻ III 3⁺–3⁺ IV; Ferreira et al. 2015). Although *Dendropsophus minimus* (Ahl, 1933) was placed in the *D. minimus* species group (sensu Faivovich et al. 2005), this species has never been included in a phylogenetic analysis and its group membership is uncertain. *Dendropsophus bilobatus* can be distinguished from *D. minimus* by having a visible tympanum and by the absence of tarsal fold (concealed tympanum and presence of tarsal fold; Ahl 1933).

Holotype description. INPA-H 41300. Adult male (Figs 3, 4A, B), SVL 18.8 mm; body moderately robust; head slightly wider than long (HW/HL = 1.08); snout truncate in dorsal and lateral views; snout short, eye-nostril distance shorter than eye diameter (END/ED = 0.68); canthus rostralis rounded in dorsal and lateral views; loreal region slightly concave; internarial area slightly depressed; nostrils barely protuberant, directed dorsolaterally; interorbital area flat, slightly depressed in the central portion; interorbital distance equal 34% of head width; eyes large, strongly protuberant, ED/TD = 3.30, ED/HL = 0.42; tympanic membrane small, round, clearly distinct, its di-

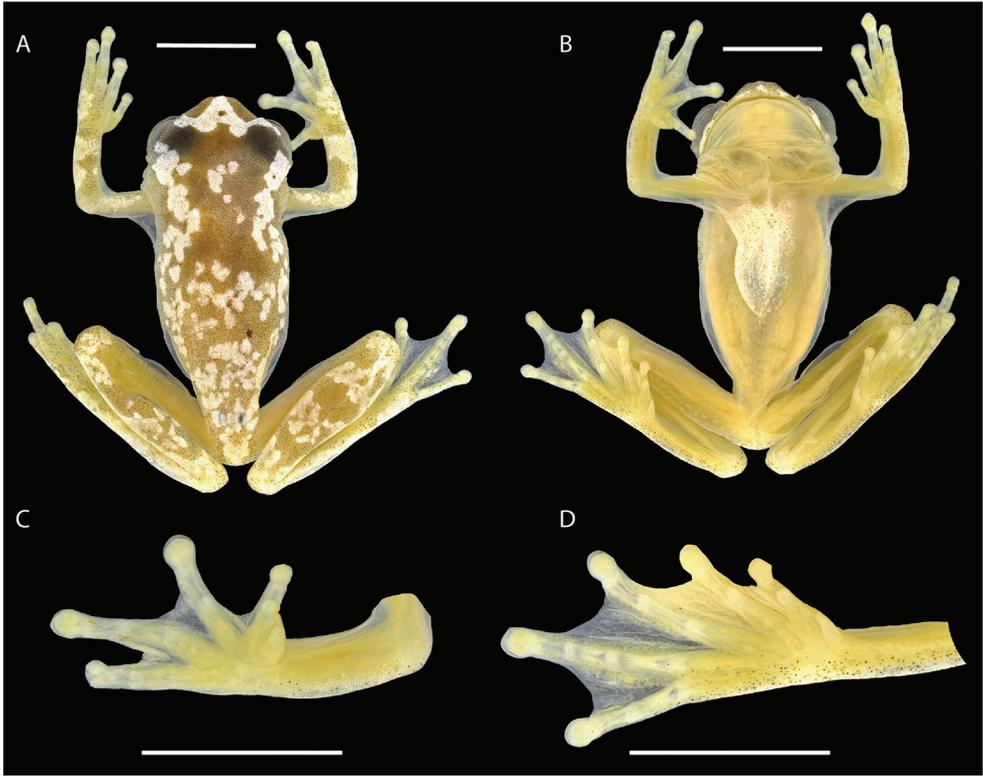


Figure 3. Dorsal view of the body (A) and ventral views of the body (B), hand (C) and foot (D) of the preserved holotype of *Dendropsophus bilobatus* sp. nov. (INPA-H 41300) from the RAPELD Jaci-Novo sampling site, east bank of the upper Madeira River, municipality of Porto Velho, Rondônia, Brazil. Scale bars: 5 mm. Photographs: Jeni Lima Magnusson.

iameter 30% of eye diameter and 13% of head length; tympanic annulus distinct ventrally and anteriorly; supratympanic fold barely evident, slightly obscuring the upper edge of the tympanum. Arms slender and not hypertrophied; ulnar tubercles and fold absent; axillary membrane reaches the second third of the upper arm; hand relatively long, about 30% of SVL, approximately the same size as the forearm; fingers long, slender, bearing small discs; finger III twice as wide medially than anteriorly; relative length of fingers I<II<IV<III; discs rounded on fingers; diameter of disc on finger III about the size of the tympanum; subarticular tubercles of fingers I and IV medium to large-sized, round, prominent, bifid in finger IV; subarticular tubercles of fingers II–III small, round, prominent; supernumerary tubercles barely evident; palmar tubercle small, flat, oval, barely evident proximally; prepollical tubercle large, flat, ovoid; nuptial pad white, glandular, covering the dorsolateral portion of the thumb but not reaching the ventral surface; webbing formula of fingers I 2⁺–2 II 1^{1/2}–2^{2/3} III 2[–]–2 IV. Legs moderately long, slender (THL/SVL = 0.55; TL/SVL = 0.56); tibia slightly longer than thigh (TL/THL = 1.02); tarsal fold and tarsal tubercles absent; calcar tubercles absent; toes moderately



Figure 4. *Dendropsophus bilobatus* sp. nov. from the Jaci-Parana River, a tributary of the east bank of the upper Madeira River, municipality of Porto Velho, Rondônia, Brazil, in life. **A, B** Holotype, INPA-H 41300, SVL = 18.8 mm, Jaci-Novo sampling site. **C–E** Adult male, INPA-H 41303, SVL = 19.9 mm, Jaci-Novo sampling site. **F** Adult male, INPA-H 41301, SVL = 18.9 mm, Jaci-Direito sampling site. Note the inflated translucent greenish bilobate vocal sac (**A, C, D**). Photographs: Albertina Pimentel Lima.

long, bearing discs slightly smaller than those on fingers; toe IV length equals 60% of foot length; relative length of toes I<II<III<V<IV; toes I, II and V slender; toes III and IV widened by elongated flat glandular structures on both sides, glandular structures

forming a continuous elongated glandular patch along toe IV, small glandular aggregations present also on fingers II and V; discs rounded on toes; diameter of the disc on toe IV equals diameter of the disc on finger III; subarticular tubercles round, prominent, penultimate tubercle on toe V bifid; supernumerary tubercles on toes III–IV small, round, barely evident; inner metatarsal tubercle elliptical, flat; outer metatarsal tubercle barely distinct; webbing formula of toes I 1⁺–2[–] II 1⁺–1^{1/2} III 1^{1/2}–2[–] IV 2[–]–1⁺ V.

Skin on head, dorsum, dorsal surfaces of limbs and flanks smooth; vocal sac and ventral surfaces of arms smooth; belly smooth laterally, coarsely granular medially; lower surfaces of thighs and surroundings of cloaca slightly granular. Cloacal opening directed posteroventrally at midlevel of thigh, covered dorsally by a wide cloacal sheath. Choanae small, vertically oval; dentigerous processes of vomers small, three vomerine teeth present on the right process, absent on the left process. Tongue cordiform, posterior third not attached to the floor of the mouth. Vocal slits long, extending from the midlateral base of the tongue to the angle of the jaw; anterior part covered by the lateral margin of the tongue. Vocal sac bilobate, subgular (Figs 3A, 4A, C, D).

In life (Fig. 4A, B), the dorsum and dorsal surfaces of the limbs are light brown with an irregular pattern of yellow spots; the head has a large triangular yellow blotch that extends from the tip of the snout to the anterior interorbital region, including the anterior margin of the upper eyelids; the lateral sides of the head are greenish brown with two white horizontally elongate subocular spots on the left side and one elongate and one round white spot on the right side. The iris is pale to dark brown with barely visible tiny brown veins; its outer edge is brown to black. Proximal dorsal surfaces of fingers I–III are greenish white to yellowish white; the proximal dorsal surface of finger IV is brown; distal dorsal surfaces of the fingers are yellowish orange; nuptial pads are white. The upper part of the flanks is a light pinkish brown; the posterior part of the flanks and the groin are pinkish white. Hidden dorsal surfaces of the thighs are yellow. The vocal sac is green when deflated but translucent greenish white when inflated. The chest and belly are yellowish white medially but translucent pinkish white laterally and posteriorly. Ventral surfaces of arms and legs are translucent pinkish white; the anteroventral side of the thigh is yellow, the posteroventral side is pinkish white; palmar surfaces are greenish yellow; plantar surfaces are orange. Bones are white.

In alcohol (Fig. 3), the head and dorsum are cream to brown with numerous tiny black melanophores and irregular white spots and blotches; dorsal surfaces of the limbs are light cream or translucent; ventral surfaces are translucent to cream, the chest and medial area of the belly are white. Bones are white.

Holotype measurements (in mm): SVL, 18.8; HL, 6.1; HW, 6.6; EN, 1.7; ED, 2.5; IOD, 2.3; TD, 0.8; 3FD, 0.8; 4TD, 0.8; TL, 14.4; THL, 10.3; TAL, 5.6; FL, 14.1.

Variation. The morphology of paratypes and paratopotypes does not deviate from that of the holotype. Morphometric measurements of all type specimens are shown in Table 3. *Dendropsophus bilobatus* sp. nov. exhibits two dorsal color patterns. The pattern of the holotype, while less common, is shared with two other specimens (INPA-H 41306 and 41307). The second and most common pattern is characterized by the dorsum and dorsal surfaces of the limbs being light brown with small irregularly dis-

Table 3. Morphometric measurements of the type series of *Dendropsophus bilobatus* sp. nov. from the east bank of the Madeira River, Municipality of Porto Velho, Rondonia, Brazil. Bold font denotes the holotype. Abbreviations: Desv., standard deviation, Min., minimum, Max., maximum. Morphometric abbreviations are described in Materials and methods.

Voucher	SVL	HL	HW	EM	ED	IOD	TD	3FD	4TD	TL	THL	TAL	FL
INPA-H 41300	18.8	6.1	6.6	1.7	2.5	2.3	0.8	0.8	0.8	10.4	10.3	5.6	8.4
INPA-H 41304	20.1	6.9	7.2	1.8	2.8	2.5	0.9	1.0	0.9	11.1	10.7	6.1	9.2
INPA-H 41305	19.4	6.4	6.8	1.9	2.6	2.4	0.9	0.9	0.9	10.7	10.2	6.1	9.0
INPA-H 41303	19.9	5.8	6.6	1.6	2.4	2.4	1.9	0.9	1.0	10.2	10.2	5.9	8.8
INPA-H 41301	18.9	6.3	6.4	1.6	2.5	2.2	0.9	0.8	0.8	10.2	9.7	5.6	8.1
INPA-H 41307	19.6	6.7	7.0	1.8	2.7	2.4	0.8	1.0	1.0	11.2	11.0	6.2	9.5
INPA-H 41306	20.8	6.6	6.8	1.7	2.8	2.1	1.0	0.9	0.9	11.5	10.9	6.0	9.4
INPA-H 41302	19.4	6.5	6.7	1.9	2.9	2.2	0.9	0.8	1.0	10.5	9.5	5.5	8.6
Mean	19.6	6.4	6.8	1.7	2.6	2.3	1.0	0.9	0.9	10.7	10.3	5.9	8.9
Desv.	0.6	0.3	0.2	0.1	0.2	0.1	0.4	0.1	0.1	0.5	0.5	0.3	0.5
Min.	18.8	5.8	6.4	1.6	2.4	2.1	0.8	0.8	0.8	10.2	9.5	5.5	8.1
Max.	20.8	6.9	7.2	1.9	2.9	2.5	1.9	1.0	1.0	11.5	11.0	6.2	9.5

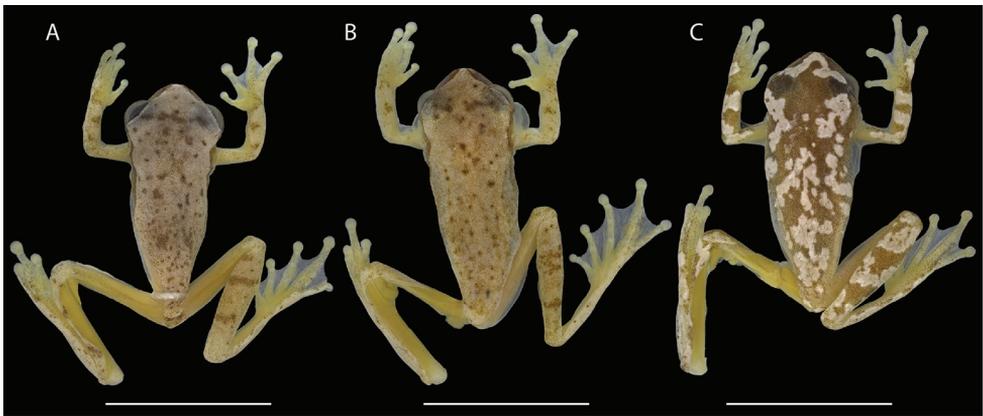


Figure 5. Preserved males of *Dendropsophus bilobatus* sp. nov. in dorsal view showing color variation. **A** INPA-H 41302, SVL = 19.4 mm **B** INPA-H 41304, SVL = 20.1 mm **C** INPA-H 41306, SVL = 20.8 mm. Scale bars: 10 mm. Photographs: Jeni Lima Magnusson.

tributed brown dots (Fig. 5A, B). Different from the holotype's pattern (Fig. 5C), the limit between the pinkish flanks and the light brown dorsum is well marked (Fig. 4C). The number of subocular light spots is variable in both patterns, ranging from 1 to 3 spots. White nuptial pads are conspicuous in all specimens but absent in paratopotype INPA-H 41306. Ventral color is similar in all specimens, as well as the color of the bilobate vocal sac. Females are unknown.

Call description. The advertisement call of *Dendropsophus bilobatus* (Fig. 6) consists of single- or multiple-note calls emitted regularly in series of 7–35 calls (19 ± 9 , $N = 12$). The most common arrangements are the single-note call ($N = 181$) and the two-note call ($N = 58$), while the rarest are the three-note ($N = 1$) and four-note calls ($N = 1$). Single-

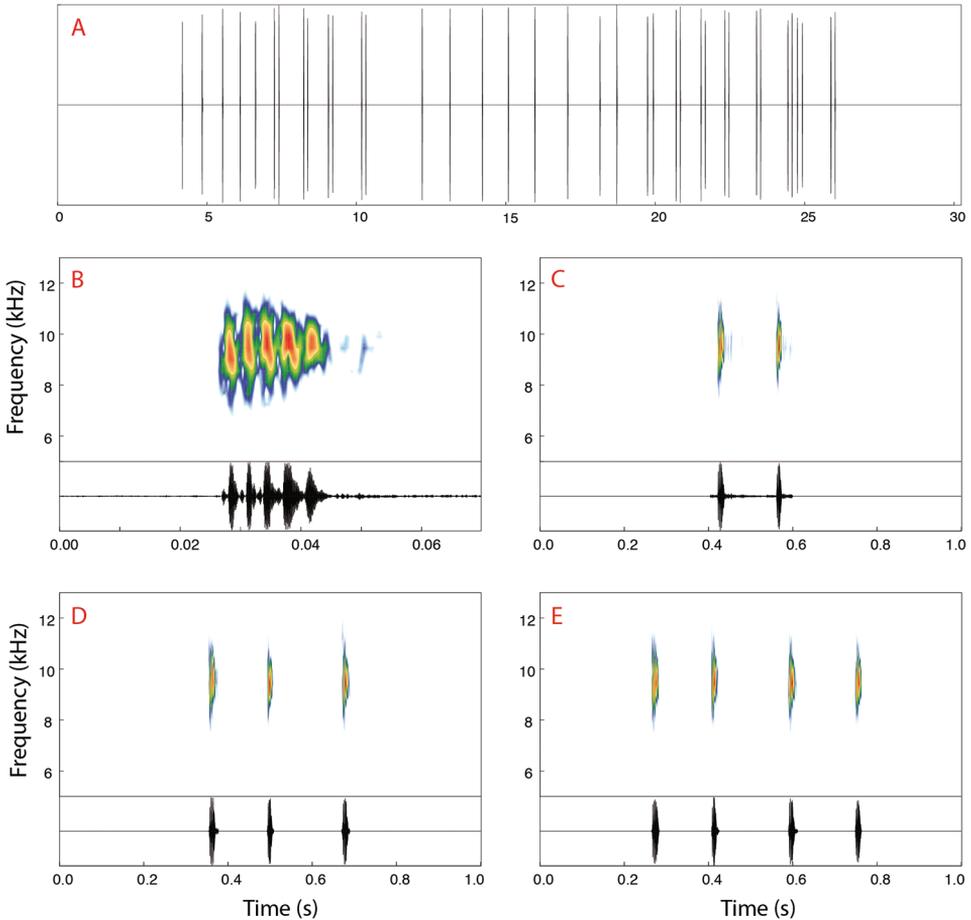


Figure 6. Advertisement call of *Dendropsophus bilobatus* sp. nov. from the RAPELD Jaci-Novo sampling site, Jaci-Parana River, a tributary of the east bank of the upper Madeira River, municipality of Porto Velho, Rondônia, Brazil. **A** Oscillogram of a call series composed of 24 calls. Spectrograms (upper graphs) and oscillograms (lower graphs) of calls formed by **B** one **C** two **D** three and **E** four pulsed notes. Recorded male: INPA-H 41303. Air temperature: 25.7 °C.

note calls have a call duration of 12–24 ms (8.2 ± 3 , $N = 30$), an inter-call interval of 483–1,284 ms (751 ± 201 , $N = 30$), and a call period of 503–1,302 ms (769 ± 202 , $N = 30$). Two-note calls have a call-duration of 155–199 ms (171 ± 13 , $N = 22$), an inter-call interval of 437–1,347 ms (816 ± 196 , $N = 19$), and a call period of 612–1,542 ms (985 ± 198 , $n = 19$). Notes in the two-note calls have a note duration of 12–22 ms (17 ± 3 , $N = 44$) and an inter-note interval of 126–165 ms (137 ± 11 , $N = 22$). The notes of both single- and multiple-note calls consist of 3–8 pulses (5 ± 1 , $N = 74$). Pulse duration is 1–2 ms (1.2 ± 0.4 , $N = 30$), inter-pulse intervals are 1–2 ms (1.5 ± 0.4 , $N = 30$). The high-pitched calls are emitted with a dominant frequency of 8,979–9,606 Hz ($9,274 \pm 195$, $N = 52$) and have a bandwidth of 7328–11517 Hz ($N = 33$).

Distribution and natural history. Our research team has sampled frogs at more than 150 permanent sampling sites distributed on both banks of the upper Madeira River and along the Purus-Madeira Interfluve. Yet, we have only observed *Dendropsophus bilobatus* in the lowland ombrophilous open forest on the east bank of the upper Madeira River. This area is close to the border between Brazil and Bolivia, and we expect that the new species also occurs in Bolivian lowland ombrophilous open forest, as do other anuran species that are known exclusively from the east bank of the upper Madeira River (e.g., *Hydrolaetare caparu* [Jansen, Gonzales-Álvarez & Köhler, 2007] and *Hamptophryne alios* [Wild, 1995]; Simões et al. 2011, Ferrão et al. 2014).

To date, specimens of *Dendropsophus bilobatus* have been observed only in the rainy season (early November to late March), which coincides with the species' breeding season. Calling males were observed in flooded areas connected to rivers of moderate (Jaci-Parana River) to large size (Madeira River). Males typically call in a large chorus while perched on leaves and tiny trunks that range in height from just a few centimeters above the water surface to ~ 2 m high. Males start calling in the crepuscule (~ 18:00 hs) and call activity has been observed at least to approximately midnight. When call activity ends remains unknown. Amplexus has not been observed. Other sympatric frogs include *Rhaebo guttatus* (Schneider, 1799), *Boana cinerascens* (Spix, 1824), *B. lanciformis* (Cope, 1871), *Scinax* sp. 6 (sensu Ferrão et al. 2016) and an uncollected *Scinax* with an advertisement call that resembles that of *S. garbei* (Miranda-Ribeiro, 1926).

Etymology. The specific name *bilobatus* is derived from the Latin noun *bilobate*. The name refers to the characteristic bilobate shape of the vocal sac of males of the new species.

Discussion

The upper Madeira River is characterized by high biodiversity; several priority areas for conservation are identified in this region (Capobianco 2001). Yet, recent studies have revealed that knowledge of the species diversity of amphibians inhabiting forests in the upper Madeira River is still incomplete, and description of new species is ongoing (e.g., Simões et al. 2010; Brcko et al. 2013; Ferrão et al. 2016; Ferrão et al. 2018). The new taxon described herein is the first species of *Dendropsophus* described from the Brazilian portion of the upper Madeira River, and at least one other new *Dendropsophus* species is awaiting formal description (work in preparation). At the same time, many of these species are highly threatened by increasing levels of deforestation caused by both illegal expansion of pastureland and infrastructure development associated with human settlements (e.g., reconstruction of the BR-319 highway and construction of large hydroelectric powerplants; Fearnside and Graça 2006; Fearnside 2015). Forests adjacent to the type locality of *D. bilobatus* sp. nov. were illegally deforested in 2016 and the paratype locality is now surrounded by pastures.

Based on general morphological similarity with specimens in the type series, we tentatively refer specimens from Jirau-Direito and Morrinhos, two localities in the east bank of the upper Madeira River, to *D. bilobatus*. However, these specimens differ

from the type material in some otherwise conserved characters (e.g., dorsal skin texture and color of iris), and there are no molecular data from them that might clarify their taxonomic relationship to the sequenced type specimens. Therefore, we have chosen not to include these specimens in the type series of *D. bilobatus* to avoid confounding specimens of the new species with what might turn out to be a second, but undescribed, cryptic species.

Intragenetic variation in vocal sac shape is not unusual in anurans, e.g., single subgular median in *Osteocephalus subtilis* and *O. oophagus*, single subgular expanded laterally in *O. vilarsi*, and paired lateral in *O. taurinus* (Jungfer and Schiesari 1995; Jungfer et al. 2013; Ferrão et al. 2019). As a result, vocal sac shape is a widely used character in anuran taxonomy and systematics. Aside from some members of the *Dendropsophus marmoratus* species group, *D. bilobatus* is the only species in the genus to possess a bilobate subgular vocal sac. All other congeners differ in the size and extent of a single subgular vocal sac, e.g., medium size in *D. bromeliaceus*, large size in *D. juliani*, well developed and extending laterally over the forearms in *D. ozzyi* (Moravec et al. 2006; Orrico et al. 2014; Ferreira et al. 2015). Given that the *D. marmoratus* and *D. microcephalus* species groups are not close relatives (Faivovich et al. 2005; Jansen et al. 2019; present study), the bilobate vocal sac evolved at least twice within *Dendropsophus*. However, this conclusion is tentative pending a fuller evaluation of the diversity and evolutionary history of vocal sac structures in *Dendropsophus* in a phylogenetic context.

Intragenetic differences in vocal sac shape have been associated with different breeding strategies in some neotropical anurans. For example, a small or indistinct single subgular vocal sac in phytotelmata-associated *Osteocephalus* is hypothesized to be a morphological adaptation for calling in small cavities relative to the large paired sacs of pond-breeding congeners (Jungfer and Hödl 2002; Moravec et al. 2009; Jungfer et al. 2013). We have not observed, however, any unusual feature of the breeding behavior or habitat of *Dendropsophus bilobatus* that might explain its remarkable bilobate vocal sac versus the single sac of most of its congeners.

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Appendix I

Additional specimens used in comparisons.

Dendropsophus sp. A: BRAZIL: Amazonas: BR-319, RAPELD Module 11 (APL 19069–71, 20806); Rondônia: Porto Velho, RAPELD Teotônio (APL 22013, 22067).

Dendropsophus sp. B: BRAZIL: Rondônia: Porto Velho, RAPELD Bufalo (APL 19281, 20563), RAPELD Pedras (APL 19310, 19311, 20556, 20557).

Dendropsophus aperomeus: PERU: Amazonas: 8 km NNE of Balzapata (KU 181812 [holotype]); Huánuco: 30 km NE Tingo María, Cordillera Azul (AMNH 91917–18 [paratypes]); San Martín: Rioja: Venceremos, 89 km NW Rioja (KU 212085–98).

Dendropsophus coffea: BOLIVIA: La Paz: 55 km on road from Caranavi to Palos Blancos (NKA 6538 [holotype], ZFMK 80590 [paratype]); 5 km N Río Beni bridge, near Sapecho (CBF 5538, ZFMK 82182 [paratypes]).

Dendropsophus delarivai: BOLIVIA: Cochabamba: road from Paractito to Cochabamba via El Palmar (CBF 3332 [holotype], CBF 3331, CBF 3336–37, KU 224700, MNCN 23696–97, ZSM 1–3/1999, ZFMK 67139–42, ZFMK 70317 [paratypes]); La Paz: Colonia Eduardo Avaroa (NKA 6539, ZFMK 80587–88).

- Dendropsophus joannae*: BOLIVIA: Pando: Cobija (CBF 3323 [holotype], CBF 3324–26, KU 224701–03, ZFMK 67119–20, ZFMK 67121–24 [paratypes]); Nacebe (NMP6V 72169/1–2).
- Dendropsophus leali*: BOLIVIA: Beni: El Porvenir (CBF 2449–50, ZFMK 62826); Totaizal (CBF 2358–61); Bosque Chimanes (CBF 1859–60); Infierno Verde (CBF 1861–62); Puerto Almacén, Río Ibaré (ZFMK 60721–22); Rurrenabaque (CBF 1080); Cochabamba: 6.5 km N Chipiriri (KU 136281–94); Pando: Bolpebra (CBF 5806, NMP6V 72562). BRAZIL: Rondônia: Forte Príncipe da Beira (KU 92058–59 [paratypes]). PERU: Loreto: Puerto Almendras (NMP6V 71183); Madre de Dios: Cuzco Amazónico, 15 km E Puerto Maldonado (KU 205488–92, 205498–590, 207577–79).
- Dendropsophus mapinguari*: BRAZIL: Amazonas: kilometer 168 of the BR-319 federal highway, Purus-Madeira Interfluve (INPA-H 41071–72).
- Dendropsophus meridianus*: BRAZIL: Rio de Janeiro: 20 km N of Rio de Janeiro (ZFMK 39499–500).
- Dendropsophus microcephalus*: COSTA RICA: Guanacaste: Colorado (ZFMK 62142–48). VENEZUELA: Sucre: Parare (ZFMK 36085–94).
- Dendropsophus minimus*: BRAZIL: Amazonas: Taperinha (near Santarem) (NMW 19436 [holotype]).
- Dendropsophus minutus*: BOLIVIA: Chuquisaca: W of Vaca Guzmán (ZFMK 66045); Santa Cruz: Samaipata (ZFMK 60403–07); Laguna de Bermejo (ZFMK 60440); Pando: Barracón (NMP6V 72803/1–2); Bolpebra (NMP6V 72566); Cobija (NMP6V 72466, ZFMK 66790); Sena (NMP6V 72802/1–4).
- Dendropsophus miyatai*: PERU: Loreto: Anguilla (NMP6V 71259).
- Dendropsophus nanus*: BOLIVIA: Beni: Puerto Almacén (ZFMK 60458–62); 6.5 km NE of Riberalta (NMP6V 70693/1–3), 2 km SW of Riberalta (NMP6V 70694); Santa Cruz: Buenavista (ZFMK 80011–14); San Ramón (ZFMK 60391–92); La Florida (ZFMK 60374–81); Santa Cruz de la Sierra (ZFMK 67001). PARAGUAY: Chaco: 23 km S of Filadelfia (ZFMK 53262–66).
- Dendropsophus praestans*: COLOMBIA: Huila: Parque Arqueológico San Agustín (MCZ-A 100216 [paratype]).
- Dendropsophus riveroi*: BOLIVIA: Beni: El Trimefo (CBF 1960–90); road San Borja–Trinidad, Río Matos (CBF 2456–57); Totaizal (CBF 2691); Cobija (ZFMK 67145–48); Santa Cruz: Buenavista (ZFMK 80015–17). COLOMBIA: Amazonas: Leticia (CM 37433 [holotype]).
- Dendropsophus rhodopeplus*: BOLIVIA: Pando: Bioceanica (CBF 5813–14, NMP6V 72568); Bolpebra (NMP6V 72569). PERU: Loreto: Puerto Almendras (NMP6V 71179). BRAZIL: AMAZONAS: Porong, Rio Juruá (INPA-H 4006, 4010).
- Dendropsophus* cf. *rubicundulus*: BOLIVIA: La Paz: Puerto Moscoso, Laguna Piraña (CBF 5360–61).
- Dendropsophus* cf. *schubarti*: BOLIVIA: La Paz: La Paz: Puerto Moscoso, Laguna Piraña (CBF 5317).
- Dendropsophus tritaeniatus*: BOLIVIA: Santa Cruz: P.N. Noel Kempff Mercado (ZFMK 72688).

Dendropsophus walfordi: BRAZIL: Amazonas: Lago Catalão (INPA-H 25291–93); Rondônia: Forte Príncipe da Beira, Costa Marques (MZUSP 73652 [holotype], INPA-H 31321, 31324–25, 31331–32).

Dendropsophus xapuriensis: BOLIVIA: Pando: Bioceanica, (CBF 5684–89, NMP6V 72571/1–6).

Appendix 2

Species, voucher numbers, GenBank accession numbers, and localities of samples used for phylogenetic analyses.

Species	Voucher	GenBank	Locality	Reference
<i>D. berthalutzae</i>	CFBH5418	AY843607	Brazil: Rio de Janeiro, Duque de Caxias	Faivovich et al. (2005)
<i>D. bifurcus</i>	QCAZA23802	KY406446	Ecuador: Morona Santiago, 4 Km N de Macas	Caminer et al. (2017)
<i>D. bipunctatus</i>	MRT5946	AY843608	Brazil: Bahia, Jussari, Serra do Teimoso	Faivovich et al. (2005)
<i>D. brevifrons</i>	QCAZA48099	KT721783	Ecuador: Pompeya	Fouquet et al. (2015)
<i>D. cachimbo</i> A	MNKA9655	JF790046	Bolivia: Santa Cruz, Ñuflo de Chavez, San Sebastián	Jansen et al. (2011)
<i>D. cachimbo</i> A	MNKA9656	JF790047	Bolivia: Santa Cruz, Ñuflo de Chavez, San Sebastián	Jansen et al. (2011)
<i>D. carnifex</i>	QCAZA39333	KY406456	Ecuador: Ecuador: Imbabura, Santa Rosa	Caminer et al. (2017)
<i>D. coffea</i>	ZFMK82181	JF790050	Bolivia: La Paz, Sur Yungas, near Sapecho	Jansen et al. (2011)
<i>D. counani</i>	MNHN2015.107	KT721771	French Guiana: Montagne tortue grande	Fouquet et al. (2015)
<i>D. decipiens</i>	CFBHT07254	KU495203	Brazil: Sao Paulo, Cananea	Lyra et al. (2017)
<i>D. elianae</i>	ITH0653	AY843661	Brazil: Sao Paulo, Buri	Faivovich et al. (2005)
<i>D. gaucheri</i>	62BM	JF973303	French Guiana: Savane Corossony	Fouquet et al. (2011)
<i>D. gaucheri</i>	UTAA61327	JF973302	Suriname: Sipaliwini	Fouquet et al. (2011)
<i>D. juliani</i>	CBF5926	JF790052	Bolivia: Pando, Madre de Dios, Borracón	Jansen et al. (2011)
<i>D. juliani</i>	NMP6V72799/3	JF790051	Bolivia: Pando, Madre de Dios, Borracón	Jansen et al. (2011)
<i>D. juliani</i> A	MNKA9579	JF790053	Bolivia: Santa Cruz, Velasco, Caparu	Jansen et al. (2011)
<i>D. juliani</i> A	MNKA9919	JX187447	Bolivia: Santa Cruz, Velasco, Caparu	Schulze et al. (2015)
<i>D. leali</i>	MNKA9706	JF790057	Bolivia: Santa Cruz, Ñuflo de Chavez, San Sebastián	Jansen et al. (2011)
<i>D. leali</i>	MNKA10358	KF723024	Bolivia	Schulze et al. (2015)
<i>D. leucophyllatus</i>	MNHN2015.127	KY406356	French Guiana: Petit-saut	Caminer et al. (2017)
<i>D. luddeckei</i>	Chiquiquira03	JF422599	Colombia	Guarnizo et al. (2012)
<i>D. manonegra</i>	MHUAA7336	KF009943	Colombia: Caqueta, Florencia, vereda Sucre	Rivera-Correa and Orrico (2013)
<i>D. mathiassoni</i>	AJC1746	KP149479	Colombia: Meta, San Juan de Arama, Caserio Miraflores	Guarnizo et al. (2015)
<i>D. mathiassoni</i>	AJC3923	KP149474	Colombia: Meta, San Juan de Arama, Caserio Miraflores	Guarnizo et al. (2015)
<i>D. melanargyreus</i>	AS682	KF723036	Bolivia	Schulze et al. (2015)
<i>D. meridianus</i>		KM390784	Brazil	Chaves et al., unpublished
<i>D. microcephalus</i>	UTAA50632	AY843643	Honduras: Atlantida, Cordillera Nombre de Dios	Faivovich et al. (2005)
<i>D. microcephalus</i>	AJC4038	KP149404	Colombia: Santander, Reserva el arboretum	Guarnizo et al. (2015)
<i>D. microcephalus</i>	AJC3887	KP149423	Colombia: Santander, Sabana de Torres, Sabana de Torres	Guarnizo et al. (2015)
<i>D. minusculus</i>	48mc	EF376061	French Guiana	Salducci et al., unpublished
<i>D. minutus</i>	MNRJ77141	KJ833250	Brazil: Rio de Janeiro, Nova Friburgo	Gehara et al. (2014)
<i>D. miyatai</i>	JPC10772	AY843647	Ecuador: Sucumbios	Faivovich et al. (2005)

Species	Voucher	GenBank	Locality	Reference
<i>D. nanus</i>	MNKA9474	JF790086	Bolivia: Santa Cruz, Sara, Buenavista	Jansen et al. (2011)
<i>D. nanus</i>	MACN37785	AY549346	Argentina: Entre Rios, Dto. Islas del Ibicuy	Faivovich et al. (2005)
<i>D. nanus</i>	SMF88421	JX187442	Bolivia: Santa Cruz, Ichilo, Buenavista	Schulze et al. (2015)
<i>D. novaisi</i>	ZUEC17858	KY053470	Brazil: Jequié, Bahia State	Teixeira et al. (2016b)
<i>D. reichlei</i>	EBT1	JF790109	Bolivia: Pando, Manuripi, Estación Biológica Tahuamanu	Jansen et al. (2011)
<i>D. reichlei</i>	EBT2	JF790108	Bolivia: Pando, Manuripi, Estación Biológica Tahuamanu	Jansen et al. (2011)
<i>D. rhodopeplus</i>	QCAZA44584	KY406466	Ecuador: Orellana, Huiririma	Camminer et al. (2017)
<i>D. rhodopeplus</i>	QCAZA44329	KY406465	Ecuador: Orellana, Chiroisla	Camminer et al. (2017)
<i>D. rozenmani</i>	MNKA9531	JF790112	Bolivia: Santa Cruz, Velasco, Caparu	Jansen et al. (2011)
<i>D. rozenmani</i>	MNKA9368	JF790115	Bolivia: Beni, Yucuma, Los Lagos	Jansen et al. (2011)
<i>D. sanborni</i>	MACN38638	AY843663	Argentina: Entre Rios, Dto. Islas del Ibicuy, Ruta 12 vieja	Faivovich et al. (2005)
<i>D. soaresi</i>	ZUEC16867	KY053471	Brazil: Barreiras, Bahia State	Teixeira et al. (2016b)
<i>D. walfordi</i>	MJH129	AY843683	Brazil	Faivovich et al. (2005)
<i>D. xapuriansis</i>	TG2812	KJ940034	Brazil: Acre, Tarauaca	Gehara et al. (2014)
<i>D. bilobatus</i> sp. nov.	INPA-H 41301	MN977834	Brazil: Rondonia, Porto Velho, Jaci Direito	This study
<i>D. bilobatus</i> sp. nov.	INPA-H 41303	MN977835	Brazil: Rondonia, Porto Velho, Jaci Novo	This study
<i>D. bilobatus</i> sp. nov.	INPA-H 41305	MN977836	Brazil: Rondonia, Porto Velho, Jaci Novo	This study
<i>D. bilobatus</i> sp. nov.	INPA-H 41300	MN977837	Brazil: Rondonia, Porto Velho, Jaci Novo	This study
<i>Dendropsophus</i> sp. A	APL19069	MN977838	Brazil: Amazonas, BR-319, RAPELD M11	This study
<i>Dendropsophus</i> sp. A	APL19070	MN977839	Brazil: Amazonas, BR-319, RAPELD M11	This study
<i>Dendropsophus</i> sp. A	APL19071	MN977840	Brazil: Amazonas, BR-319, RAPELD M11	This study
<i>Dendropsophus</i> sp. A	APL20806	MN977841	Brazil: Amazonas, BR-319, RAPELD M11	This study
<i>Dendropsophus</i> sp. A	APL22013	MN977842	Brazil: Rondônia, Porto Velho, RAPELD Teotônio	This study
<i>Dendropsophus</i> sp. A	APL22067	MN977843	Brazil: Rondônia, Porto Velho, RAPELD Teotônio	This study
<i>Dendropsophus</i> sp. B	APL19281	MN977844	Brazil: Rondônia, Porto Velho, RAPELD Bufalo	This study
<i>Dendropsophus</i> sp. B	APL19310	MN977845	Brazil: Rondônia, Porto Velho, RAPELD Pedras	This study
<i>Dendropsophus</i> sp. B	APL19311	MN977846	Brazil: Rondônia, Porto Velho, RAPELD Pedras	This study
<i>Dendropsophus</i> sp. B	APL20556	MN977847	Brazil: Rondônia, Porto Velho, RAPELD Pedras	This study
<i>Dendropsophus</i> sp. B	APL20557	MN977848	Brazil: Rondônia, Porto Velho, RAPELD Pedras	This study
<i>Dendropsophus</i> sp. B	APL20563	MN977849	Brazil: Rondônia, Porto Velho, RAPELD Bufalo	This study
<i>Xenohyla truncata</i>	CFBH7600	AY843775	Brazil: Rio de Janeiro, Restinga de Marica	Faivovich et al. (2005)

Four new species of Asian horned toads (Anura, Megophryidae, *Megophrys*) from southern China

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Abstract

Recent phylogenetic analysis encompassing multilocus nuclear-gene and matrilineal mtDNA genealogy has revealed a series of cryptic species of the subgenus *Panophrys* within genus *Megophrys* from southern and eastern China. This study demonstrates that the *Panophrys* specimens from the hilly areas among Guangdong, Guangxi and Hunan can be morphologically distinguished from all recognized congeners, thereby providing additional supports for the recognitions of four new species of *Panophrys*, namely *Megophrys* (*Panophrys*) *mirabilis* Lyu, Wang & Zhao, **sp. nov.** from northeastern Guangxi, *Megophrys* (*Panophrys*) *shimentaina* Lyu, Liu & Wang, **sp. nov.** from northern Guangdong, and *Megophrys* (*Panophrys*) *xiangnanensis* Lyu, Zeng & Wang, **sp. nov.** and *Megophrys* (*Panophrys*) *yangmingensis* Lyu, Zeng & Wang, **sp. nov.** from southern Hunan. The descriptions of these species take the number of *Megophrys* species to 101, 46 of which belong to the subgenus *Panophrys*.

Keywords

cryptic species, diversity, morphology, *Panophrys*, taxonomy

Introduction

The Asian horned toad genus *Megophrys* Kuhl & Van Hasselt, 1822 within the family Megophryidae Bonaparte, 1850, is a typical representative for Oriental fauna, spreading throughout southern China, southern and eastern Himalayas, across Indochina to islands of the Sunda Shelf and the Philippines (Mahony et al. 2017; Liu et al. 2018; Frost 2020). Although morphological identifications on *Megophrys* species are not easy (Li et al. 2014; Liu et al. 2018), with the progress in integrative taxonomy, a large number of new species have been recognized in the last decade, and takes the species number of genus *Megophrys* sensu lato to 97 (Frost 2020).

During our herpetological surveys in the hilly areas among Guangdong, Guangxi and Hunan, southern China (Fig. 1), a series of specimens of horned toads were collected. These specimens morphologically belong to genus *Megophrys* but could not be assigned to any recognized species by the combinations of characteristics. Furthermore, the phylogenetic analysis encompassing multilocus nuclear-gene and matrilineal mtDNA genealogy conducted by Liu et al. (2018) has indicated that these specimens should be regarded as four cryptic species of the subgenus *Panophrys*, i.e., *M. sp29* from northern Guangdong, *M. sp25* from northeastern Guangxi, and *M. sp2* and *M. sp28* from southwestern Hunan. In this study, as a follow-up work on this series of specimens, we provide the additional morphological comparisons and descriptions to substantiate the recognition of these four cryptic species of *Panophrys* from southern China.

Materials and methods

Taxonomic system. The higher systematics of Asian horned toads has been in intensive debates for decades (Delorme et al. 2006; Frost et al. 2006; Li and Wang 2008; Chen et al. 2017; Deuti et al. 2017; Mahony et al. 2017; Liu et al. 2018; Li et al. 2020). In this study, not involving in the controversy of generic relationship in subfamily Megophryinae, we followed the most recent taxonomic arrangement (Mahony et al. 2017; Liu et al. 2018; Frost 2020), in which the genus *Megophrys* is considered to include seven subgenera: *Atympanophrys* Tian & Hu, 1983, *Brachytarsophrys* Tian & Hu, 1983, *Megophrys*, *Ophryophryne* Boulenger, 1903, *Pelobatrachus* Beddard, 1908, *Panophrys* Rao & Yang, 1997, and *Xenophrys* Günther, 1864. Since the subgenus *Panophrys* has been unanimously considered as a monophyletic group that is significantly divergent from other subgenera, we perform the analyses and comparisons on the undescribed specimens with *Panophrys* congeners in this study.

Phylogeny. Two mitochondrial genes, namely partial 16S ribosomal RNA gene (16S) and partial cytochrome C oxidase 1 gene (CO1), were used for phylogenetic analysis. All sequences were attained from GenBank, encompassing 17 samples of the unnamed species (originally submitted by Liu et al. 2018) and 40 samples from 40 recognized *Panophrys* congeners. Besides, two samples of subgenus *Xenophrys* were incorporated into our dataset as out-groups. Detailed information of these materials is given in Table 1.

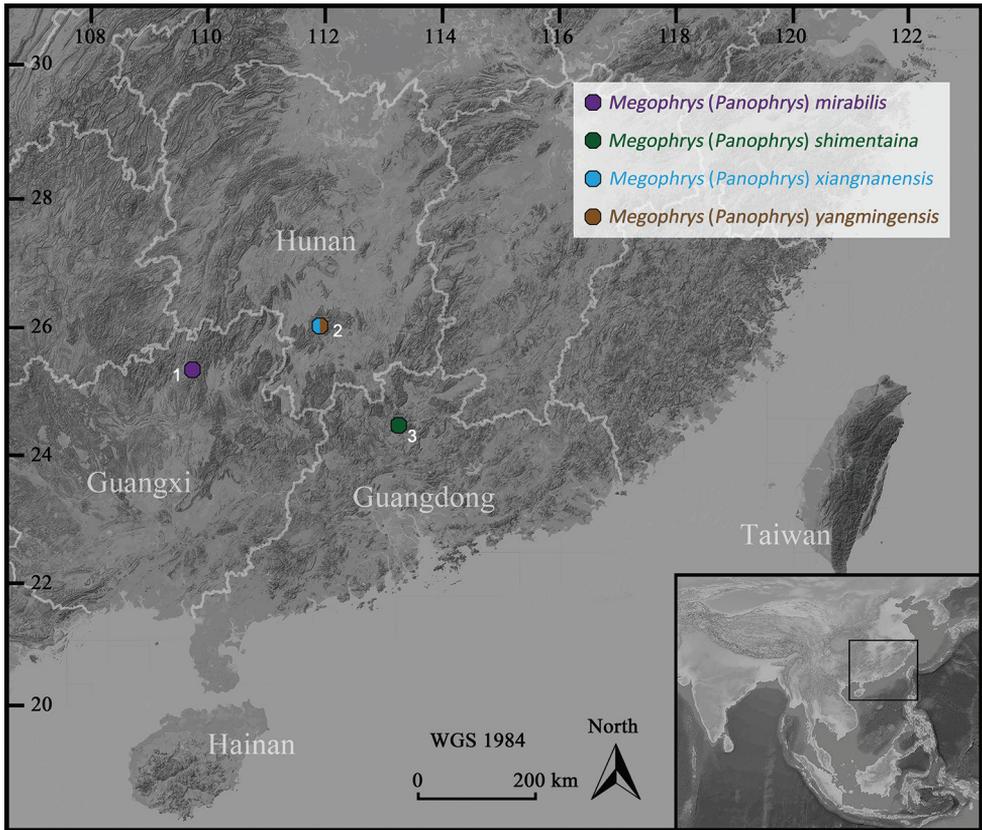


Figure 1. Map showing the collecting location of the new species. **1** Huaping Nature Reserve, Lingui District, Guilin City, Guangxi **2** Mt Yangming, Shuangpai County, Yongzhou City, Hunan **3** Shimentai Nature Reserve, Yingde City, Qingyuan City, Guangdong.

DNA sequences were aligned by the Clustal W algorithm with default parameters (Thompson et al. 1997) and trimmed with the gaps partially deleted in MEGA 6 (Tamura et al. 2013). Two gene segments, 632 base pairs (bp) of CO1 and 541 bp of 16S, were concatenated seriatim into a 1173-bp sequence, and were further tested in jmodeltest v2.1.2 with Akaike and Bayesian information criteria, all resulting the best-fitting nucleotide substitution models of GTR+I+G. Sequenced data was analyzed using Bayesian inference (BI) in MrBayes 3.2.4 (Ronquist et al. 2012). Two independent runs were conducted in a BI analysis, each of which was performed for 10,000,000 generations and sampled every 1000 generations with the first 25% samples were discarded as burn-in, resulting a potential scale reduction factor (PSRF) of < 0.005. Mean genetic distances of 16S gene between and within species were calculated in MEGA 6 using the uncorrected *p*-distance model.

Bioacoustics. Advertisement calls of the unnamed species were recorded in the field by a SONY PCM-D50 digital sound recorder. The sound files in wave format

Table I. Localities, voucher information, and GenBank numbers for all samples used in this study.

ID	Species	Localities	Voucher number	16s	co1
Megophrys (Panophrys)					
1	<i>M. (Pa.) mirabilis</i> sp. nov.	China: Guangxi: Huaping Nature Reserve	SYS a002192	MH406669	MH406109
2	<i>M. (Pa.) mirabilis</i> sp. nov.	China: Guangxi: Huaping Nature Reserve	SYS a002193	MH406670	MH406110
3	<i>M. (Pa.) mirabilis</i> sp. nov.	China: Guangxi: Huaping Nature Reserve	SYS a002289	MH406681	MH406127
4	<i>M. (Pa.) mirabilis</i> sp. nov.	China: Guangxi: Huaping Nature Reserve	SYS a002917	MH406724	MH406176
5	<i>M. (Pa.) shimentaina</i> sp. nov.	China: Guangdong: Shimentai Nature Reserve	SYS a002077	MH406655	MH406092
6	<i>M. (Pa.) shimentaina</i> sp. nov.	China: Guangdong: Shimentai Nature Reserve	SYS a002081	MH406656	MH406093
7	<i>M. (Pa.) shimentaina</i> sp. nov.	China: Guangdong: Shimentai Nature Reserve	SYS a004172	MH406787	MH406249
8	<i>M. (Pa.) shimentaina</i> sp. nov.	China: Guangdong: Shimentai Nature Reserve	SYS a004173	MH406788	MH406250
9	<i>M. (Pa.) xiangnanensis</i> sp. nov.	China: Hunan: Mt Yangming	SYS a002874	MH406713	MH406165
10	<i>M. (Pa.) xiangnanensis</i> sp. nov.	China: Hunan: Mt Yangming	SYS a002875	MH406714	MH406166
11	<i>M. (Pa.) xiangnanensis</i> sp. nov.	China: Hunan: Mt Yangming	SYS a002876	MH406715	MH406167
12	<i>M. (Pa.) xiangnanensis</i> sp. nov.	China: Hunan: Mt Yangming	SYS a002878	MH406717	MH406169
13	<i>M. (Pa.) xiangnanensis</i> sp. nov.	China: Hunan: Mt Yangming	SYS a002879	MH406718	MH406170
14	<i>M. (Pa.) yangmingensis</i> sp. nov.	China: Hunan: Mt Yangming	SYS a002877	MH406716	MH406168
15	<i>M. (Pa.) yangmingensis</i> sp. nov.	China: Hunan: Mt Yangming	SYS a002888	MH406719	MH406171
16	<i>M. (Pa.) yangmingensis</i> sp. nov.	China: Hunan: Mt Yangming	SYS a002889	MH406720	MH406172
17	<i>M. (Pa.) yangmingensis</i> sp. nov.	China: Hunan: Mt Yangming	SYS a002890	MH406721	MH406173
18	<i>M. (Pa.) acuta</i>	China: Guangdong: Heishiding Nature Reserve	SYS a002266	KJ579119	MH406122
19	<i>M. (Pa.) baolongensis</i>	China: Chongqing: Baolong Town	KIZ 019216	KX811813	KX812093
20	<i>M. (Pa.) binchuanensis</i>	China: Yunnan: Mt. Jizu	KIZ 019441	KX811849	KX812112
21	<i>M. (Pa.) binlingensis</i>	China: Sichuan: Mt. Wawu	SYS a005313	MH406892	MH406354
22	<i>M. (Pa.) boetgeri</i>	China: Fujian: Mt. Wuyi	SYS a004149	MF667878	MH406247
23	<i>M. (Pa.) brachykolos</i>	China: Hong Kong	SYS a002258	KJ560403	MH406120
24	<i>M. (Pa.) caudoprocta</i>	China: Hunan: Badagongshan Nature Reserve	SYS a004281	MH406795	MH406257
25	<i>M. (Pa.) cheni</i>	China: Hunan: Taoyuandong Nature Reserve	SYS a002142	KJ560398	MH406098
26	<i>M. (Pa.) daweimontis</i>	China: Yunnan: Mt. Dawei	KIZ 048997	KX811867	KX812125
27	<i>M. (Pa.) dongguanensis</i>	China: Guangdong: Mt. Yinping	SYS a001973	MH406647	MH406083
28	<i>M. (Pa.) fansipanensis</i>	Vietnam: Lao Cai: Sa Pa	VNMN 2018.01	MH514886	/
29	<i>M. (Pa.) hoanglienensis</i>	Vietnam: Lao Cai: Sa Pa	VNMN 07034	MH514890	/
30	<i>M. (Pa.) huangshanensis</i>	China: Anhui: Mt. Huangshan	SYS a002703	MF667883	MH406161
31	<i>M. (Pa.) insularis</i>	China: Guangdong: Nan'ao Island	SYS a002169	MF667887	MF667924
32	<i>M. (Pa.) jiangi</i>	China: Guizhou: Kuankuoshui Nature Reserve	CIB KKS20180722006	MN107743	MN107748
33	<i>M. (Pa.) jingdongensis</i>	China: Yunnan: Mt. Wuliang	SYS a003928	MH406773	MH406232
34	<i>M. (Pa.) jinggangensis</i>	China: Jiangxi: Mt. Jinggang	SYS a004028	MH406780	MH406239
35	<i>M. (Pa.) jiulianensis</i>	China: Jiangxi: Mt. Jiulian	SYS a004219	MH406791	MH406253
36	<i>M. (Pa.) kuatunensis</i>	China: Fujian: Mt. Wuyi	SYS a003449	MF667881	MH406206
37	<i>M. (Pa.) leishanensis</i>	China: Guizhou: Mt. Leigong	SYSa002213	MH406673	MH406113
38	<i>M. (Pa.) liboensis</i>	China: Guizhou: Libo Country	20150813001	MF285253	/
39	<i>M. (Pa.) lini</i>	China: Hunan: Taoyuandong Nature Reserve	SYS a002381	MF667874	MH406135
40	<i>M. (Pa.) lishuiensis</i>	China: Zhejiang: Lishui City	WYF00169	KY021418	/
41	<i>M. (Pa.) minor</i>	China: Sichuan: Mt. Qingcheng	SYS a003209	MF667862	MH406194
42	<i>M. (Pa.) mufumontana</i>	China: Hunan: Mt. Mufu	SYS a006390	MK524104	MK524135
43	<i>M. (Pa.) nankunensis</i>	China: Guangdong: Mt. Nankun	SYS a004501	MH406822	MH406284
44	<i>M. (Pa.) nanlingensis</i>	China: Guangdong: Nanling Nature Reserve	SYS a001964	MH406646	MH406082
45	<i>M. (Pa.) obesa</i>	China: Guangdong: Heishiding Nature Reserve	SYS a002271	KJ579121	MH406123
46	<i>M. (Pa.) ombraphila</i>	China: Fujian: Mt. Wuyi	WUYI2015101	KX856397	/
47	<i>M. (Pa.) omeimontis</i>	China: Sichuan: Mt. Emei	SYS a005301	MH406887	MH406349
48	<i>M. (Pa.) palpebralespinosa</i>	Vietnam: Thanh Hoa: Pu Hu Nature Reserve	KIZ 011650	KX811889	KX812138
49	<i>M. (Pa.) rubrimeru</i>	Vietnam: Lao Cai: Sa Pa	VNMN 2017.002	MF536420	/
50	<i>M. (Pa.) sangzhiensis</i>	China: Hunan: Badagongshan Nature Reserve	SYS a004306	MH406797	MH406259
51	<i>M. (Pa.) shunhuangensis</i>	China: Hunan: Nanshan Forest Park	HNNU 18NS01	MK836023	MK977594
52	<i>M. (Pa.) spinata</i>	China: Guizhou: Mt. Leigong	SYS a002226	MH406675	MH406115
53	<i>M. (Pa.) ruberognanulatus</i>	China: Hunan: Badagongshan Nature Reserve	SYS a004310	MH406801	MH406263
54	<i>M. (Pa.) wugongensis</i>	China: Jiangxi: Mt. Wugong	SYS a004800	MH406853	MH406315
55	<i>M. (Pa.) wuliangshanensis</i>	China: Yunnan: Mt. Wuliang	SYS a003924	MH406771	MH406230
56	<i>M. (Pa.) wushanensis</i>	China: Hubei: Shennongjia Nature Reserve	SYS a003008	MH406732	MH406184
57	<i>M. (Pa.) xianjuensis</i>	China: Zhejiang: Xianju County	CIB XJ190505	MN563753	MN563769
Megophrys (Xenophrys)					
58	<i>M. (X.) glandulosa</i>	China: Yunnan: Mt. Gaoligong	SYS a003758	MH406755	MH406214
59	<i>M. (X.) mangshanensis</i>	China: Guangdong: Mt. Sanyue	SYS a002177	MH406666	MH406106

were sampled at 48 kHz with 24 bits in depth. Raven pro 1.5 (Cornell Lab of Ornithology, 2003–2014) was used to output the spectrograms and to measure interrelated parameters with Fast Fourier transform of 256 points and a 50% overlap. The following measurements were performed: call/note duration (the difference between begin time and end time for a selected call/note), notes per call, inter-note intervals (the difference between end time for a selected note and begin time for the next selected note), peak frequency (the frequency at which peak power occurs within the selected call), high frequency (the highest frequency of the selected call), low frequency (the lowest frequency of the selected call), bandwidth 90% (the difference between the 5% and 95% frequencies of a selected call).

Morphology. Thirty-six unnamed specimens from the hilly areas among Guangdong, Guangxi and Hunan, southern China were examined, 17 of which have been used in the phylogenetic analysis. All examined specimens were fixed in 10% buffered formalin and later transferred to 70% ethanol. All studied specimens are deposited in The Museum of Biology, Sun Yat-sen University (**SYS**), and Chengdu Institute of Biology, Chinese Academy of Sciences (**CIB**), China.

External measurements were made for the unnamed specimens with digital calipers (Neiko 01407A Stainless Steel 6-Inch Digital Caliper, USA) to the nearest 0.1 mm. Mean and standard deviation (SD) were calculated in R 3.3.2 (R Core Team 2016). These measurements were as follows:

- ED** eye diameter (from the anterior corner of the eye to posterior corner of the eye);
- FTL** foot length (from distal end of shank to the tip of digit IV);
- HDL** head length (from tip of snout to the articulation of the jaw);
- HDW** head width (head width at the commissure of the jaws);
- HND** hand length (from the proximal border of the outer palmar tubercle to the tip of digit III);
- IND** internasal distance (distance between nares);
- IOD** interorbital distance (minimum distance between upper eyelids);
- RAD** radio-ulna length (from the flexed elbow to the proximal border of the outer palmar tubercle);
- SNT** snout length (from tip of snout to the anterior corner of the eye);
- SVL** snout-vent length (from tip of snout to posterior margin of vent);
- TD** tympanum diameter (horizontal diameter of tympanum);
- TED** tympanum-eye distance (from anterior edge of tympanum to posterior corner of the eye);
- TIB** tibial length (from the outer surface of the flexed knee to the heel).

Sex was determined by secondary sexual characters, i.e., the presence of vocal sac, nuptial pads/spines in males (Fei et al. 2016).

Morphological characters of all 42 recognized congeners of subgenus *Panophrys* for comparisons were based on the examination of museum specimens listed in Appendix I and on information available in the literature (Table 2).

Table 2. Literature for morphological characters of 42 recognized species of *Megophrys* (*Panophrys*).

ID	Species	References
1	<i>M. (Pa.) acuta</i> Wang, Li & Jin, 2014	Li et al. 2014
2	<i>M. (Pa.) baolongensis</i> Ye, Fei & Xie, 2007	Ye et al. 2007; Fei and Ye 2016
3	<i>M. (Pa.) binchuanensis</i> Ye & Fei, 1995	Fei and Ye 2016
4	<i>M. (Pa.) binlingensis</i> Jiang, Fei & Ye, 2009	Fei and Ye 2016
5	<i>M. (Pa.) boettgeri</i> (Boulenger, 1899)	Fei and Ye 2016
6	<i>M. (Pa.) brachykolos</i> Inger & Romer, 1961	Fei and Ye 2016
7	<i>M. (Pa.) caudoprocta</i> Shen, 1994	Fei and Ye 2016
8	<i>M. (Pa.) cheni</i> (Wang & Liu, 2014)	Wang et al. 2014
9	<i>M. (Pa.) daweimontis</i> Rao & Yang, 1997	Fei and Ye 2016
10	<i>M. (Pa.) dongguanensis</i> Wang & Wang	Wang et al. 2019a
11	<i>M. (Pa.) fansipanensis</i> Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018	Tapley et al. 2018
12	<i>M. (Pa.) hoanglienensis</i> Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018	Tapley et al. 2018
13	<i>M. (Pa.) huangshanensis</i> Fei & Ye, 2005	Fei and Ye 2016
14	<i>M. (Pa.) insularis</i> (Wang, Liu, Lyu, Zeng & Wang, 2017)	Wang et al. 2017a
15	<i>M. (Pa.) jiangi</i> Liu, Li, Wei, Xu, Cheng, Wang & Wu, 2020	Liu et al. 2020
16	<i>M. (Pa.) jingdongensis</i> Fei & Ye, 1983	Fei and Ye 2016
17	<i>M. (Pa.) jinggangensis</i> (Wang, 2012)	Wang et al. 2012
18	<i>M. (Pa.) jiulianensis</i> Wang, Zeng, Lyu & Wang	Wang et al. 2019a
19	<i>M. (Pa.) kuatunensis</i> Pope, 1929	Fei and Ye 2016
20	<i>M. (Pa.) latidactyla</i> Orlov, Poyarkov & Nguyen, 2015	Orlov et al. 2015
21	<i>M. (Pa.) leishanensis</i> Li, Xu, Liu, Jiang, Wei & Wang, 2018	Li et al. 2018
22	<i>M. (Pa.) liboensis</i> (Zhang, Li, Xiao, Li, Pan, Wang, Zhang & Zhou, 2017)	Zhang et al. 2017
23	<i>M. (Pa.) lini</i> (Wang & Yang, 2014)	Wang et al. 2014
24	<i>M. (Pa.) lishuiensis</i> (Wang, Liu & Jiang, 2017)	Wang et al. 2017b
25	<i>M. (Pa.) minor</i> Stejneger, 1926	Fei and Ye 2016
26	<i>M. (Pa.) mufumontana</i> Wang, Lyu & Wang	Wang et al. 2019a
27	<i>M. (Pa.) nankunensis</i> Wang, Zeng & Wang	Wang et al. 2019a
28	<i>M. (Pa.) nanlingensis</i> Lyu, Wang, Liu & Wang	Wang et al. 2019a
29	<i>M. (Pa.) obesa</i> Wang, Li & Zhao, 2014	Li et al. 2014
30	<i>M. (Pa.) ombrophila</i> Messenger & Dahn, 2019	Messenger et al. 2019
31	<i>M. (Pa.) omeimontis</i> Liu, 1950	Fei and Ye 2016
32	<i>M. (Pa.) palpebralespinosa</i> Bourret, 1937	Fei and Ye 2016
33	<i>M. (Pa.) robrimera</i> Tapley, Cutajar, Mahony, Chung, Dau, Nguyen, Luong & Rowley, 2017	Tapley et al. 2017
34	<i>M. (Pa.) sangzhiensis</i> Jiang, Ye & Fei, 2008	Jiang et al. 2008; Fei and Ye 2016
35	<i>M. (Pa.) shuichengensis</i> Tian & Sun, 1995	Tian et al. 2000; Fei and Ye 2016
36	<i>M. (Pa.) shunbuangensis</i> Wang, Deng, Liu, Wu & Liu, 2019	Wang et al. 2019b
37	<i>M. (Pa.) spinata</i> Liu & Hu, 1973	Fei and Ye 2016
38	<i>M. (Pa.) tubero granulatus</i> Shen, Mo & Li, 2010	Mo et al. 2010; Fei and Ye 2016
39	<i>M. (Pa.) wugongensis</i> Wang, Lyu & Wang	Wang et al. 2019a
40	<i>M. (Pa.) wuliangshanensis</i> Ye & Fei, 1995	Fei and Ye 2016
41	<i>M. (Pa.) wushanensis</i> Ye & Fei, 1995	Fei and Ye 2016
42	<i>M. (Pa.) xianjuensis</i> Wang, Wu, Peng, Shi, Lu & Wu, 2020	Wang et al. 2020

Results

The BI phylogenetic result is shown in Fig. 2 with Bayesian posterior probabilities (BPP) for major nodes > 0.90. The mean *p*-distances of 16S gene among all in-group and out-group species used in this study are given in Table 3. The diagnostic characters separating all 42 recognized species of the subgenus *Panophrys* are given in Table 4.

The unnamed samples from Huaping Nature Reserve, Guangxi (samples ID 1–4 in Table 1), are clustered into a monophyletic clade with strong node supports (BPP

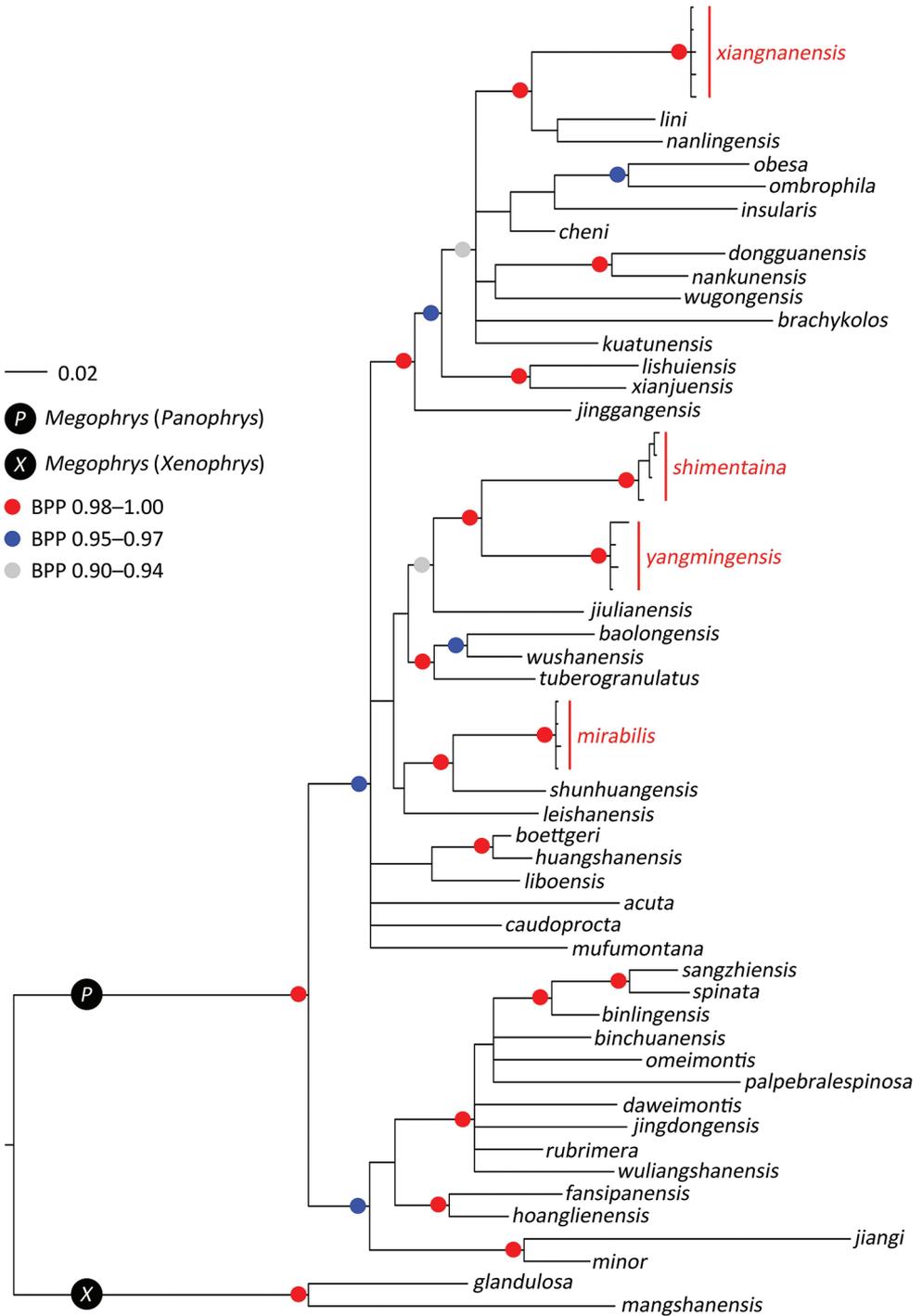


Figure 2. Phylogenetic tree of *Megophrys* (*Panophrys*) inferred from mitochondrial genes by Bayesian inference.

ID	Species	1–4	5–8	9–13	14–17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57			
54	<i>M. (Pa.) wuyangensis</i>	3.9	4.2	3.9	3.9	8.9	4.2	4.6	4.5	3.6	6.0	4.7	2.8	4.0	4.3	4.2	4.0	3.7	4.5	8.1	4.3	3.6	3.4	4.3	3.7	4.7	4.5	6.0	5.3	4.9	4.3	4.5	5.7	4.9	4.5	6.5	4.2	4.5	3.7	4.1	3.4	/	/	/	/			
55	<i>M. (Pa.) wuliangshanensis</i>	3.9	5.5	4.7	4.9	9.2	4.6	3.2	3.4	4.1	6.3	4.5	4.5	2.9	5.8	4.7	4.6	4.5	5.8	8.3	3.0	4.7	4.7	4.3	3.7	4.9	5.4	6.5	5.4	4.7	5.2	6.2	7.0	5.8	3.0	4.6	2.8	4.3	3.7	3.7	3.7	4.9	/	/	/	/		
56	<i>M. (Pa.) wushanensis</i>	3.6	3.6	3.6	2.8	8.1	2.1	4.0	3.9	2.6	4.9	3.4	3.2	3.6	3.9	3.8	3.6	3.0	3.6	7.7	4.3	3.2	3.0	3.2	2.4	3.4	4.5	4.0	6.0	3.2	3.7	5.0	6.0	4.3	3.7	5.0	3.8	4.5	2.6	4.5	1.7	3.9	3.9	/	/	/	/	
57	<i>M. (Pa.) xianjiansis</i>	3.6	4.4	4.0	3.2	7.3	3.2	4.0	4.4	2.3	4.9	3.6	3.0	3.6	4.2	4.8	3.8	3.0	4.2	7.6	4.4	4.0	3.4	3.0	2.7	3.2	4.6	2.8	5.5	3.4	4.0	5.1	5.1	4.6	3.8	5.1	3.8	5.3	2.9	5.0	2.9	4.8	4.4	3.2	/	/	/	/

Table 4. Diagnostic characters separating all 46 species of the *Megophrys* (*Panophrys*).

ID	Species	SVL in males (in mm)	SVL in females (in mm)	Horn-like tubercle at upper eyelids: slightly large (2), small (1)	Vomerine teeth: present (1), or absent (0)	Tongue: notched (1), or not notched (0)	Lateral fringes on toes: wide (2), narrow (1), or lacking (0)	Webs on toes: more than one-fourth (2), rudimentary (1), or lacking (0)	TD/ED	TIB/SVL
1	<i>M. (Pa.) mirabilis</i> sp. nov.	55.8–61.4	68.5–74.8	2	0	0	1	1	0.49–0.63	0.45–0.47
2	<i>M. (Pa.) shimentatina</i> sp. nov.	28.0–30.6	/	1	0	0	1	1	0.57–0.66	0.44–0.53
3	<i>M. (Pa.) xianguanensis</i> sp. nov.	38.6–42.0	44.4	1	0	0	2	1	0.38–0.49	0.41–0.46
4	<i>M. (Pa.) yangningensis</i> sp. nov.	33.2–37.1	45.2	1	0	0	1	1	0.42–0.50	0.44–0.51
5	<i>M. (Pa.) acuta</i>	27.1–33.0	28.1–33.6	2	0	0	1	1	0.57–0.71	0.38–0.45
6	<i>M. (Pa.) baolongensis</i>	42.0–45.0	/	1	0	1	0	0	0.41	0.46
7	<i>M. (Pa.) binshuanensis</i>	32.0–36.0	40.2–42.5	1	0	1 or 0	2	1	0.33–0.50	0.46–0.48
8	<i>M. (Pa.) binlingensis</i>	45.1–51.0	/	1	0	/	/	1	0.47–0.52	0.52–0.53
9	<i>M. (Pa.) boetgeri</i>	34.5–37.8	39.7–46.8	1	0	1	2	1	0.40–0.67	0.45–0.49
10	<i>M. (Pa.) brachykolos</i>	33.7–39.3	33.9–45.9	1	0	0	0	1	> 0.50	0.37–0.42
11	<i>M. (Pa.) caudoprocta</i>	81.3	/	2	1	0	/	1	0.5	0.51
12	<i>M. (Pa.) cheni</i>	26.2–29.5	31.8–34.1	1	0	1	2	1	0.41–0.54	0.50–0.54
13	<i>M. (Pa.) dauvimonitis</i>	34.0–37.0	40.0–46.0	1	1	/	0	0	/	0.54
14	<i>M. (Pa.) dongguanensis</i>	30.2–39.3	/	1	1	0	0	1	0.42–0.60	0.41–0.46
15	<i>M. (Pa.) fenshanensis</i>	30.9–44.3	41.7–42.5	1	1	1	0	0	0.53–0.80	0.49–0.59
16	<i>M. (Pa.) huangtienensis</i>	37.4–47.6	59.6	1	1	1	0	0	0.54–0.75	0.44–0.63
17	<i>M. (Pa.) huangshuanensis</i>	36.0–41.6	44.2	1	0	1	0	0	< 0.50	0.42–0.45
18	<i>M. (Pa.) insularis</i>	36.8–41.2	47.1	1	1	1	0	1	0.46–0.57	0.40–0.43
19	<i>M. (Pa.) jiangi</i>	34.4–39.2	39.5–40.4	1	0	0	0	1	/	/
20	<i>M. (Pa.) jingdongensis</i>	53.0–56.5	63.5	1	1	1	2	2	/	0.58–0.59
21	<i>M. (Pa.) jingguangensis</i>	35.1–36.7	38.4–41.6	2	1	0	1	1	0.73–0.88	0.47–0.50
22	<i>M. (Pa.) jiuhtanensis</i>	30.4–33.9	34.1–37.5	1	1	1	0	1	0.50–0.59	0.44–0.48
23	<i>M. (Pa.) kuatuensis</i>	26.2–29.6	37.4	1	1	1	1	0	0.44	0.38–0.48
24	<i>M. (Pa.) latidactyla</i>	38.9	/	2	1	0	2	1	0.85	0.52
25	<i>M. (Pa.) leishanensis</i>	30.4–38.7	42.3	1	0	0	0	1	/	/
26	<i>M. (Pa.) libbensis</i>	60.5–67.7	60.8–70.6	2	1	1	2	1	0.48–0.78	0.44–0.61
27	<i>M. (Pa.) lini</i>	34.1–39.7	37.0–39.9	1	0	0	2	1	0.40–0.60	0.46–0.53
28	<i>M. (Pa.) lishanensis</i>	30.7–34.7	36.9–40.4	1	0	0	0	0	/	/
29	<i>M. (Pa.) minor</i>	34.5–41.2	/	1	0	1	0	1	0.8–0.83	0.46–0.48
30	<i>M. (Pa.) mufhimontana</i>	30.1–30.8	36.3	1	0	0	1	1	0.51–0.58	0.47–0.53
31	<i>M. (Pa.) nankunensis</i>	29.9–34.9	39.4–41.9	1	1	0	0	1	0.43–0.61	0.35–0.42
32	<i>M. (Pa.) nanlingensis</i>	30.5–37.3	/	1	1	1	1	1	0.43–0.57	0.45–0.51
33	<i>M. (Pa.) obesa</i>	35.6	37.5–41.2	1	0	0	0	1	0.51–0.66	0.41–0.47
34	<i>M. (Pa.) ombrophila</i>	27.4–34.5	32.8–35.0	1	0	0	0	0	0.53–0.69	0.33–0.41

ID	Species	SVL in males (in mm)	SVL in females (in mm)	Horn-like tubercle at upper eyelid: slightly large (2), small (1)	Vomerine teeth: present (1), or absent (0)	Tongue: notched (1), or not notched (0)	Lateral fringes on toes: wide (2), narrow (1), or lacking (0)	Webs on toes: more than one-fourth (2), rudimentary (1), or lacking (0)	TD/ED	TIB/SVL
35	<i>M. (Pa.) omeimontis</i>	56.0–59.5	68.0–72.5	1	1	1	1	1	/	0.52–0.56
36	<i>M. (Pa.) palpebratospinosa</i>	36.2–38.0	/	2	1	0	2	2	/	0.55
37	<i>M. (Pa.) rubrimera</i>	26.7–30.5	/	1	1	1	1	0	0.58–0.76	0.48–0.56
38	<i>M. (Pa.) sangzhiensis</i>	54.7	/	1	1	1	1	1	0.62	0.59
39	<i>M. (Pa.) shuchengensis</i>	102.0–118.3	99.8–115.6	2	0	1	2	2	0.67	0.43–0.47
40	<i>M. (Pa.) shunhuangensis</i>	30.3–33.7	37.6	1	0	0	0	1	0.40–0.63	0.50–0.55
41	<i>M. (Pa.) spinata</i>	47.2–54.4	54.0–55.0	1	0	1	2	2	0.43	0.56–0.58
42	<i>M. (Pa.) tubogranulatus</i>	33.2–39.0	50.5	1	0	0	0	1	0.5	0.45–0.51
43	<i>M. (Pa.) wangongensis</i>	31.0–34.1	38.5–42.8	1	0	0	0	1	0.45–0.53	0.37–0.44
44	<i>M. (Pa.) wuliangshanensis</i>	27.3–31.6	41.3	1	0	1 or 0	0	0	0.5	0.50–0.51
45	<i>M. (Pa.) wushanensis</i>	30.4–35.5	38.4	1	0	0	0	1	0.5	0.47–0.48
46	<i>M. (Pa.) xianjiensis</i>	31.0–36.3	41.6	1	0	0	1	1	0.48–0.60	0.40–0.50

1.00) and almost have no molecular divergences (p -distances 0.0), which was defined as a cryptic species *Megophrys* sp25 in Liu et al. (2018); this population can be further distinguished from all recognized and undescribed species by a combination of distinctive morphological characters (see Taxonomic accounts below). Therefore, the population from Huaping Nature Reserve represents a separately evolving lineage, and is described as a new species, *Megophrys (Panophrys) mirabilis* sp. nov.

The samples from Shimentai Nature Reserve, Guangxi (samples ID 5–8 in Table 1), are grouped into a monophyletic clade with strong node supports (BPP 1.00) and almost have no molecular divergences (p -distances 0.0), which was defined as a cryptic species *Megophrys* sp29 in Liu et al. (2018); samples (ID 14–17 in Table 1) from Mt Yangming, Hunan, are clustered into a monophyletic clade with strong node supports (BPP 1.00) and have small molecular divergences (p -distances 0.3), which was defined as a cryptic species *M. sp28* in Liu et al. (2018). These two populations are sister taxa to each other with significant genetic divergences (p -distances 4.1), and can be distinguished from all congeners by a combination of distinctive morphological characters (see Taxonomic accounts below). Therefore, the populations from Shimentai Nature Reserve and Mt Yangming represent two separately evolving lineages, and are described as new species, *Megophrys (Panophrys) shimentaina* sp. nov. and *Megophrys (Panophrys) yangmingensis* sp. nov., respectively.

The other samples from Mt Yangming, Hunan (samples ID 9–13 in Table 1), cluster into a monophyletic clade with strong node supports (BPP 1.00) and almost have no molecular divergences (p -distances 0.0), which was defined as a cryptic species *Megophrys* sp2 in Liu et al. (2018). This clade is conspicuously distant from the sympatric species *Megophrys (Panophrys) yangmingensis* sp. nov. in phylogeny. Furthermore, this population can be distinguished from all congener species by a combination of distinctive morphological characters (see Taxonomic accounts below). Therefore, this population from Mt Yangming represents a separately evolving lineage, and is described as a new species, *Megophrys (Panophrys) xiangnanensis* sp. nov.

Taxonomic accounts

Megophrys (Panophrys) mirabilis Lyu, Wang & Zhao, sp. nov.

<http://zoobank.org/E624C3F8-5522-4A3C-B376-3519B7E5A377>

Figures 3, 4A

Chresonymy. *Megophrys* sp25 (SYS a002192–93, 2289, 2917 in Liu et al. 2018).

Type material. Holotype. SYS a002917 (Figs 3, 4A), adult male, collected on 16 June 2014 by Yu-Long Li and Ying-Yong Wang from Huaping Nature Reserve (25.5554N, 109.9490E; ca 1300 m a.s.l.), Lingui District, Guilin City, Guangxi Zhuang Autonomous Region, PR China.

Paratypes. Three adult specimens from the same locality as the holotype: male SYS a002192 and female SYS a002193 collected on 10 July 2013 by Jian Zhao and Yu-Long Li; female SYS a002289 collected on 9 September 2013 by Zu-Yao Liu.

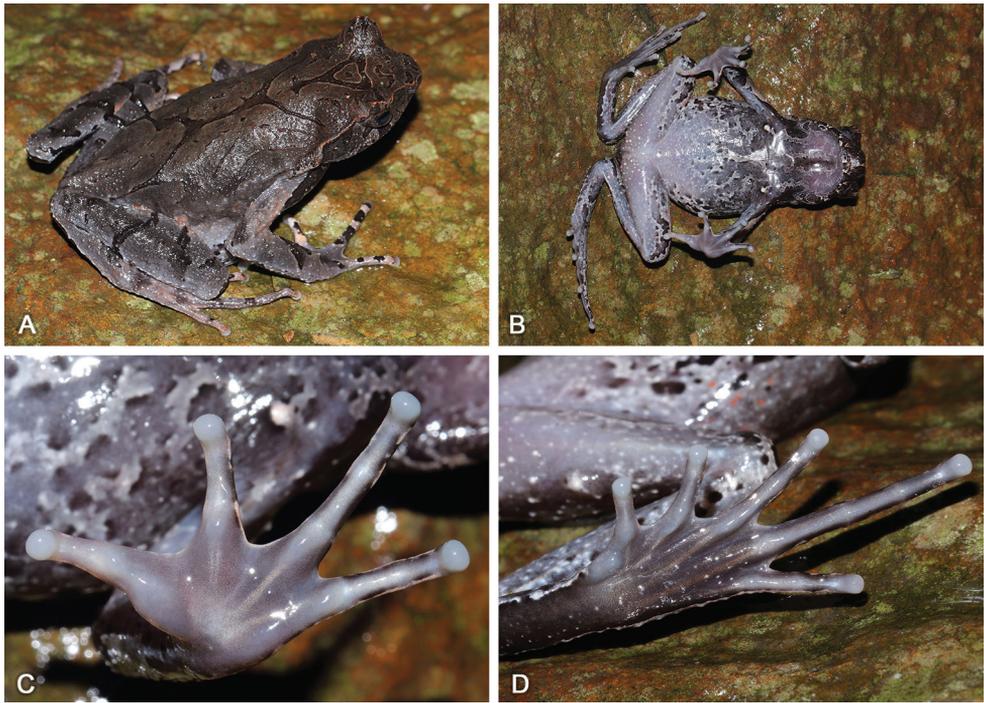


Figure 3. Adult male holotype SYS a002917 of *Megophrys (Panophrys) mirabilis* sp. nov. in life **A** dorso-lateral view **B** ventral view **C** hand **D** foot.

Etymology. The specific epithet *mirabilis* means marvelous, referring to its distinctive habitus and color pattern of this species within the subgenus *Panophrys*.

Common name. Huaping Horned Toad (in English) / Huā Píng Jiǎo Chán (花坪角蟾 in Chinese)

Diagnosis. (1) Body size relatively large, SVL 55.8–61.4 mm ($N = 2$) in adult males and SVL 68.5–74.8 ($N = 2$) mm in adult females; (2) snout rounded in dorsal view; (3) internasal distance smaller than interorbital distance; (4) tympanum clear, moderate size, TD/ED 0.49–0.63; (5) absence of vomerine ridge and vomerine teeth; (6) tongue small, majorly attached to the mandible, free margin small and rounded, not notched behind; (7) hindlimbs slender, heels overlapping and tibio-tarsal articulation reaching forward at the central eye; (8) fingers with distinct lateral fringes, presence of indistinct subarticular tubercles at the bases; (9) toes with distinct lateral fringes and rudiment of webs, presence of indistinct subarticular tubercles at the bases; (10) presence of slightly large horn-like tubercle at the edge of upper eyelid; (11) dorsal skin smooth with granules, (12) skin on flanks flabby, with spiny tubercles; (13) supratympanic fold distinct, with dense tubercles, forming an extremely swollen large shoulder gland above insertion of arm; (14) grayish brown above, tinged with blue in males, but dorsum of head and body reddish brown in females; (15) ventral surface of throat and chest with grayish blue latticed patches and black spots in males, but with

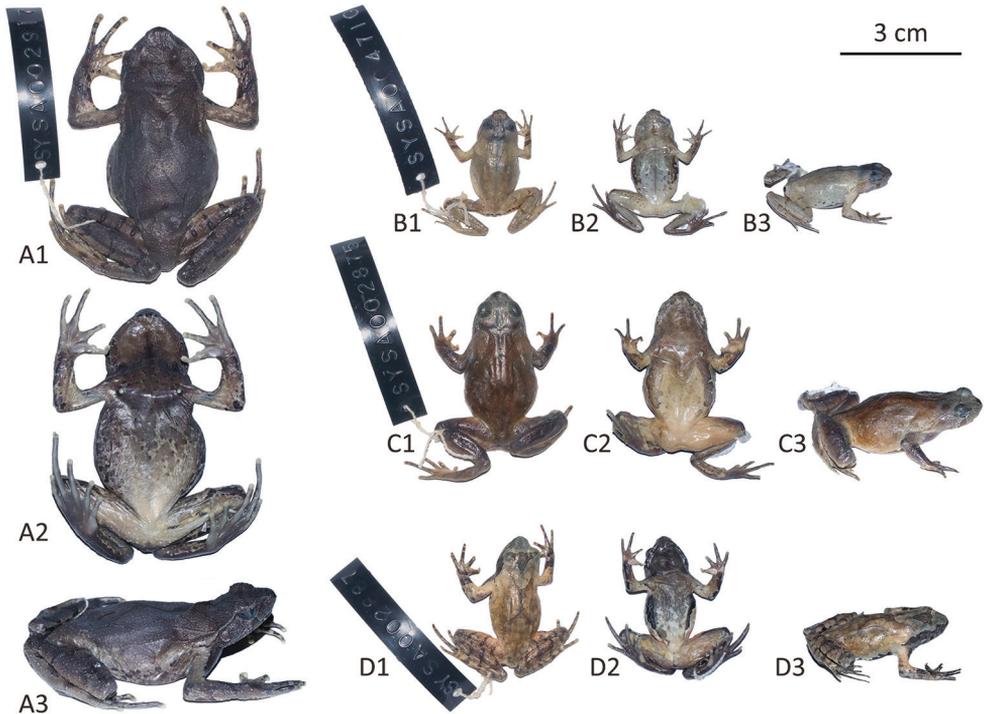


Figure 4. The holotype specimen of each new species in preservative **A** *Megophrys (Panophrys) mirabilis* sp. nov. **B** *M. (Pa.) shimentaina* sp. nov. **C** *M. (Pa.) xiangnanensis* sp. nov. **D** *M. (Pa.) yangmingensis* sp. nov. **1** dorsal view **2** ventral view **3** lateral view.

orange latticed patches and black spots in females; (16) presence of underdeveloped nuptial pads on the dorsal surface of the first finger in adult males.

Comparison. *Megophrys (Panophrys) mirabilis* sp. nov. can be easily distinguished from all recognized congeners, by having a small tongue, majorly attached to the mandible, flank skin flabby with spiny tubercles, and supratympanic fold with dense tubercles forming an extremely swollen large shoulder gland above insertion of arm.

Further, detailed comparative data of *Megophrys (Panophrys) mirabilis* sp. nov. with 42 recognized congeners of *Panophrys* are given in Table 4.

Five *Panophrys* species were previously recorded from the hilly areas among Guangdong, Guangxi, and Hunan, namely *Megophrys (Panophrys) acuta*, *M. (Pa.) brachykolos*, *M. (Pa.) nanlingensis*, *M. (Pa.) obesa*, and *M. (Pa.) shunhuangensis*. *M. (Pa.) mirabilis* sp. nov. differs from *M. (Pa.) acuta* by the larger body size, SVL 55.8–61.4 mm in males and 68.5–74.8 mm in females (vs. 27.1–33.0 mm in males and 28.1–33.6 in females), snout rounded in dorsal view (vs. strongly remarkably pointed), fingers with distinct lateral fringes (vs. absent), and overlapping heels (vs. not meeting). *M. (Pa.) mirabilis* sp. nov. differs from *M. (Pa.) brachykolos* by the larger body size, SVL 55.8–61.4 mm in males and 68.5–74.8 mm in females (vs. 33.7–39.3 mm in males and 33.9–45.9 in females), slightly large horn-like tubercle at upper eyelid (vs. small),

fingers and toes with distinct lateral fringes (vs. all absent), overlapping heels (vs. not meeting). *M. (Pa.) mirabilis* sp. nov. differs from *M. (Pa.) nanlingensis* by the larger body size, SVL 55.8–61.4 mm in males (vs. 30.5–37.3 mm), slightly large horn-like tubercle at upper eyelid (vs. small), absence of vomerine ridge and vomerine teeth (vs. both present), tongue not notched behind (vs. notched), and fingers with distinct lateral fringes (vs. absent). *M. (Pa.) mirabilis* sp. nov. differs from *M. (Pa.) obesa* by larger body size, SVL 55.8–61.4 mm in males and 68.5–74.8 mm in females (vs. 35.6 mm in male and 37.5–41.2 in females), slightly large horn-like tubercle at upper eyelid (vs. small), absence of vomerine ridge (vs. present), fingers and toes with distinct lateral fringes (vs. all absent), and overlapping heels (vs. not meeting). *M. (Pa.) mirabilis* sp. nov. differs from *M. (Pa.) shunhuangensis* by larger body size, SVL 55.8–61.4 mm in males and 68.5–74.8 mm in females (vs. 30.3–33.7 mm in males and 37.6 in female), slightly large horn-like tubercle at upper eyelid (vs. small), and fingers and toes with lateral fringes (vs. all absent).

With a large body size, SVL 55.8–61.4 mm in adult males and 68.5–74.8 mm in adult females, *Megophrys (Panophrys) mirabilis* sp. nov. is significantly different from 30 congeners whose SVL < 50 mm in males or < 60 mm in females, namely *M. (Pa.) baolongensis*, *M. (Pa.) binchuanensis*, *M. (Pa.) boettgeri*, *M. (Pa.) cheni*, *M. (Pa.) daweimontis*, *M. (Pa.) dongguanensis*, *M. (Pa.) fansipanensis*, *M. (Pa.) hoanglienensis*, *M. (Pa.) huangshanensis*, *M. (Pa.) insularis*, *M. (Pa.) jiangi*, *M. (Pa.) jinggangensis*, *M. (Pa.) jiulianensis*, *M. (Pa.) kuatunensis*, *M. (Pa.) latidactyla*, *M. (Pa.) leishanensis*, *M. (Pa.) lini*, *M. (Pa.) lishuiensis*, *M. (Pa.) minor*, *M. (Pa.) mufumontana*, *M. (Pa.) nankunensis*, *M. (Pa.) ombrophila*, *M. (Pa.) palpebralespinosa*, *M. (Pa.) rubrimera*, *M. (Pa.) spinata*, *M. (Pa.) tubero granulatus*, *M. (Pa.) wugongensis*, *M. (Pa.) wuliangshanensis*, *M. (Pa.) wushanensis*, and *M. (Pa.) xianjuensis*.

Megophrys (Panophrys) mirabilis sp. nov. can be further distinguished from the remaining seven congeners by the following characteristics: SVL 55.8–61.4 mm in adult males and 68.5–74.8 mm in adult females [vs. SVL 45.1–51.0 mm in adult males in *M. (Pa.) binlingensis*; vs. SVL 81.3 mm in adult male in *M. (Pa.) caudoprocta*; vs. SVL 63.5 mm in adult female in *M. (Pa.) jingdongensis*; vs. SVL 102.0–118.3 mm in adult males and 99.8–115.6 mm in adult females in *M. (Pa.) shuichengensis*]; slightly large horn-like tubercle at upper eyelid [vs. small in *M. (Pa.) binlingensis*, *M. (Pa.) jingdongensis*, *M. (Pa.) omeimontis*, and *M. (Pa.) sangzhiensis*]; vomerine teeth absent [vs. present in *M. (Pa.) caudoprocta*, *M. (Pa.) jingdongensis*, *M. (Pa.) liboensis*, *M. (Pa.) omeimontis*, and *M. (Pa.) sangzhiensis*]; tongue not notched behind [vs. notched in *M. (Pa.) binlingensis*, *M. (Pa.) jingdongensis*, *M. (Pa.) liboensis*, *M. (Pa.) omeimontis*, *M. (Pa.) sangzhiensis*, and *M. (Pa.) shuichengensis*]; lateral fringes on toes narrow [vs. wide in *M. (Pa.) jingdongensis*, *M. (Pa.) liboensis*, and *M. (Pa.) shuichengensis*]; rudimentary webs on toes [vs. more than one-fourth webs in *M. (Pa.) jingdongensis* and *M. (Pa.) shuichengensis*].

Description of holotype. Adult male. Body size large, SVL 61.4 mm; head width slightly larger than head length, HDW/HDL 1.02; snout rounded in dorsal view, projecting, sloping backward to mouth in profile, protruding well beyond margin of lower jaw; top of head flat; eyes large, ED 0.31 of HDL, pupil vertical; nostril oblique-

ovoid; canthus rostralis well developed; loreal region slightly oblique; internasal distance smaller than interorbital distance; tympanum clear, TD/ED 0.49; large ovoid choanae at the base of the maxilla; absence of vomerine ridge and vomerine teeth; tongue small, majority attached at the mouth, margin rounded, not notched behind; absence of vocal sac.

Radio-ulna length 0.26 of SVL and hand 0.28 of SVL; hand without webs, fingers with distinct lateral fringes, relative finger length $II < I < IV < III$; tips of fingers slightly dilated, round; one indistinct subarticular tubercle at the bases of each finger; metacarpal tubercles indistinct, the inner one observably enlarged and the outer one smaller; presence of underdeveloped nuptial pad on the dorsal surface of the first finger, without nuptial spines. Hindlimbs slender, tibio-tarsal articulation reaching forward at the central eye when hindlimb is stretched along the side of the body; heels overlapping when the flexed hindlimbs are held at right angles to the body axis; tibia length 0.47 of SVL and foot length 0.71 of SVL; relative toe length $I < II < V < III < IV$; tips of toes round and slightly dilated; toes with narrow lateral fringes and rudiment of webs; one indistinct subarticular tubercle at the bases of each toe; inner metatarsal tubercle long ovoid and the outer one absent.

Dorsal skin smooth with sparse granules; flanks flabby with spiny tubercles; distinct supratympanic fold curving postero-ventrally from posterior corner of eye to a level above insertion of arm; small tubercles arranged from above the nostril, along the canthus rostralis, edge of upper eyelid and supratympanic fold, to the posterior margin of temporal region; a distinct horn-like prominent tubercle on the edge of upper eyelid; a discontinuous X-shaped ridge with several short ridges on two sides on the back; transverse skin ridges on the dorsal shank and thigh; ventral surface smooth; several tubercles on posterior hindlimbs; small pectoral gland closer to axilla; a single large femoral gland on rear of thigh.

Coloration. Grayish brown above in life; an dark interorbital triangle with light colored center and edge; a dark X-shaped marking with light edge on the central of dorsum; dark brown transverse bands on forearms and hindlimbs; supratympanic fold light gray; dark vertical band below the eye; iris grayish brown; ventral surface grayish white; throat and chest with grayish blue latticed patches and black spots; ventral hands and feet grayish white, tips of digits creamy white, metacarpal tubercle and metatarsal tubercle grayish white; pectoral gland and femoral gland white.

Variations. Measurement data of type series are listed in Table 5. All paratypes are similar to the holotype. Females (SVL 68.5–74.8 mm) are significantly larger than males (SVL 55.8–61.4 mm). Dorsal surfaces reddish brown and ventral surfaces with orange latticed patches and black spots in females SYS a002193, 2289.

Distribution and ecology. Currently, *Megophrys (Panophrys) mirabilis* sp. nov. is only known from Huaping Nature Reserve, northeastern Guangxi. The individuals were found on shrubbery branches near trail paths between elevations of 1300–1330 m a.s.l. from June to September. Males were not calling when found, but the collected female specimens bear mature yellowish oocytes. Tadpoles have not been found and ecological information remains unknown.

Table 5. Measurements (in mm) of the type series of *Megophrys (Panophrys) mirabilis* sp. nov., * for the holotype.

	SYS a002917 *	SYS a002192	SYS a002193	SYS a002289
Sex	Male	Male	Female	Female
SVL	61.4	55.8	74.8	68.5
HDL	21.4	18.8	23.7	22.6
HDW	21.8	18.8	23.9	22.4
SNT	7.8	7.1	9.0	8.8
IND	6.7	5.9	7.5	6.8
IOD	7.2	6.5	8.1	7.6
ED	6.7	5.9	8.1	6.8
TD	3.3	3.2	4.3	4.3
TED	3.3	3.2	4.2	3.7
HND	17.3	15.3	20.2	19.5
RAD	15.9	13.9	18.3	17.6
FTL	43.7	37.8	48.8	43.2
TIB	28.9	26.3	33.8	30.5

***Megophrys (Panophrys) shimentaina* Lyu, Liu & Wang, sp. nov.**

<http://zoobank.org/E9F8A869-8923-4C0F-8750-181EE0843A07>

Figures 4B, 5, 6A

Chresonymy. *Megophrys* sp29 (SYS a002077, 2081, 4172–4173 in Liu et al. 2018)

Type material. Holotype. SYS a004710 (Figs 4B, 5), adult male, collected on 27 April 2016 by Zhi-Tong Lyu and Yuan-Qiu Li from Shimentai Nature Reserve (24.4095N, 113.1095E; ca 370 m a.s.l.), Yingde City, Qingyuan City, Guangdong Province, PR China.

Paratypes. Eleven adult males from the same locality as the holotype: SYS a002077, 2081–2085, collected on 25–26 April 2013 by Run-Lin Li and Yuan-Qiu Li; SYS a004172–4173, collected on 27 July 2015 by Ying-Yong Wang and Yuan-Qiu Li; SYS a005448/CIB 110015 collected on 19 August 2016 and SYS a005992–5993 collected on 20 June 2017 by Zhi-Tong Lyu and Yong-You Zhao.

Etymology. The specific epithet *shimentaina* refers to its type locality, Shimentai Nature Reserve.

Common name. Shimentai Horned Toad (in English) / Shí Mén Tǎi Jiǎo Chán (石 门 台 角 蟾 in Chinese)

Diagnosis. (1) Body size small, SVL 28.0–30.6 (28.9 ± 0.9, *N* = 12) mm in adult males; (2) snout rounded in dorsal view; (3) tympanum clear, TD/ED 0.57–0.66; (4) presence of weak vomerine ridge and vomerine teeth; (5) margin of tongue rounded, not notched behind; (6) hindlimbs slender, heels overlapping and tibio-tarsal articulation reaching forward between tympanum to anterior corner of eye; (7) tibia 0.44–0.53 of SVL and foot 0.62–0.76 of SVL; (8) fingers with narrow lateral fringes, presence of indistinct subarticular tubercles at the bases; (9) toes with narrow lateral fringes and rudiment of webs, absence of subarticular tubercle; (10) presence of a small horn-like tubercle at the edge of upper eyelid; (11) presence of tiny, barely visible, black to dark brown spines on the whole dorsal skin, flanks, dorsal limbs, the



Figure 5. Adult male holotype SYS a004710 of *Megophrys (Panophrys) shimentaina* sp. nov. in life **A** dorso-lateral view **B** ventral view **C** hand **D** foot **E** posterior view **F** large warts on the flanks and absence of conical spines on supratympanic fold.

region around cloaca, and rear of hindlimbs; (12) dorsal skin rough, a discontinuous “/ \”-shaped ridge with two discontinuous dorsolateral ridges on two sides on the back; (13) several large warts on the flanks; (14) supratympanic fold distinct and white, with tiny spines; (15) light brown above, a dark brown stripe on each upper eyelid; (16) single subgular vocal sac in males; (17) weak nuptial pads with serried olive nuptial spines, on the dorsal surface of the first and second fingers in adult males.

Comparison. Comparative data of *Megophrys (Panophrys) shimentaina* sp. nov. with *M. (Pa.) mirabilis* sp. nov. and 42 recognized congeners of *Panophrys* are given in Table 4.

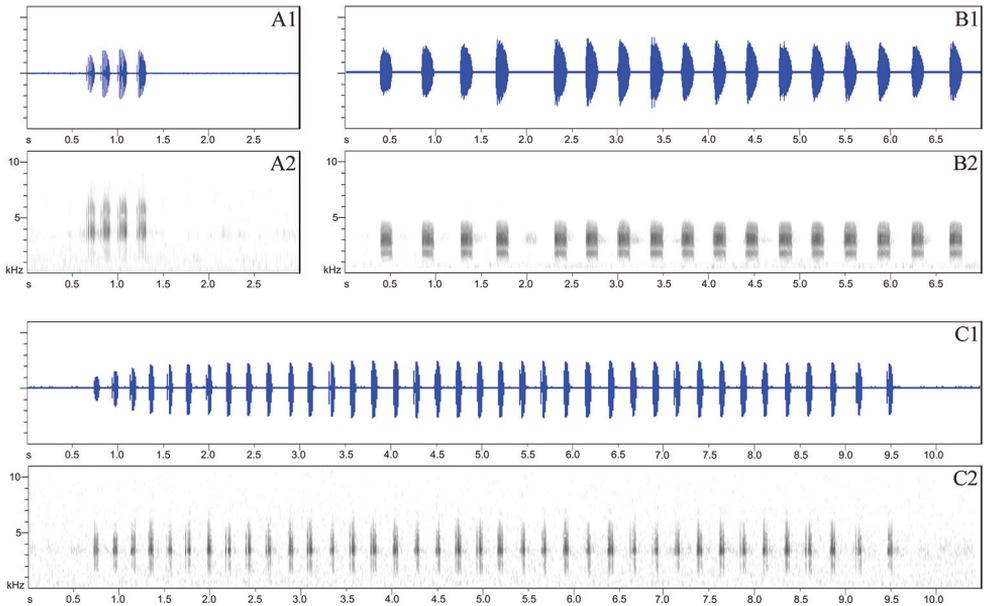


Figure 6. Advertisement calls spectrograms **A** *Megophrys (Panophrys) shimentaina* sp. nov. **B** *M. (Pa.) xiangananensis* sp. nov. **C** *M. (Pa.) yangmingensis* sp. nov. **1** sonogram **2** waveform.

Megophrys (Panophrys) shimentaina sp. nov. differs from *M. (Pa.) mirabilis* sp. nov. by the smaller body size, SVL 28.0–30.6 mm in males (vs. 55.8–61.4 mm in males), small horn-like tubercle at upper eyelid (vs. slightly large), presence of vomerine teeth (vs. absent), the presence of tiny spines on the whole dorsal skin, flanks, dorsal limbs, the region around cloaca, and rear of hindlimbs (vs. such spines absent), presence of vocal sac in males (vs. absent), and presence of nuptial spines in males (vs. absent).

Compared with the five *Panophrys* species previously recorded from the hilly areas among Guangdong, Guangxi, and Hunan, *Megophrys (Panophrys) shimentaina* sp. nov. differs from *M. (Pa.) acuta* by the small horn-like tubercle at upper eyelid (vs. slightly large), snout rounded in dorsal view (vs. strongly remarkably pointed), presence of vomerine teeth (vs. absent), presence of tiny spines on the whole dorsal skin, flanks, dorsal limbs, the region around cloaca, and rear of hindlimbs (vs. such spines absent), and overlapping heels (vs. not meeting). *M. (Pa.) shimentaina* sp. nov. differs from *M. (Pa.) brachykolos* by the smaller body size SVL 28.0–30.6 mm in males (vs. 33.7–39.3 mm in males), presence of vomerine teeth (vs. absent), presence of tiny spines on the whole dorsal skin, flanks, dorsal limbs, the region around cloaca, and rear of hindlimbs (vs. such spines absent), narrow lateral fringes on toes (vs. absent), and overlapping heels (vs. not meeting). *M. (Pa.) shimentaina* sp. nov. differs from *M. (Pa.) nanlingensis* by the presence of tiny spines on the whole dorsal skin, flanks, dorsal limbs, the region around cloaca, and rear of hindlimbs (vs. such spines absent), and tongue not notched behind (vs. notched). *M. (Pa.) shimentaina* sp. nov. differs from *M. (Pa.) obesa* by the smaller body size SVL 28.0–30.6 mm in males (vs. 35.6 mm in

single male), presence of vomerine teeth (vs. absent), presence of tiny spines on the whole dorsal skin, flanks, dorsal limbs, the region around cloaca, and rear of hindlimbs (vs. such spines absent), narrow lateral fringes on toes (vs. absent), and overlapping heels (vs. not meeting). *M. (Pa.) shimentaina* sp. nov. differs from *M. (Pa.) shunhuanensis* by the presence of vomerine teeth (vs. absent), tibio-tarsal articulation reaching forward between tympanum to anterior corner of eye (vs. at the eye), and the presence of tiny spines on the whole dorsal skin, flanks, dorsal limbs, the region around cloaca, and rear of hindlimbs (vs. such spines absent).

With a small body size, SVL 28.0–30.6 mm in adult males, *Megophrys (Panophrys) shimentaina* sp. nov. is significantly different from 15 congeners whose SVL > 35 mm in males, namely *M. (Pa.) baolongensis*, *M. (Pa.) binlingensis*, *M. (Pa.) caudoprocta*, *M. (Pa.) hoanglienensis*, *M. (Pa.) huangshanensis*, *M. (Pa.) insularis*, *M. (Pa.) jingdongensis*, *M. (Pa.) jinggangensis*, *M. (Pa.) latidactyla*, *M. (Pa.) liboensis*, *M. (Pa.) omeimontis*, *M. (Pa.) palpebralespinosa*, *M. (Pa.) sangzhiensis*, *M. (Pa.) shuichengensis*, and *M. (Pa.) spinata*.

Megophrys (Panophrys) shimentaina sp. nov. can be further distinguished from the remaining 22 congeners by the following characteristics: vomerine teeth present [vs. absent in *M. (Pa.) binchuanensis*, *M. (Pa.) boettgeri*, *M. (Pa.) cheni*, *M. (Pa.) jiangi*, *M. (Pa.) kuatunensis*, *M. (Pa.) leishanensis*, *M. (Pa.) lini*, *M. (Pa.) lishuiensis*, *M. (Pa.) minor*, *M. (Pa.) mufumontana*, *M. (Pa.) ombrophila*, *M. (Pa.) tuberogranulatus*, *M. (Pa.) wugongensis*, *M. (Pa.) wuliangshanensis*, *M. (Pa.) wushanensis*, and *M. (Pa.) xianjuensis*]; tongue not notched behind [vs. notched in *M. (Pa.) cheni*, *M. (Pa.) boettgeri*, *M. (Pa.) fansipanensis*, *M. (Pa.) jiulianensis*, *M. (Pa.) kuatunensis*, *M. (Pa.) minor*, and *M. (Pa.) rubrimera*]; lateral fringes on toes narrow [vs. absent in *M. (Pa.) daweimontis*, *M. (Pa.) dongguanensis*, *M. (Pa.) fansipanensis*, *M. (Pa.) jiangi*, *M. (Pa.) jiulianensis*, *M. (Pa.) leishanensis*, *M. (Pa.) lishuiensis*, *M. (Pa.) minor*, *M. (Pa.) nankunensis*, *M. (Pa.) ombrophila*, *M. (Pa.) tuberogranulatus*, *M. (Pa.) wugongensis*, and *M. (Pa.) wuliangshanensis*; wide in *M. (Pa.) binchuanensis*, *M. (Pa.) boettgeri*, *M. (Pa.) cheni*, and *M. (Pa.) lini*; vs. absent in females while wide in males in *M. (Pa.) wushanensis*]; rudimentary webs on toes [vs. lacking webs in *M. (Pa.) daweimontis*, *M. (Pa.) fansipanensis*, *M. (Pa.) kuatunensis*, *M. (Pa.) lishuiensis*, *M. (Pa.) ombrophila*, *M. (Pa.) rubrimera*, and *M. (Pa.) wuliangshanensis*].

Description of holotype. Adult male. Body size small, SVL 28.4 mm; head width slightly smaller than head length, HDW/HDL 0.95; snout rounded in dorsal view, projecting, sloping backward to mouth in profile, protruding well beyond margin of lower jaw; top of head flat; eyes large, ED 0.33 of HDL, pupil vertical; nostril oblique-ovoid; canthus rostralis well developed; loreal region slightly oblique; internasal distance slightly larger than interorbital distance; tympanum clear, in medium size, TD/ED 0.61; large ovoid choanae at the base of the maxilla; presence of weak vomerine ridge and vomerine teeth; margin of tongue rounded, not notched behind; presence of a single subgular vocal sac, a pair of slit-like openings at posterior of jaw.

Radio-ulna length 0.22 of SVL and hand 0.26 of SVL; hand without webs, fingers with narrow lateral fringes, relative finger length I \approx II < IV < III; tips of fingers slightly dilated, round; one indistinct subarticular tubercle at the bases of each finger;

inner metacarpal tubercle observably enlarged and the outer one smaller; nuptial pads with serried olive nuptial spines on the dorsal surface of the first and second fingers. Hindlimbs slender, tibio-tarsal articulation reaching forward to the posterior corner of eye when hindlimb is stretched along the side of the body; heels overlapping when the flexed hindlimbs are held at right angles to the body axis; tibia length 0.47 of SVL and foot length 0.67 of SVL; relative toe length I < II < V < III < IV; tips of toes round and slightly dilated; toes with distinct lateral fringes and rudiment of webs, without subarticular tubercle; inner metatarsal tubercle long ovoid and the outer one absent.

Dorsal skin rough; numerous granules densely arranged on the top of head, loreal region, lips, temporal region, dorsal body, flanks and dorsal limbs; several tubercles on upper eyelid, including a horn-like prominent tubercle on the edge; all granules and tubercles bearing tiny, barely visible spines; clear supratympanic fold with tiny spines, curving postero-ventrally from posterior corner of eye to a level above insertion of arm; tubercles and granules forming discontinuous “/ \”-shaped ridge and two discontinuous dorsolateral ridges on two sides at the central back; large tubercles and warts on the flanks; ventral surface smooth; several granules bearing black spines on the region around cloaca and rear of hindlimbs; small pectoral gland closer to axilla; a single large femoral gland on rear of thigh.

Coloration. Light brown above in life; a dark brown stripe on dorsal surface of each eye; narrow dark brown transverse bands on forearms and hindlimbs; supratympanic fold white; dark vertical band below the eye; iris reddish brown; all spines black or dark brown; ventral surface pale; throat flesh color; scarlet spots on the chest; a large white blotch on the belly; a pair of lateroventral longitudinal broad black stripes with several white tubercles on two sides; ventral limbs flesh color with white spots; ventral hands and ventral feet brown, tips of digits pale brown; metacarpal tubercle and metatarsal tubercle reddish; pectoral gland and femoral gland white.

Variations. Measurement data of type series are listed in Table 6. All paratypes are extremely similar to the holotype but SYS a002082 has an “X” pattern on its back.

Table 6. Measurements (in mm) of the type series of *Megophrys (Panophrys) shimentaina* sp. nov., * for the holotype.

	SYS a004710*	SYS a002077	SYS a002081	SYS a002082	SYS a002083	SYS a002084	SYS a002085	SYS a004172	SYS a004173	SYS a005448 / CIB 110015	SYS a005992	SYS a005993
Sex	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male
SVL	28.4	28.5	28.1	30.6	29.0	29.2	28.8	28.0	30.4	28.0	29.3	28.7
HDL	10.0	10.1	9.9	10.4	10.1	10.1	10.0	9.9	10.5	10.7	10.1	10.1
HDW	9.6	9.5	9.5	10.0	9.6	9.7	9.5	9.8	10.0	10.3	9.8	9.9
SNT	3.3	3.4	3.3	3.5	3.3	3.4	3.4	3.3	3.4	3.2	3.4	3.4
IND	3.0	3.0	3.0	3.1	3.1	3.1	3.0	3.1	3.2	2.8	3.1	3.0
IOD	2.6	2.8	2.8	2.9	2.8	2.8	2.7	2.8	2.9	3.1	2.8	2.6
ED	3.3	3.2	3.3	3.4	3.3	3.3	3.3	3.4	3.4	3.4	3.4	3.4
TD	2.0	1.8	1.9	2.2	2.0	1.9	2.0	1.9	2.1	2.1	2.2	2.1
TED	1.6	1.6	1.5	1.6	1.7	1.5	1.5	1.4	1.6	1.5	1.5	1.5
HND	7.4	7.2	7.3	7.5	7.5	7.4	7.3	6.8	7.2	7.8	7.1	7.5
RAD	6.2	6.1	6.2	6.4	6.2	6.2	6.0	5.5	6.1	6.3	6.0	6.4
FTL	19.1	19.9	19.0	20.5	19.4	19.3	19.3	17.9	18.7	21.4	19.4	20.3
TIB	13.5	14.2	13.2	14.9	13.4	14.3	13.6	12.8	13.3	14.9	13.4	14.6

Distribution and ecology. Currently, *Megophrys (Panophrys) shimentaina* sp. nov. is known only from Shimentai Nature Reserve, northern Guangdong. This toad is uncommon in its distribution areas. All individuals were found from two slowly flowing mountain streams between elevations of 210–500 m a.s.l. Males call on plant leaves from April to August, suggesting their breeding season corresponds to this period. Females and tadpoles have not been found.

Vocalization. The advertisement calls of *Megophrys (Panophrys) shimentaina* sp. nov. were recorded from four males at 18–20 °C air temperature on 27 April 2016. Thirty calls with 96 notes are measured and the spectrograms are shown in Fig. 6A. The advertisement call is made up of 3.8 ± 0.4 (3–4, $N = 30$) continuous click notes. Each call lasts 0.50 ± 0.07 s (0.36–0.58 s, $N = 30$) and each note lasts 85 ± 8 ms (64–101 ms, $N = 96$) with an interval of 67 ± 14 ms (44–121 ms, $N = 71$) between every two notes. The peak frequency measures at 4895 ± 124 Hz (4688–5156 Hz, $N = 96$).

***Megophrys (Panophrys) xiangnanensis* Lyu, Zeng & Wang, sp. nov.**

<http://zoobank.org/F27079DE-C1AF-4B00-900F-1E1783C58762>

Figures 4C, 6B, 7

Chresonymy. *Megophrys* sp2 (SYS a002874–76, 2878–79 in Liu et al. 2018)

Holotype. SYS a002875 (Figs 4C, 7), adult male, collected on 12 June 2014 by Yu-Long Li and Ying-Yong Wang from Mt Yangming (26.1177N, 111.8945E; ca 1360 m a.s.l.), Shuangpai County, Yongzhou City, Hunan Province, PR China.

Paratypes. Eleven adult specimens, female SYS a002874 and males SYS a002876/CIB 116072 and SYS a002878–2886, collected at the same time from the same locality as the holotype.

Etymology. The specific epithet *xiangnanensis* is an adjective derived from Chinese Pinyin Xiāng Nán, which means southern Hunan, for the distribution area of this species.

Common name. Southern Hunan Horned Toad (in English) / Xiāng Nán Jiǎo Chán (湘南角蟾 in Chinese)

Diagnosis. (1) Moderate body size, SVL 38.6–42.0 mm (40.3 ± 1.3 , $N = 11$) in adult males and SVL 44.4 mm in adult female; (2) snout rounded in dorsal view; (3) tympanum clear, TD/ED 0.38–0.49; (4) presence of weak vomerine ridge, absence of vomerine teeth; (5) margin of tongue rounded, not notched behind; (6) hindlimbs slender, heels just meeting and tibio-tarsal articulation reaching forward between eye and tympanum; (7) tibia 0.41–0.46 of SVL and foot 0.57–0.62 of SVL; (8) fingers without lateral fringes, presence of distinct subarticular tubercles at the bases; (9) toes with relatively wide lateral fringes and rudiment of webs, presence of distinct subarticular tubercles at the bases; (10) presence of small horn-like tubercle at the edge of upper eyelid; (11) dorsal skin smooth with sparse granules, a discontinuous X-shaped ridge with two discontinuous dorsolateral ridges on two side on the back; (12) sparse tubercles on the flanks; (13) supratympanic fold light colored; (14) single subgular vocal sac in males; (15) presence of nuptial pads on the dorsal surface of the first and second fingers in adult males.

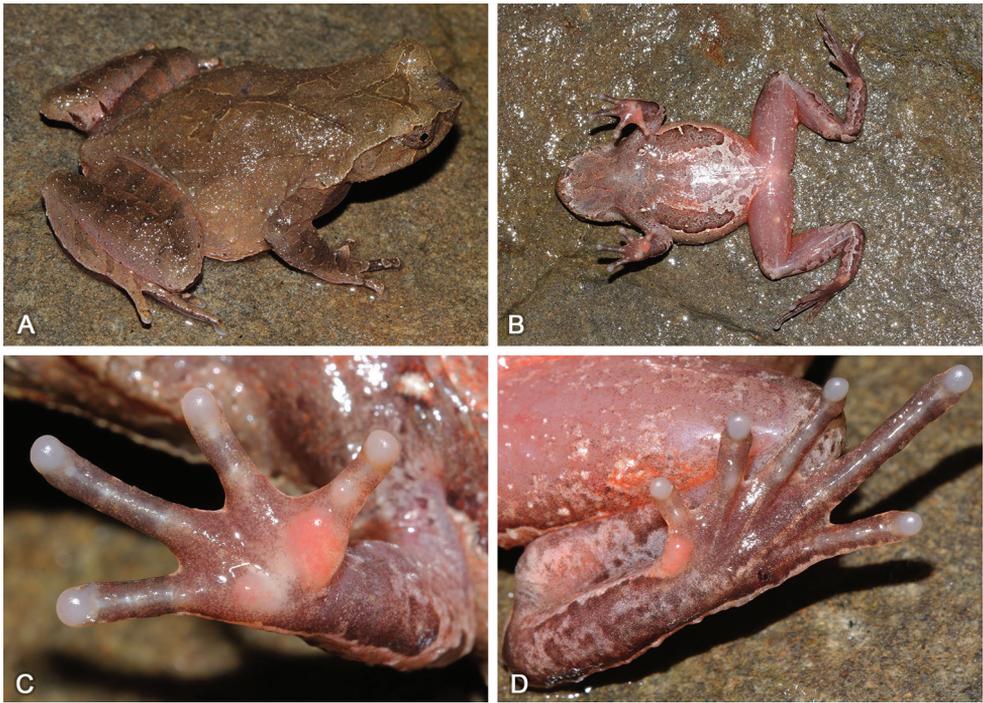


Figure 7. Adult male holotype SYS a002875 of *Megophrys (Panophrys) xiangnanensis* sp. nov. in life **A** dorsolateral view **B** ventral view **C** hand **D** foot.

Comparison. Comparative data of *Megophrys (Panophrys) xiangnanensis* sp. nov. with *M. (Pa.) mirabilis* sp. nov., *M. (Pa.) shimentaina* sp. nov., and 42 recognized congeners of *Panophrys* are given in Table 4.

Megophrys (Panophrys) xiangnanensis sp. nov. differs from *M. (Pa.) mirabilis* sp. nov. by the smaller body size, SVL 38.6–42.0 mm in males and 44.4 mm in single female (vs. 55.8–61.4 mm in males and 68.5–74.8 in females), small horn-like tubercle at upper eyelid (vs. slightly large), wide lateral fringes on toes (vs. narrow), heels just meeting (vs. overlapping), presence of vocal sac in males (vs. absent), and presence of nuptial spines in males (vs. absent).

Megophrys (Panophrys) xiangnanensis sp. nov. differs from *M. (Pa.) shimentaina* sp. nov. by the larger body size, SVL 38.6–42.0 mm in males (vs. 28.0–30.6 mm in males), absence of vomerine teeth (vs. present), wide lateral fringes on toes (vs. narrow), and heels just meeting (vs. overlapping).

Compared with the five *Panophrys* species previously recorded from the hilly areas among Guangdong, Guangxi and Hunan, *Megophrys (Panophrys) xiangnanensis* sp. nov. differs from *M. (Pa.) acuta* by the larger body size, SVL 38.6–42.0 mm in males and 44.4 mm in single female (vs. 27.1–33.0 mm in males and 28.1–33.6 mm in females), small horn-like tubercle at upper eyelid (vs. slightly large), snout rounded in dorsal view (vs. strongly remarkably pointed), wide lateral fringes on toes (vs. narrow),

and heels just meeting (vs. not meeting). *M. (Pa.) xiangnanensis* sp. nov. differs from *M. (Pa.) brachykolos* by the wide lateral fringes on toes (vs. absent), and heels just meeting (vs. not meeting). *M. (Pa.) xiangnanensis* sp. nov. differs from *M. (Pa.) nanlingensis* by the larger body size, SVL 38.6–42.0 mm in males (vs. 30.5–37.3 mm in males), absence of vomerine teeth (vs. present), tongue not notched behind (vs. notched), wide lateral fringes on toes (vs. narrow), and heels just meeting (vs. overlapping). *M. (Pa.) xiangnanensis* sp. nov. differs from *M. (Pa.) obesa* by the larger body size, SVL 38.6–42.0 mm in males and 44.4 mm in single female (vs. 35.6 mm in single male and 37.5–41.2 mm in females), wide lateral fringes on toes (vs. absent), and heels just meeting (vs. not meeting). *M. (Pa.) xiangnanensis* sp. nov. differs from *M. (Pa.) shunhuangensis* by the larger body size, SVL 38.6–42.0 mm in males and 44.4 mm in single female (vs. 30.3–33.7 mm in males and 37.6 in female), wide lateral fringes on toes (vs. absent), and heels just meeting (vs. overlapping).

With a moderate body size, SVL 38.6–42.0 mm in adult males, *Megophrys (Panophrys) xiangnanensis* sp. nov. is significantly different from 18 congeners whose SVL < 35 mm or > 45 mm in males, namely *M. (Pa.) binlingensis*, *M. (Pa.) caudoprocta*, *M. (Pa.) cheni*, *M. (Pa.) jingdongensis*, *M. (Pa.) jiulianensis*, *M. (Pa.) kuatunensis*, *M. (Pa.) liboensis*, *M. (Pa.) lishuiensis*, *M. (Pa.) mufumontana*, *M. (Pa.) nankunensis*, *M. (Pa.) ombrophila*, *M. (Pa.) omeimontis*, *M. (Pa.) rubrimera*, *M. (Pa.) sangzhiensis*, *M. (Pa.) shuichengensis*, *M. (Pa.) spinata*, *M. (Pa.) wugongensis*, and *M. (Pa.) wuliangshanensis*.

Megophrys (Panophrys) xiangnanensis sp. nov. can be further distinguished from the remaining 19 congeners by the following characteristics: small horn-like tubercle at upper eyelid [vs. slightly large in *M. (Pa.) jinggangensis*, *M. (Pa.) latidactyla*, and *M. (Pa.) palpebralespinosa*]; vomerine teeth absent [vs. present in *M. (Pa.) daweimontis*, *M. (Pa.) dongguanensis*, *M. (Pa.) fansipanensis*, *M. (Pa.) hoanglienensis*, *M. (Pa.) insularis*, *M. (Pa.) jinggangensis*, *M. (Pa.) latidactyla*, and *M. (Pa.) palpebralespinosa*]; tongue not notched behind [vs. notched in *M. (Pa.) baolongensis*, *M. (Pa.) boettgeri*, *M. (Pa.) fansipanensis*, *M. (Pa.) hoanglienensis*, *M. (Pa.) huangshanensis*, *M. (Pa.) insularis*, and *M. (Pa.) minor*]; lateral fringes on toes wide [vs. absent in *M. (Pa.) baolongensis*, *M. (Pa.) daweimontis*, *M. (Pa.) dongguanensis*, *M. (Pa.) fansipanensis*, *M. (Pa.) hoanglienensis*, *M. (Pa.) huangshanensis*, *M. (Pa.) insularis*, *M. (Pa.) jiangi*, *M. (Pa.) leishanensis*, *M. (Pa.) minor*, and *M. (Pa.) tuberogranulatu*; vs. narrow in *M. (Pa.) jinggangensis* and *M. (Pa.) xianjuensis*; vs. absent in females while wide in males in *M. (Pa.) wushanensis*]; rudimentary webs on toes [vs. more than one-fourth webs in *M. (Pa.) palpebralespinosa*; vs. lacking webs in *M. (Pa.) baolongensis*, *M. (Pa.) daweimontis*, *M. (Pa.) fansipanensis*, *M. (Pa.) hoanglienensis*, and *M. (Pa.) huangshanensis*].

Description of holotype. Adult male. Moderate body size, SVL 40.9 mm; head width slightly larger than head length, HDW/HDL 1.02; snout rounded in dorsal view, projecting, sloping backward to mouth in profile, protruding well beyond margin of lower jaw; top of head flat; eyes large, ED 0.41 of HDL, pupil vertical; nostril oblique-ovoid; canthus rostralis well developed; loreal region slightly oblique; internasal distance slightly larger than interorbital distance; tympanum clear, TD/ED 0.44; large ovoid choanae at the base of the maxilla; presence of weak vomerine ridge, ab-

sence of vomerine teeth; margin of tongue rounded, not notched behind; presence of a single subgular vocal sac, a pair of slit-like openings at posterior of jaw.

Radio-ulna length 0.22 of SVL and hand 0.23 of SVL; hand without webs, fingers without lateral fringes, relative finger length $I < II < IV < III$; tips of fingers slightly dilated, round; one distinct subarticular tubercle at the bases of each finger; inner metacarpal tubercle observably enlarged and the outer one smaller; a single nuptial pad on the dorsal surface of the first and second fingers. Hindlimbs slender, tibio-tarsal articulation reaching forward between eye and tympanum when hindlimb is stretched along the side of the body; heels just meeting when the flexed hindlimbs are held at right angles to the body axis; tibia length 0.42 of SVL and foot length 0.58 of SVL; relative toe length $I < II < V < III < IV$; tips of toes round and slightly dilated; toes with relatively wide lateral fringes and rudiment of webs; one distinct subarticular tubercle at the bases of each toe; inner metatarsal tubercle long ovoid and the outer one absent.

Dorsal skin smooth with sparse granules; sparse tubercles on the flanks; a horn-like prominent tubercle on the edge; clear supratympanic fold curving postero-ventrally from posterior corner of eye to a level above insertion of arm; a discontinuous X-shaped ridge and two discontinuous dorsolateral ridges on two sides at the central back; sparse tubercles on the dorsal shank and thigh; ventral surface smooth; several tubercles on posterior hindlimbs; small pectoral gland closer to axilla; a single large femoral gland on rear of thigh.

Coloration. Yellowish brown above in life; a dark interorbital triangle with light colored center and edge; a dark X-shaped making with light edge on the central of dorsum; dark brown transverse bands on forearms and hindlimbs; supratympanic fold light colored; dark vertical band below the eye; iris light brown with net-like stripes; throat and anterior chest reddish gray; a longitudinal stripe on the throat; a large white blotch with scarlet spots on the belly; one pair of lateroventral longitudinal broad reddish stripes on two sides; ventral limbs flesh color; ventral hands purplish, tips of fingers pale-grey, metacarpal tubercle reddish; ventral feet purplish brown, tips of fingers pale grey, metatarsal tubercle reddish; pectoral gland and femoral gland white.

Variations. Measurement data of type series are listed in Table 7. All paratypes are similar to the holotype. Female (SVL 44.4 mm) are slightly larger than males (SVL 38.6–42.0 mm).

Distribution and ecology. *Megophrys (Panophrys) xiangnanensis* sp. nov. is currently known only from Mt Yangming, southwestern Hunan. This toad inhabits areas near slowly flowing mountain streams surrounded by moist subtropical secondary evergreen broadleaf forests between elevations of 900–1400 m a.s.l. Males call from May to July, and during this time the males bear nuptial pads. Only one female individual was found, and tadpoles and other ecological information remain unknown.

Vocalization. The advertisement calls of *Megophrys (Panophrys) xiangnanensis* sp. nov. were recorded from the Holotype at 16 °C air temperature on 12 June 2014. Four calls with 98 notes are measured and the spectrograms are shown in Fig. 6B. The advertisement call is made up of 24.5 ± 4.7 (17–29, $N = 4$) continuous click notes. Each

Table 7. Measurements (in mm) of the type series of *Megophrys (Panophrys) xiangnanensis* sp. nov., * for the holotype.

	SYS a002875*	SYS a002876 / CIB 116072	SYS a002878	SYS a002879	SYS a002880	SYS a002881	SYS a002882	SYS a002883	SYS a002884	SYS a002885	SYS a002886	SYS a002874
Sex	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Female
SVL	40.9	38.7	39.0	40.2	38.6	40.5	41.7	41.5	42.0	41.0	39.1	44.4
HDL	13.2	12.6	12.3	13.1	12.9	13.0	13.1	13.2	13.4	13.0	12.8	14.0
HDW	13.5	12.5	12.6	13.3	12.8	13.2	13.1	13.2	14.0	13.1	13.0	14.3
SNT	4.5	4.4	4.3	4.4	4.3	4.6	4.2	4.5	4.7	4.6	4.3	5.0
IND	4.5	4.2	4.2	4.5	4.3	4.0	4.4	4.6	4.5	4.5	4.3	4.5
IOD	3.7	3.6	4.0	4.0	3.8	3.7	3.7	4.0	3.8	3.9	3.8	44.3
ED	5.4	4.6	5.0	5.1	4.8	5.0	5.0	5.1	5.1	5.0	5.1	5.5
TD	2.4	2.1	2.0	2.4	2.1	1.9	2.2	2.1	2.5	2.2	2.2	2.7
TED	2.3	2.4	2.0	2.3	2.1	2.9	2.3	2.5	2.1	2.5	2.2	2.4
HND	9.3	9.0	8.8	9.0	8.9	10.3	9.3	8.9	9.0	9.3	9.2	9.8
RAD	9.0	8.7	8.8	8.8	8.9	9.8	9.3	8.9	9.0	9.3	9.2	9.8
FTL	23.9	23.0	24.3	22.9	23.1	24.8	24.2	24.3	24.3	23.5	23.8	27.6
TIB	17.0	17.9	17.8	17.0	17.9	18.2	18.2	18.2	17.3	17.4	17.7	19.1

call lasts 9.46 ± 1.77 s (6.39–10.53 s, $N = 4$) and each note lasts 151 ± 12 ms (113–177 ms, $N = 98$) with an interval of 240 ± 95 ms (148–631 ms, $N = 94$) between every two notes. The peak frequency measures at 3033 ± 123 Hz (2813–3188 Hz, $N = 98$).

***Megophrys (Panophrys) yangmingensis* Lyu, Zeng & Wang, sp. nov.**

<http://zoobank.org/D466B824-AE2D-4EAA-94D1-A6BF39534942>

Figures 4D, 6C, 8

Chresonymy. *Megophrys* sp28 (SYS a002877, 2888–2890 in Liu et al. 2018)

Holotype. SYS a002887 (Figs 4D, 8), adult male, collected on 12 June 2014 by Yu-Long Li and Ying-Yong Wang from Mt Yangming (26.1177N, 111.8945E; ca 1360 m a.s.l.), Shuangpai County, Yongzhou City, Hunan Province, PR China.

Paratypes. Seven adult specimens from the same locality as the holotype: female SYS a002877, and males SYS a2888–2889, 2891–2892, collected at the same time as the holotype; male SYS a002307 and SYS a002310/CIB 116073, collected on 8 September 2013 by Zu-Yao Liu.

Etymology. The specific epithet *yangmingensis* refers to its type locality, Mt Yangming.

Common name. Mt Yangming Horned Toad (in English) / Yáng Míng Shān Jiǎo Chán (阳明山角蟾 in Chinese)

Diagnosis. (1) Body size small, SVL 33.2–37.1 mm (35.3 ± 1.4 , $N = 7$) in adult males and SVL 45.2 mm in adult female; (2) snout rounded in dorsal view; (3) tympanum clear, TD/ED 0.42–0.50; (4) presence of weak vomerine ridge, absence of vomerine teeth; (5) margin of tongue rounded, not notched behind; (6) hindlimbs slender, heels overlapping and tibio-tarsal articulation reaching forward at the anterior corner of the eye; (7) tibia 0.47–0.51 of SVL and foot 0.64–0.69 of SVL in males, while tibia 0.44 of SVL and foot 0.51 of SVL in female; (8) fingers without lateral



Figure 8. Adult male holotype SYS a002887 of *Megophrys (Panophrys) yangmingensis* sp. nov. in life **A** dorsolateral view **B** ventral view **C** hand **D** foot **E** villiform black nuptial spines **F** single subgular vocal sac.

fringes, presence of distinct subarticular tubercles at the bases; (9) toes with lateral fringes and rudiment of webs, presence of subarticular tubercles at the bases; (10) presence of small horn-like tubercle at the edge of upper eyelid; (11) dorsal skin rough with sparse granules, a discontinuous X-shaped ridge with two discontinuous dorsolateral ridges on two side on the back; (12) sparse tubercles on the flanks; (13) orange-brown or light brown above, a dark interorbital triangle with light colored center and edge, a dark X-shaped making with light edge on the central of dorsum; (14) single subgular vocal sac in males; (15) presence of villiform black nuptial spines on the dorsal surface of the first and second fingers in adult males.

Comparison. Comparative data of *Megophrys* (*Panophrys*) *yangmingensis* sp. nov. with *M. (Pa.) mirabilis* sp. nov., *M. (Pa.) shimentaina* sp. nov., *M. (Pa.) xiangnanensis* sp. nov., and 42 recognized congeners of *Panophrys* are given in Table 4.

Megophrys (*Panophrys*) *yangmingensis* sp. nov. differs from *M. (Pa.) mirabilis* sp. nov. by the smaller body size, SVL 33.2–37.1 mm in males and 45.2 mm in single female (vs. 55.8–61.4 mm in males and 68.5–74.8 in females), small horn-like tubercle at upper eyelid (vs. slightly large), presence of vocal sac in males (vs. absent), and presence of nuptial spines in adult males (vs. absent).

Megophrys (*Panophrys*) *yangmingensis* sp. nov. differs from *M. (Pa.) shimentaina* sp. nov. by the larger body size, SVL 33.2–37.1 mm in males (vs. 28.0–30.6 mm in males), absence of vomerine teeth (vs. present), and absence of tiny spines on the whole dorsal skin, flanks, dorsal limbs, the region around cloaca, and rear of hindlimbs (vs. such spines present).

Megophrys (*Panophrys*) *yangmingensis* sp. nov. differs from *M. (Pa.) xiangnanensis* sp. nov. by the smaller body size, SVL 33.2–37.1 mm in males (vs. 38.6–42.0), heels overlapping (vs. just meeting), tibio-tarsal articulation reaching forward at the anterior corner of the eye (vs. between eye and tympanum), and narrow lateral fringes on toes (vs. wide).

Compared with the five *Panophrys* species previously recorded from the hilly areas among Guangdong, Guangxi and Hunan, *Megophrys* (*Panophrys*) *yangmingensis* sp. nov. differs from *M. (Pa.) acuta* by the larger body size, SVL 33.2–37.1 mm in males and 45.2 mm in single female (vs. 27.1–33.0 mm in males and 28.1–33.6 mm in females), small horn-like tubercle at upper eyelid (vs. slightly large), snout rounded in dorsal view (vs. strongly remarkably pointed), and heels overlapping (vs. not meeting). *M. (Pa.) yangmingensis* sp. nov. differs from *M. (Pa.) brachykolos* by the narrow lateral fringes on toes (vs. absent), and heels overlapping (vs. not meeting). *M. (Pa.) yangmingensis* sp. nov. differs from *M. (Pa.) nanlingensis* by the absence of vomerine teeth (vs. present), and tongue not notched behind (vs. notched). *M. (Pa.) yangmingensis* sp. nov. differs from *M. (Pa.) obesa* by the narrow lateral fringes on toes (vs. absent), and heels overlapping (vs. not meeting). *M. (Pa.) yangmingensis* sp. nov. differs from *M. (Pa.) shunhuangensis* sp. nov. by the presence of vomerine ridge (vs. absence).

With a small body size, SVL 33.2–37.1 mm in adult males, *Megophrys* (*Panophrys*) *yangmingensis* sp. nov. is significantly different from nine congeners whose SVL > 40 mm in males, namely *M. (Pa.) baolongensis*, *M. (Pa.) binlingensis*, *M. (Pa.) caudo-procta*, *M. (Pa.) jingdongensis*, *M. (Pa.) liboensis*, *M. (Pa.) omeimontis*, *M. (Pa.) sangzhiensis*, *M. (Pa.) shuichengensis*, and *M. (Pa.) spinata*.

Megophrys (*Panophrys*) *yangmingensis* sp. nov. can be further distinguished from the remaining 28 congeners by the following characteristics: small horn-like tubercle at upper eyelid [vs. slightly large in *M. (Pa.) jinggangensis*, *M. (Pa.) latidactyla*, and *M. (Pa.) palpebralespinosa*]; vomerine teeth absent [vs. present in *M. (Pa.) daweimontis*, *M. (Pa.) dongguanensis*, *M. (Pa.) fansipanensis*, *M. (Pa.) hoanglienensis*, *M. (Pa.) insularis*, *M. (Pa.) jinggangensis*, *M. (Pa.) jiulianensis*, *M. (Pa.) latidactyla*, *M. (Pa.) nankunensis*, *M. (Pa.) palpebralespinosa*, and *M. (Pa.) rubrimera*]; tongue not notched behind [vs. notched in *M. (Pa.) cheni*, *M. (Pa.) boettgeri*, *M. (Pa.) fansipanensis*, *M. (Pa.) hoanglienensis*,

M. (Pa.) huangshanensis, *M. (Pa.) insularis*, *M. (Pa.) jiulianensis*, *M. (Pa.) kuatunensis*, *M. (Pa.) minor*, and *M. (Pa.) rubrimera*]; lateral fringes on toes narrow [vs. absent in *M. (Pa.) daweimontis*, *M. (Pa.) dongguanensis*, *M. (Pa.) fansipanensis*, *M. (Pa.) hoanglienensis*, *M. (Pa.) huangshanensis*, *M. (Pa.) insularis*, *M. (Pa.) jiangi*, *M. (Pa.) jiulianensis*, *M. (Pa.) leishanensis*, *M. (Pa.) lishuiensis*, *M. (Pa.) minor*, *M. (Pa.) nankunensis*, *M. (Pa.) ombrophila*, *M. (Pa.) tuberogranulatus*, *M. (Pa.) wugongensis*, and *M. (Pa.) wuliangshanensis*; vs. wide in *M. (Pa.) binchuanensis*, *M. (Pa.) boettgeri*, *M. (Pa.) cheni*, *M. (Pa.) latidactyla*, *M. (Pa.) lini*, and *M. (Pa.) palpebralespinosa*; vs. absent in females while wide in males in *M. (Pa.) wushanensis*]; rudimentary webs on toes [vs. more than one-fourth webs in *M. (Pa.) palpebralespinosa*; vs. lacking webs in *M. (Pa.) daweimontis*, *M. (Pa.) fansipanensis*, *M. (Pa.) hoanglienensis*, *M. (Pa.) huangshanensis*, *M. (Pa.) kuatunensis*, *M. (Pa.) lishuiensis*, *M. (Pa.) ombrophila*, *M. (Pa.) rubrimera*, and *M. (Pa.) wuliangshanensis*]; tympanum clear with distinct edge [vs. upper 1/4 of tympanum concealed by supratympanic fold in *M. (Pa.) mufumontana*]; tibio-tarsal articulation reaching forward at the anterior corner of the eye [vs. between tympanum and eye in *M. (Pa.) xianjuensis*].

Description of holotype. Adult male. Body size moderate, SVL 35.1 mm; head width slightly larger than head length, HDW/HDL 1.01; snout rounded in dorsal view, projecting, protruding well beyond margin of lower jaw; top of head flat; eyes large, ED 0.43 of HDL, pupil vertical; nostril oblique-ovoid; canthus rostralis well developed; loreal region slightly oblique; internasal distance slightly larger than inter-orbital distance; tympanum clear, TD/ED 0.43; large ovoid choanae at the base of the maxilla; presence of weak vomerine ridge, absence of vomerine teeth; margin of tongue rounded, not notched behind; presence of a single subgular vocal sac, a pair of slit-like openings at posterior of jaw.

Radio-ulna length 0.24 of SVL and hand 0.23 of SVL; hand without webs, fingers without lateral fringes, relative finger length $II < I < IV < III$; tips of fingers slightly dilated, round; one distinct subarticular tubercle at the bases of each finger; inner metacarpal tubercle observably enlarged and the outer one smaller; villiform black nuptial spines on the dorsal surface of the first and second fingers. Hindlimbs slender, tibio-tarsal articulation reaching forward at the anterior corner of eye when hindlimb is stretched along the side of the body; heels overlapping when the flexed hindlimbs are held at right angles to the body axis; tibia length 0.51 of SVL and foot length 0.67 of SVL; relative toe length $I < II < V < III < IV$; tips of toes round and slightly dilated; toes with lateral fringes and rudiment of webs; one subarticular tubercle at the bases of each toe; inner metatarsal tubercle long ovoid and the outer one absent.

Dorsal skin rough with sparse granules; sparse tubercles on the flanks and hindlimbs; several tubercles on upper eyelid, including a horn-like prominent tubercle on the edge; clear supratympanic fold curving postero-ventrally from posterior corner of eye to a level above insertion of arm; a discontinuous X-shaped ridge and two discontinuous dorsolateral ridges on two sides at the central back; four transverse skin ridges on the dorsal shank and thigh; ventral surface smooth; several granules on posterior hindlimbs; small pectoral gland closer to axilla; a single large femoral gland on rear of thigh.

Table 8. Measurements (in mm) of the type series of *Megophrys (Panophrys) yangmingensis* sp. nov., * for the holotype.

	SYS a002887 *	SYS a002307	SYS a002310/ CIB 116073	SYS a002888	SYS a002889	SYS a002891	SYS a002892	SYS a002877
Sex	Male	Male	Male	Male	Male	Male	Male	Female
SVL	35.1	34.5	36.6	33.2	37.1	36.4	34.5	45.2
HDL	11.3	11.6	11.7	11.2	11.9	11.5	11.2	13.6
HDW	11.4	11.9	11.7	11.1	11.8	11.5	11.3	13.5
SNT	4.0	4.4	4.2	3.8	4.4	4.3	4.2	4.8
IND	3.9	3.6	4.0	3.8	3.9	3.8	3.8	4.2
IOD	3.4	3.8	3.8	3.4	3.8	3.6	3.7	4.4
ED	4.9	4.7	4.6	4.8	4.7	5.0	4.7	5.6
TD	2.1	2.2	2.3	2.2	2.2	2.1	2.3	2.8
TED	1.5	1.4	1.4	1.4	2.1	1.7	1.6	2.1
HND	8.0	8.2	8.2	7.0	8.2	8.2	7.9	10.1
RAD	8.5	8.2	8.2	7.0	9.2	8.4	8.0	9.9
FTL	24.2	23.3	23.4	21.1	24.1	24.1	23.2	23.0
TIB	17.8	17.2	17.3	15.5	17.3	17.6	17.0	19.9

Coloration. Orange-brown above in life; a triangular marking with light edge between eyes; a dark X-shaped marking with light edge on the central of dorsum; supratympanic fold light brown; dark vertical band below the eye; iris orange-brown; throat and anterior chest purplish brown; belly dark gray with a large white blotch on the central; ventral limbs purplish; ventral hands reddish brown with dark stripes, tips of fingers pale-grey, metacarpal tubercle reddish; ventral feet purplish, tips of fingers pale-grey, metatarsal tubercle reddish; pectoral gland and femoral gland white.

Variations. Measurement data of type series are listed in Table 8. All paratypes are similar to the holotype. The single female (SVL 45.2 mm) are distinctly larger than males (SVL 33.2–37.1 mm), while with relatively shorter hindlimbs. Dorsal surfaces lighter brown in SYS a002877, 2888–2889, 2891–2892.

Distribution and ecology. Currently, *Megophrys (Panophrys) yangmingensis* sp. nov. is only known from Mt Yangming, southwestern Hunan. This toad inhabits near flowing mountain streams over 1300 m a.s.l. Males call from early June to early September. Males found in early June bear well developed nuptial spines, while the spines are absent in males found in early September, suggesting the breeding season of this toad is before September. Only one female was found, and tadpoles and more ecological information remain unknown.

Vocalization. The advertisement calls of *Megophrys (Panophrys) yangmingensis* sp. nov. were recorded from the Holotype at 16 °C air temperature on 12 June 2014. Five calls with 160 notes are measured and the spectrograms are shown in Fig. 6C. The advertisement call is made up by 31.6 ± 9.0 (22–46, $N = 5$) continuous click notes. Each call lasts 7.38 ± 2.08 s (4.61–10.58 s, $N = 5$) and each note lasts 75 ± 5 ms (64–94 ms, $N = 160$) with an interval of 160 ± 31 ms (120–366 ms, $N = 155$) between every two notes. The peak frequency measures at 3424 ± 82 Hz (3375–3563 Hz, $N = 160$).

Discussion

The phylogenetic analysis encompassing multilocus nuclear-gene and matrilineal mtDNA genealogy (Liu et al. 2018) has revealed 41 cryptic species within the subgenus *Panophrys*. Subsequently, eight of them were described as seven new species (Li et al. 2018; Wang et al. 2019a, b). It is worth noting that the cryptic species *M. sp6* and *M. sp7* revealed based on molecular data were suggested to be the same species and is described as *M. (Pa.) nanlingensis* after detailed morphological examination (Wang et al. 2019a). In our present study, we propose four new species, on the basis of detailed morphological evidences combined with previous phylogenetic data. There are 29 undescribed cryptic species remaining according to Liu et al. (2018), nevertheless, the recognitions from molecular data still require validation from detailed morphological characteristics to substantiate.

The genus *Panophrys* was established by Rao and Yang (1997) but was controversially considered as a subgenus or synonymy of *Xenophrys* or *Megophrys* by different subsequent morphological researches (Dubois and Ohler 1998; Delorme et al. 2006; Li and Wang 2008; Fei et al. 2009). Based on multilocus nuclear-gene and matrilineal mtDNA genealogy, three recent studies have revealed highly similar phylogenetic relationships within Megophryinae, which is unanimously considered to contain the following monophyletic groups: Pelobatrachus, Megophrys, Xenophrys, Panophrys, Brachytarsophrys, Ophryophryne, Atympanophrys (Chen et al. 2017; Mahony et al. 2017; Liu et al. 2018). However, the taxonomic proposals for these groups are in conflict by different authors. Chen et al. (2017) considered that subfamily Megophryinae is valid and composed of five genera: *Atympanophrys*, *Brachytarsophrys*, *Megophrys*, *Ophryophryne* and *Xenophrys* (including *Panophrys* as a subgenus). Mahony et al. (2017) treated the entire subfamily Megophryinae as a single genus *Megophrys* with containing seven subgenera (corresponding to the seven molecularly resolved clades). To resolve these conflicts, Li et al. (2020) suggested to elevate the seven monophyletic subgenera to genus levels, which fulfills the following three criteria to be descriptively useful: reasonably compact, monophyletic, and ecologically, morphologically or biogeographically distinct (Gill et al. 2005). Li et al.'s suggestion was based on the review of *Brachytarsophrys*, which shows significant differences against other groups. Therefore, the recognition of genus *Brachytarsophrys* must be accepted, while further supported evidences for other genera are needed.

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Appendix I

Specimens examined

- Megophrys (Panophrys) acuta* (10): **China: Guangdong:** Fengkai: Heishiding Nature Reserve (type locality): SYS a000168, 0517, 0521, 3257, 2159, 2266–2269, 2276.
- Megophrys (Panophrys) binlingensis* (2): **China: Sichuan:** Hongya: Mt. Wawu (type locality): SYS a005313–5314.
- Megophrys (Panophrys) boettgeri* (16): **China: Fujian:** Wuyishan: Mt. Wuyi (type locality): SYS a002480, 4149–4151; **Jiangxi:** Guixi: Yangjifeng Nature Reserve: SYS a000312, 0315, 0328–0330, 0376, 0378; Guangfeng: Tongboshan Nature Reserve: SYS a001671–1673, 1683, 1700.
- Megophrys (Panophrys) brachykolos* (2): **China: Hong Kong** (type locality): SYS a001502–1503.
- Megophrys (Panophrys) caudoprocta* (3): **China: Hunan:** Sangzhi: Badagongshan Nature Reserve (type locality): SYS a004281, 4308–4309.
- Megophrys (Panophrys) cheni* (19): **China: Jiangxi:** Jinggangshan: Mt. Jinggang (type locality): SYS a001427–1429, 1871–1873; **Hunan:** Yanling: Taoyuandong Nature Reserve: SYS a002123–2127, 2140–2145.

- Megophrys (Panophrys) huangshanensis* (13): **China: Anhui:** Huangshan: Mt. Huangshan (type locality): SYS a002702–2707; **Jiangxi:** Wuyuan: Mt. Dazhang: SYS a001622–1623, 3705–3707; **Zhejiang:** Lin'an: Mt. Tianmu: SYS a002684–2685.
- Megophrys (Panophrys) jingdongensis* (14): **China: Yunnan:** Jingdong: Mt. Wuliang (type locality): SYS a003909, 3928–3929; Zhenyuan: Mt. Ailao: SYS a001778, 2988, 2989–2991, 2993–2994, 3005–3006, 3903–3904.
- Megophrys (Panophrys) jinggangensis* (11): **China: Jiangxi:** Jinggangshan: Mt. Jinggang (type locality): SYS a001413–1416, 1430, 4028; **Hunan:** Yanling: Taoyuandong Nature Reserve: SYS a001859–1863.
- Megophrys (Panophrys) kuatunensis* (3): **China: Fujian:** Wuyishan: Guadun: SYS a001579, 1590; **Jiangxi:** Guixi: Yangjifeng Nature Reserve: SYS a000241.
- Megophrys (Panophrys) lini* (27): **China: Hunan:** Yanling: Taoyuandong Nature Reserve (type locality): SYS a002128; **Jiangxi:** Jinggangshan: Mt. Jinggang: SYS a001417–1424, 2375–2386; Suichuan: Nanfengmian Nature Reserve: SYS a002369–2374.
- Megophrys (Panophrys) minor* (5): **China: Sichuan:** Dujiangyan: Mt. Qingcheng (type locality): SYS a003209–3213.
- Megophrys (Panophrys) obesa* (4): **China: Guangdong:** Fengkai: Heishiding Nature Reserve (type locality): SYS a002270–2272, 3047.
- Megophrys (Panophrys) omeimontis* (11): **China: Sichuan:** Emeishan: Mt. Emei (type locality): SYS a001798–1801, 1940–1941, 5301; Hongya: Mt. Wawu: SYS a005330–5331; Pingshan: Mt. Laojun: SYS a002740–2741.
- Megophrys (Panophrys) sangzhiensis* (6): **China: Hunan:** Sangzhi: Badagongshan Nature Reserve (type locality): SYS a004306–4307, 4313–4316.
- Megophrys (Panophrys) spinata* (2): **China: Guizhou:** Leishan: Mt. Leigong (type locality): SYS a002226–2227.
- Megophrys (Panophrys) tubero granulatus* (1): **China: Hunan:** Sangzhi: Badagongshan Nature Reserve (type locality): SYS a004310.
- Megophrys (Panophrys) wushanensis* (5): **China: Hubei:** Shennongjia: Shennongjia Nature Reserve: SYS a003008–3011, 3013.
- Megophrys (Panophrys) wuliangshanensis* (5): **China: Yunnan:** Jingdong: Mt. Wuliang (type locality): SYS a003924–3925; Zhenyuan: Mt. Ailao: SYS a002983–29
- Megophrys (Xenophrys) glandulosa* (13): **China: Yunnan:** Jingdong: Mt. Wuliang (type locality): SYS a003907–3908, 3923; Tengchong: Gaoligong Nature Reserve: SYS a002944–2946, 3757–3758, 3762, 3792–3795.
- Megophrys (Xenophrys) cf. major* (3): **China: Yunnan:** Mengla: Zhushihe: SYS a002961–2962, 3955.
- Megophrys (Xenophrys) mangshanensis* (10): **China: Guangdong:** Ruyuan: Nanling Nature Reserve: SYS a000493–0496, 0586; Renhua: Danxiashan Geological Park: SYS a000288; Huaiji: Mt. Sanyue: SYS a002177; Shaoguan: Mt. Longtou: SYS a002749; **Jiangxi:** Longnan: Jiulianshan Nature Reserve: SYS a000996–0997.
- Megophrys (Xenophrys) medogensis* (3): **China: Xizang:** Motuo: Beibeng (type locality): SYS a002932–2933, 2935.
- Megophrys (Xenophrys) pachyproctus* (1): **China: Xizang:** Motuo: Beibeng (type locality): SYS a002934.

A new species of the *Rana japonica* group (Anura, Ranidae, *Rana*) from China, with a taxonomic proposal for the *R. johnsi* group

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Abstract

Rana jiulingensis **sp. nov.**, a new species from Hunan and Jiangxi, southeastern China, is described. The new species is assigned to the *R. japonica* group. The clade comprising *R. jiulingensis* **sp. nov.** and *R. dabieshanensis* from Anhui is the sister taxon of *R. omeimontis* from Sichuan. *Rana jiulingensis* **sp. nov.** can be distinguished by the significant divergences in the 16S and COI genes, and the combination of following morphological characters: body size medium, SVL 48.3–57.8 mm in adult males and 48.2–57.5 mm in adult females; dorsolateral fold straight; digits without circummarginal grooves; dorsal skin smooth; tibio-tarsal articulation reaching forward beyond the tip of snout; heels overlapping; webbing formula of toes: I $1\frac{1}{3}$ – 2 II $1\frac{1}{3}$ – $2\frac{1}{3}$ III $1\frac{1}{2}$ – $2\frac{2}{3}$ IV 3 – $1\frac{1}{3}$ V; absence of vocal sacs in males; and presence of creamy white nuptial pad with tiny hoar spines on the finger I and reddish tubercles on loreal and temporal regions in breeding males. Furthermore, based on our results and the previous literature, *R. zhengi* is synonymized with *R. sangzhiensis*, and a new species group, the *Rana johnsi* group, is proposed for the *R. johnsi* and *R. sangzhiensis*. Currently, the subgenus *Rana* contains 41 recognized species, and the phylogenetic placements of several species remain unresolved.

* Contributed equally as the first authors.

Keywords

morphology, phylogeny, *Rana jiulingensis* sp. nov., *Rana sangzhiensis*, *Rana zhengi*

Introduction

As the type genus of the family Ranidae Batsch, 1796, the concept of the true-frog genus *Rana* Linnaeus, 1758 has been discussed for a long time (Frost 2020). In a recent phylogenetic analysis (Yuan et al. 2016), *Rana* sensu lato was considered to be composed of nine clades, namely the subgenera *Rana*, *Amerana* Dubois, 1992, *Liuhurana* Fei, Ye, Jiang, Dubois & Ohler, 2010, *Aquarana* Dubois, 1992, *Lithobates* Fitzinger, 1843, *Zweifelia* Dubois, 1992, *Pantherana* Dubois, 1992, *Pseudorana* Fei, Ye & Huang, 1990, and an unnamed monotypic clade containing *R. sylvatica* (LeConte, 1825). However, this classification is still controversial, especially for the recognitions of the genera *Lithobates* and *Pseudorana* (Frost 2020). Nevertheless, the subgenus *Rana*, which is currently well recognized, contains 41 known species distributed from Europe to southeastern Asia. Among them, 23 species occur in China (AmphibiaWeb 2019). Recent researches on this subgenus have discovered new species from China and revised several taxonomic errors, indicating that the diversity and taxonomy of the subgenus *Rana* are still insufficiently understood (Yan et al. 2011; Zhou et al. 2015, 2017; Yuan et al. 2016; Wang et al. 2017; Yang et al. 2017; Zhao et al. 2017).

Based on morphological comparisons and geographical conditions, Fei et al. (2009) proposed three species groups for the Chinese species of the subgenus *Rana*: *R. longicrus* group, *R. chensinensis* group, and *R. amurensis* group. Subsequent phylogenetic analyses have revised several memberships of these groups (Yan et al. 2011; Zhou et al. 2015, 2017; Yuan et al. 2016; Wang et al. 2017; Zhao et al. 2017), and the nomenclature of the *R. longicrus* group was replaced by the *R. japonica* group (Yang et al. 2017). Currently, 16 Chinese species are recognized as members of the three species groups. The *R. japonica* group contains nine species: *R. (R.) chaochiaoensis* Liu, 1946; *R. (R.) chevronta* Hu & Ye, 1978; *R. (R.) culaiensis* Li, Lu & Li, 2008; *R. (R.) dabieshanensis* Wang, Qian, Zhang, Guo, Pan, Wu, Wang & Zhang, 2017; *R. (R.) hanluica* Shen, Jiang & Yang, 2007; *R. (R.) jiemuxiensis* Yan, Jiang, Chen, Fang, Jin, Li, Wang, Murphy, Che & Zhang, 2011; *R. (R.) longicrus* Stejneger, 1898; *R. (R.) omeimontis* Ye & Fei, 1993; and *R. (R.) zhenhaiensis* Ye, Fei & Matsui, 1995. The *R. chensinensis* group contains four species: *R. (R.) chensinensis* David, 1875; *R. (R.) dybowskii* Günther, 1876; *R. (R.) huanrenensis* Liu, Zhang & Liu, 1993; and *R. (R.) kukunoris* Nikol'skii, 1918. The *R. amurensis* group has three species: *R. (R.) amurensis* Boulenger, 1886; *R. (R.) coreana* Okada, 1928; and *R. (R.) luanchuanensis* Zhao & Yuan, 2017. However, species groups have not yet been proposed to accommodate the remaining seven species: *R. (R.) arvalis* Nilsson, 1842; *R. (R.) asiatica* Bedriaga, 1898; *R. (R.) maershanensis* Lu, Li & Jiang, 2007; *R. (R.) sauteri* Boulenger, 1909; *R. (R.) johnsi* Smith, 1921; *R. (R.) sangzhiensis* Shen, 1986; and *R. (R.) zhengi* Zhao, 1999.

During herpetofaunal surveys in the Luoxiao Range, which is situated between the Jiangxi and Hunan provinces (Fig. 1), a series of *Rana* specimens was collected

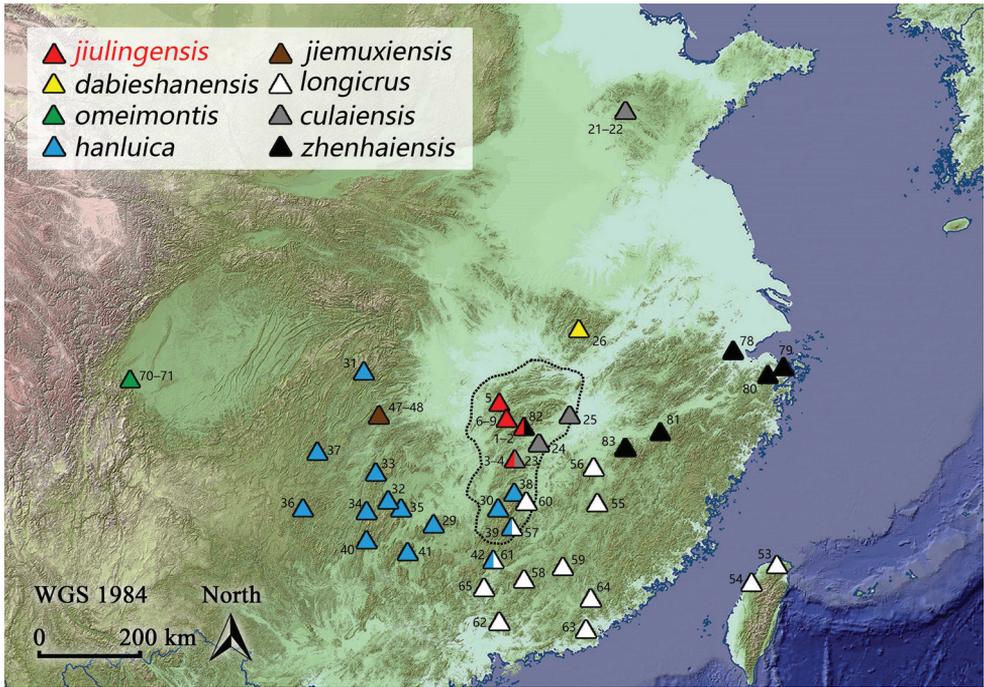


Figure 1. Collecting localities of *Rana* samples used in this study. Dotted line shows the Luoxiao Range, where five *Rana* species are recorded. Numbers correspond to the ID in Table 1.

which can be assigned to the *R. japonica* group based on morphological characteristics. However, detailed examination of these specimens showed significant differences from all known congeners. Additional molecular analysis well supported the morphological identifications, demonstrating that these specimens formed an unnamed lineage within the *R. japonica* group. Therefore, we describe this series of specimens as a new species. Additionally, as revealed from our results and the previous literature, we suggest that *R. zhengi* should be synonymized with *R. sangzhiensis*, and we also propose a new species group, the *Rana johnsi* group, for the species *R. johnsi* and *R. sangzhiensis*.

Materials and methods

Sampling and morphological analyses

Eight unnamed specimens were collected from multiple localities of Jiangxi and Hunan provinces. All specimens were fixed in 10% buffered formalin, later transferred to 70% ethanol, and deposited in the Museum of Biology, Sun Yat-sen University (SYS) and Chengdu Institute of Biology, Chinese Academy of Sciences (CIB), PR China. External measurements were made for the unnamed specimens with digital calipers (Neiko 01407A Stainless Steel 6-Inch Digital Caliper, USA) to the nearest 0.1 mm. These measurements are as follows:

- SVL** snout–vent length (from tip of snout to posterior margin of vent);
HL head length (from tip of snout to the articulation of the jaw);
HW head width (head width at the commissure of the jaws);
SL snout length (from tip of snout to the anterior corner of the eye);
IN internasal distance (distance between nares);
IO interorbital distance (minimum distance between upper eyelids);
ED eye diameter (from the anterior corner of the eye to posterior corner of the eye);
TD tympanum diameter (horizontal diameter of tympanum);
TED tympanum–eye distance (from anterior edge of tympanum to posterior corner of the eye);
HND hand length (from the proximal border of the outer palmar tubercle to the tip of digit III);
RAD radio-ulna length (from the flexed elbow to the proximal border of the outer palmar tubercle);
FTL foot length (from distal end of shank to the tip of digit IV);
TIB tibial length (from the outer surface of the flexed knee to the heel).

The morphological description follows the consistent definition by Fei et al. (2009). Sex and age were determined by examining the gonads. Webbing formula was based on Savage (1975). Comparison characters of known congeners were obtained from the literature (Stejneger 1898; Liu 1946; Liu et al. 1993; Ye et al. 1993, 1995; Lu et al. 2007; Shen et al. 2007; Li et al. 2008; Fei et al. 2009, 2012; Yan et al. 2011; Wang et al. 2017; Zhao et al. 2017) and 80 examined museum specimens listed in the Appendix 1.

DNA Extraction, PCR amplification, and sequencing

A total of 56 muscular samples of *Rana* were used, encompassing nine samples of the undescribed specimens, and 47 samples from 12 recognized species. All samples were attained from euthanasia specimens and then preserved in 95% ethanol and stored at -40°C . Genomic DNA were extracted from muscle tissue samples, using DNA extraction kit from Tiangen Biotech (Beijing) Co., Ltd. Two mitochondrion genes, namely partial 16S ribosomal RNA gene (16S) and partial cytochrome c oxidase 1 gene (COI), were amplified. Primers used for 16S were L3975 (5'-CGCCTGTTTAC-CAAAAACAT-3') and H4551 (5'-CCGGTCTGAACTCAGATCACGT-3') following Simon et al. (1994), and L2A (5'-CCAAACGAGCCTAGTGATAGCTGGTT-3') and H10 (5'-TGATTACGCTACCTTTGCACGGT-3') following Chen et al. (2013), for COI were dgLCO (5'-GGTCAACAAATCATAAAGAYATYGG-3') and dgHCO (5'-AAACTTCAGGGTGACCAARAAYCA-3') following Meyer et al. (2005). PCR amplifications were processed with the cycling conditions that initial denaturing step at 95°C for 4 min, 35 cycles of denaturing at 94°C for 40 s, annealing at 53°C (for 16S) / 48°C (for COI) for 40 s and extending at 72°C for 60 s, and a final extending step at 72°C for 10 min. PCR products were purified with spin columns and then

Table 1. Localities, voucher information, and GenBank numbers for all samples of the genus *Rana* used in this study (* = type localities).

ID	Species	Localities	Voucher no.	16S	COI
1	<i>R. jiulingensis</i>	China: Jiangxi: Mt Guanshan *	SYS a005519	MT408985	MT418647
2	<i>R. jiulingensis</i>	China: Jiangxi: Mt Guanshan *	SYS a006999	MT408994	MT418656
3	<i>R. jiulingensis</i>	China: Jiangxi: Mt Wugong	SYS a002584	MT408964	MT418626
4	<i>R. jiulingensis</i>	China: Jiangxi: Mt Wugong	SYS a002585	MT408965	MT418627
5	<i>R. jiulingensis</i>	China: Hunan: Mt Mufu	SYS a005511	MT408984	MT418646
6	<i>R. jiulingensis</i>	China: Hunan: Mt Dawei	SYS a006451	MT408989	MT418651
7	<i>R. jiulingensis</i>	China: Hunan: Mt Dawei	SYS a006494	MT408990	MT418652
8	<i>R. jiulingensis</i>	China: Hunan: Mt Dawei	SYS a006495	MT408991	MT418653
9	<i>R. jiulingensis</i>	China: Hunan: Mt Dawei	SYS a006496	MT408992	MT418654
10	<i>R. amurensis</i>	China: Heilongjiang: Taiyang Island	SYNU 11100267	KF020589	KF020603
11	<i>R. amurensis</i>	China: Liaoning, Zhangwu	SYNU 11100268	KU343216	KU343216
12	<i>R. arvalis</i>	Germany: Lower Saxony	No voucher	AY147938	/
13	<i>R. asiatica</i>	China: Xinjiang: 47tuan	KIZ XJ0251	KX269200	/
14	<i>R. chaochiaoensis</i>	China: Sichuan: Zhaojue *	SYS a001815	MT409007	MT418669
15	<i>R. chaochiaoensis</i>	China: Sichuan: Zhaojue *	SYS a001816	MT408957	MT418619
16	<i>R. chensinensis</i>	China: Shaanxi: Huxian *	KIZ RD05SHX01	KX269186	JF939080
17	<i>R. chensinensis</i>	China: Henan: Mt Yawu	SYS a002392	MT408962	MT418624
18	<i>R. chensinensis</i>	China: Henan: Mt Yawu	SYS a002393	MT408963	MT418625
19	<i>R. coreana</i>	South Korea	MMS 223	KX269202	MF149928
20	<i>R. coreana</i>	China: Shandong: Mt Kunyu	SYNU 08090641	MT409004	MT418666
21	<i>R. culaiensis</i>	China: Shandong: Mt Culai *	KIZ SD080501	KX269190	JF939082
22	<i>R. culaiensis</i>	China: Shandong: Mt Culai *	SYNU 08090549	MT409006	MT418668
23	<i>R. culaiensis</i>	China: Jiangxi: Mt Wugong	SYS a002634	MT408966	MT418628
24	<i>R. culaiensis</i>	China: Jiangxi: Shanggao	SYS a002641	MT408967	MT418629
25	<i>R. culaiensis</i>	China: Jiangxi: Mt Meiling	SYS a004239	MT408971	MT418633
26	<i>R. dabieshanensis</i>	China: Anhui: Dabie Mountains area *	AHU 2016R001	MF172963	/
27	<i>R. dybowskii</i>	Russia: Primorye: Khasanskii	MSUZP-IVM-1d	KX269188	/
28	<i>R. dybowskii</i>	China: Jilin: Mt Laoling	SYNU 11070163	MT409005	MT418667
29	<i>R. hanluica</i>	China: Hunan: Mt Yangming *	SYS a001137	MT408956	MT418618
30	<i>R. hanluica</i>	China: Hunan: Mt Bamian	SYS a004086	MT408969	MT418631
31	<i>R. hanluica</i>	China: Hunan: Mt Badagong	SYS a004298	MT408973	MT418635
32	<i>R. hanluica</i>	China: Hunan: Mt Yunshan	SYS a004359	MT408977	MT418639
33	<i>R. hanluica</i>	China: Hunan: Mt Xuefeng	SYS a007216	MT408999	MT418661
34	<i>R. hanluica</i>	China: Hunan: Suining	SYS a007250	MT409000	MT418662
35	<i>R. hanluica</i>	China: Hunan: Mt Shunhuang	SYS a007259	MT409001	MT418663
36	<i>R. hanluica</i>	China: Guizhou: Mt Leigong	SYS a002233	MT408959	MT418621
37	<i>R. hanluica</i>	China: Guizhou: Mt Fanjing	SYS a004346	MT408976	MT418638
38	<i>R. hanluica</i>	China: Jiangxi: Mt Jinggang	SYS a004033	MT408968	MT418630
39	<i>R. hanluica</i>	China: Jiangxi: Mt Qiyun	SYS a004087	MT408970	MT418632
40	<i>R. hanluica</i>	China: Guangxi: Longsheng	SYS a002286	MT408960	MT418622
41	<i>R. hanluica</i>	China: Guangxi: Mt Dupangling	SYS a005087	MT408980	MT418642
42	<i>R. hanluica</i>	China: Guangdong: Renhua	SYS a007100	MT408998	MT418660
43	<i>R. huanrenensis</i>	China: Liaoning: Huanren *	SYNU 07040035	KF204642	KX139725
44	<i>R. huanrenensis</i>	China: Liaoning: Huanren *	y-d20130058	KT588071	KT588071
45	<i>R. japonica</i>	Japan: Isumi-shi: Chiba Prefecture	KIZ YPX11775	KX269220	JF939101
46	<i>R. japonica</i>	Japan: Isumi-shi: Chiba Prefecture	NNRj	AB728192	/
47	<i>R. jiemuxiensis</i>	China: Hunan: Jiemuxi *	SYS a004318	MT408975	MT418637
48	<i>R. jiemuxiensis</i>	China: Hunan: Jiemuxi *	SYS a004319	MT409008	MT418670
49	<i>R. johnsi</i>	Vietnam: Lam Dong: Loc Bao	ABV 00203	KX269182	/
50	<i>R. kukunoris</i>	China: Qinghai: Qinghai Lake *	KIZ CJ06102001	KX269185	JF939073

ID	Species	Localities	Voucher no.	16S	COI
51	<i>R. kukunoris</i>	China: Sichuan: Hongyuan	SYS a006652	MT409009	MT418671
52	<i>R. kukunoris</i>	China: Sichuan: Hongyuan	SYS a006653	MT408993	MT418655
53	<i>R. longicrus</i>	China: Taiwan: Taipei *	Not given	AB058881	/
54	<i>R. longicrus</i>	China: Taiwan: Miaoli: Xiangtianhu	NMNS 15022	KX269189	/
55	<i>R. longicrus</i>	China: Fujian: Mt Yashu	SYS a005905	MT408987	MT418649
56	<i>R. longicrus</i>	China: Jiangxi: Mt Magu	SYS a007038	MT408996	MT418658
57	<i>R. longicrus</i>	China: Jiangxi: Mt Qiyun	SYS a002355	MT408961	MT418623
58	<i>R. longicrus</i>	China: Jiangxi: Mt Jiulian	SYS a004487	MT408978	MT418640
59	<i>R. longicrus</i>	China: Jiangxi: Mt Sanbai	SYS a005892	MT408986	MT418648
60	<i>R. longicrus</i>	China: Jiangxi: Suichuan	SYS a007097	MT408997	MT418659
61	<i>R. longicrus</i>	China: Guangdong: Renhua	SYS a000735	MT408954	MT418616
62	<i>R. longicrus</i>	China: Guangdong: Mt Nankun	SYS a000754	MT408955	MT418617
63	<i>R. longicrus</i>	China: Guangdong: Pu'ning	SYS a004605	MT408979	MT418641
64	<i>R. longicrus</i>	China: Guangdong: Mt Tonggu	SYS a005218	MT408981	MT418643
65	<i>R. longicrus</i>	China: Guangdong: Yingde	SYS a007519	MT409003	MT418665
66	<i>R. maoershanensis</i>	China: Guangxi: Mt Maoershan *	SYNU 08030061	HQ228162	/
67	<i>R. maoershanensis</i>	China: Guangxi: Mt Maoershan *	SYNU 08030062	HQ228163	/
68	<i>R. luanchuanensis</i>	China: Henan: Luanchuan *	KIZ 047452	/	MF149923
69	<i>R. luanchuanensis</i>	China: Henan: Luanchuan *	KIZ 047393	/	MF149924
70	<i>R. omeimontis</i>	China: Sichuan: Mt Emei *	SYS a005304	MT408982	MT418644
71	<i>R. omeimontis</i>	China: Sichuan: Mt Emei *	SYS a005305	MT408983	MT418645
72	<i>R. sangzhiensis</i>	China: Hunan: Mt Tianping *	SYS a004286	MT408972	MT418634
73	<i>R. sangzhiensis</i>	China: Hunan: Mt Tianping *	SYS a004299	MT408974	MT418636
74	<i>R. zhengi</i>	China: Sichuan: Hongya: Zhangcun *	SCUM 0405190CJ	KX269206	MF149929
75	<i>R. zhengi</i>	China: Sichuan: Hongya: Zhangcun *	KIZ YP06057	DQ289104	/
76	<i>R. sauteri</i>	China: Taiwan: Kaohsiung *	SCUM 0405175CJ	KX269204	/
77	<i>R. shuchinae</i>	China: Sichuan: Zhaojue	CIB HUI040009	KX269210	/
78	<i>R. zhenhaiensis</i>	China: Zhejiang: Hangzhou	SYNU 08040100	KF020599	KF020613
79	<i>R. zhenhaiensis</i>	China: Zhejiang: Zhenhai *	KIZ 0803271	KX269218	JF939065
80	<i>R. zhenhaiensis</i>	China: Zhejiang: Fenghua	SYS a006208	MT408988	MT418650
81	<i>R. zhenhaiensis</i>	China: Jiangxi: Mt Tongbo	SYS a001952	MT408958	MT418620
82	<i>R. zhenhaiensis</i>	China: Jiangxi: Mt Guanshan	SYS a007000	MT408995	MT418657
83	<i>R. zhenhaiensis</i>	China: Jiangxi: Mt Yangjifeng	SYS a007422	MT409002	MT418664

sequenced with both forward and reverse primers using BigDye Terminator Cycle Sequencing Kit per the guidelines, on an ABI Prism 3730 automated DNA sequencer by Shanghai Majorbio Bio-pharm Technology Co., Ltd. All sequences were deposited in GenBank (Table 1).

Phylogenetic analyses

For phylogenetic analyses, 26 additional sequences from all known Chinese congeners of the subgenus *Rana* (except *R. (R.) chevronata*) and an out-group sequence of *R. (Liuhurana) shuchinae* Liu, 1950 were obtained from GenBank and incorporated into our dataset. Detailed information of these materials is shown in Table 1 and Figure 1. DNA sequences were aligned respectively by the Clustal W algorithm with default

parameters (Thompson et al. 1997). For GenBank sequences that lack information for part of the segments, we filled the blank sites with “N”. The aligned data was trimmed for allowing no gap positions and default parameters in Gblocks version 0.91b (Castresana 2000). All newly obtained sequences were deposited in GenBank (Table 1). PartitionFinder2 was used to test the best partitioning scheme and jModelTest v2.1.2 was used to test the best fitting nucleotide substitution models, resulting in the best fit models for the partitions of COI and 16S as GTR + I + G. Sequenced data were analyzed using Bayesian inference (BI) in MrBayes 3.2.4 (Ronquist et al. 2012), and maximum likelihood (ML) in RaxmlGUI 1.3 (Silvestro and Michalak 2012). Two independent runs were conducted in a BI analysis, each of which was performed for 10,000,000 generations and sampled every 1000 generations with the first 25% samples discarded as burn-in, resulting in a potential scale reduction factor (PSRF) of <0.005. In ML analysis, the bootstrap consensus tree inferred from 1000 replicates was used to represent the evolutionary history of the taxa analyzed. Pairwise distances were respectively calculated in MEGA 6 using the uncorrected *p*-distance model.

Results

Morphological comparison

The unnamed specimens from Jiangxi and Hunan are assigned to the *Rana japonica* group based on the following combined characteristics: digits without circummarginal grooves, and dorsolateral fold distinct, extending straight from the posterior margin of the upper eyelid to above the groin. Therefore, we compare the new species with the species of the *R. japonica* group.

The new species differs from *Rana dabieshanensis* in the following characters: head length significantly larger than head width, HW/HL 0.82 in males and 0.85 in females (vs almost equal); supratympanic fold absent (vs distinct); tympanum diameter significantly smaller than eye diameter with TD/ED = 0.63–0.87 (vs equal); relative toe lengths I < II < III < V < IV (vs I < II < V < III < IV); toe webbing formula I 1½ – 2 II 1½ – 2⅓ III 1½ – 2⅓ IV 3 – 1⅓ V (vs I 2 – 1 II 2+ – 1+ III 3 – 2 IV 2 – 2+ V); and nuptial pad creamy white in breeding males (vs gray-blackish).

The new species differs from *R. omeimontis* as follows: body size smaller, SVL = 48.2–57.5 mm in adult females (vs 61.7–70.3 mm in females); head length significantly larger than head width, HW/HL = 0.82 in males and 0.85 in females (vs head length slightly larger than head width, HW/HL = 0.94 in males and 0.92 in females); and supernumerary tubercles present below the bases of each finger (vs absent).

The new species further differs from *R. hanluica* as follows: supratympanic fold absent (vs present); toe webbing formula I 1½ – 2 II 1½ – 2⅓ III 1½ – 2⅓ IV 3 – 1⅓ V (vs I 1½ – 1⅓ II 1 – 2 III 1½ – 2⅓ IV 2⅓ – 1 V); reddish tubercles present on loreal and temporal regions in breeding males (vs absent, but white horny spines present around loreal and temporal regions, upper eyelids, and snout in breeding males). The new

species differs from *R. longicrus* in having: internarial distances larger than interorbital distances (vs smaller) and toe webbing formula I $1\frac{1}{3}$ – 2 II $1\frac{1}{3}$ – $2\frac{1}{3}$ III $1\frac{1}{2}$ – $2\frac{2}{3}$ IV $3 - 1\frac{1}{3}$ V (vs I $1\frac{2}{3}$ – $2\frac{1}{3}$ II $1\frac{1}{2}$ – $2\frac{2}{3}$ III $1\frac{2}{3}$ – $3\frac{1}{2}$ IV $3\frac{1}{3}$ – $1\frac{1}{2}$ V); from *R. zhenhaiensis*: supratympanic fold absent (vs present), dorsolateral fold extending straight from posterior margin of upper eyelid to above groin (vs dorsolateral fold slightly curved above tympanum), two outer metacarpal tubercles distinctly separated (vs merged at base), tibio-tarsal articulation reaching forward beyond tip of snout (vs around nostril), and nuptial pad creamy white in breeding males (vs gray or gray-brownish); from *R. culaiensis*: dorsolateral fold extending straight from posterior margin of upper eyelid to above groin (vs dorsolateral fold slightly curved above tympanum), and tibio-tarsal articulation reaching forward beyond tip of snout (vs at nostril); from *R. jiemuxiensis*: dorsolateral fold extending straight from posterior margin of upper eyelid to above groin (vs dorsolateral fold slightly curved above tympanum), head length significantly larger than head width (vs slightly larger), internarial distances larger than interorbital distances (vs smaller), and two outer metacarpal tubercles distinctly separated (vs merged at base); from *R. chaochiaensis*: supratympanic fold absent (vs present), internarial distances larger than interorbital distances (vs smaller), and toe webbing formula I $1\frac{1}{3}$ – 2 II $1\frac{1}{3}$ – $2\frac{1}{3}$ III $1\frac{1}{2}$ – $2\frac{2}{3}$ IV $3 - 1\frac{1}{3}$ V (vs I $1 - 1\frac{2}{3}$ II $1\frac{1}{3}$ – 2 III $1\frac{1}{2}$ – $2\frac{1}{2}$ IV $2\frac{2}{3}$ – 1 V); from *R. japonica*: outer metacarpal tubercles present (vs absent), tibio-tarsal articulation reaching forward beyond tip of snout (vs reaching or beyond tip of snout in males, reaching at center of eye or beyond nostril in females), nuptial pad creamy white and divided into three parts (vs nuptial pads grayish brown or yellowish brown and divided into two parts).

From *Rana chevronta*, which lacks molecular data, the new species can be distinguished by its larger body size, SVL = 48.3–57.8 mm in adult males (vs 39.7–44.0 mm), head length significantly larger than head width (vs almost equal), relative finger lengths I < II < IV < III (vs II < IV < I < III), and nuptial pad creamy white and divided into three parts in breeding males (vs purplish gray and undivided).

Phylogenetic analyses

The ML and BI analyses resulted in essentially identical topologies and are integrated in Figure 2, in which the major nodes are sufficiently supported with the Bayesian posterior probabilities (BPP) >0.95 and the bootstrap supports (BS) for maximum likelihood analysis >85. The pairwise distances based on COI and 16S genes among all samples are given in the Supplementary material, Tables S1 and S2, respectively.

The *Rana* samples representing the new species are grouped in a distinct and robust monophyletic lineage with high support (BPP = 1.00 and BS = 100) and low divergence (mean 0.3%, ranging 0.0–0.6% in COI, and mean 0.1%, ranging 0.0–0.5% in 16S); they form a separate evolutionary lineage within the *R. japonica* group. This lineage from Jiangxi and Hunan is close to *R. dabieshanensis* from Anhui and *R. omeimontis* from Sichuan. The smallest genetic distance between this lineage and a previously recognized species is 3.4–4.0% in COI (with *R. omeimontis*) and 1.6–2.0% in 16S

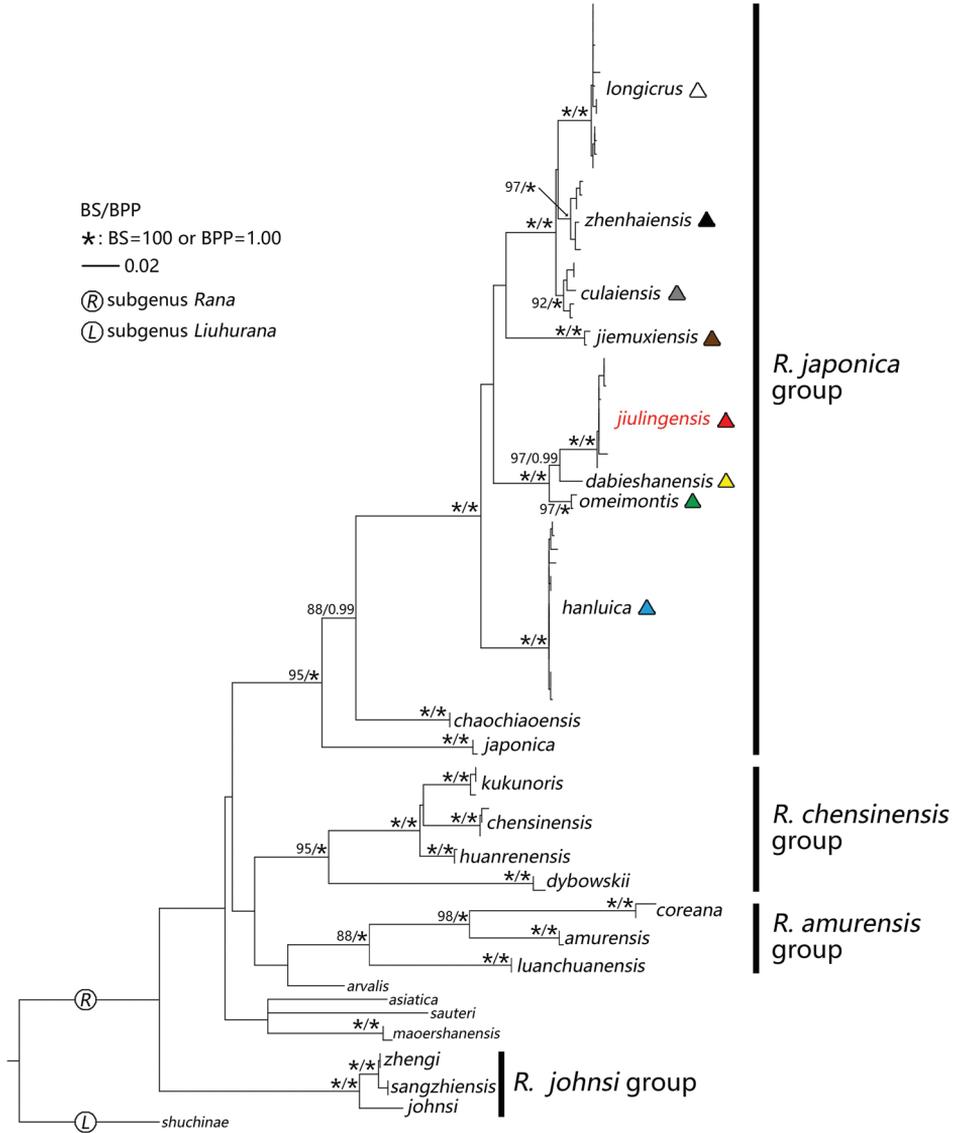


Figure 2. Bayesian inference and maximum-likelihood phylogenies based on mitochondrial 16S and COI genes.

(with *R. dabieshanensis*), which are significant when compared to all other recognized species (e.g. 2.8–3.6% in COI between *R. longicrus* and *R. culaiensis*; 1.2–1.3% in 16S between *R. dabieshanensis* and *R. omeimontis*).

Therefore, based on the significant morphological differences and phylogenetic divergence, these specimens from Jiangxi and Hunan represent a distinct evolutionary lineage and are described as a new species, *Rana jiulingensis* sp. nov.

Taxonomic account

***Rana (Rana) jiulingensis* Wan, Lyu & Wang, sp. nov.**

<http://zoobank.org/2E012E54-EFA3-4AA3-9B9F-0F884305AABD>

Holotype. SYS a005519 (Fig. 3), adult male, collected by Zhi-Tong Lyu, Jian Wang and Hai-Long He on 14 September 2016 from Guanshan Nature Reserve (28.5535N, 114.5878E; ca 300 m a.s.l.), Yifeng County, Jiangxi province, PR China.

Paratypes. Seven adult specimens. Females SYS a002584–2585 collected by Jian Zhao on 8 May 2014 from Mt Wugong (27.4607N, 114.2059E; ca 1100 m a.s.l.), Anfu



Figure 3. Morphological features of the adult male holotype SYS a005519 of *Rana jiulingensis* sp. nov. in life. **A** dorsolateral view **B** dorsal view **C** ventral view **D** grey nuptial pad **E** left hand **F** left foot.

County, Jiangxi province. Male SYS a005511 collected by Zhi-Tong Lyu, Jian Wang and Hai-Long He on 13 September 2016 from Mt Mufu (28.9750N, 113.8304E; ca 1200 m a.s.l.), Pingjiang County, Hunan province. Males SYS a006494, SYS a006495/CIB 110014, and females SYS a006451, 6496, collected by Zhi-Tong Lyu on 5–6 August 2017 from Mt Dawei (28.4250N, 114.0805E; ca 800 m a.s.l.), Liuyang City, Hunan province.

Etymology. The specific name *jiulingensis* is in reference to the type locality, Guanshan Nature Reserve in Jiuling Mountains.

Suggested common name. Jiuling Mountains Brown Frog (in English), Jiu Ling Shan Lin Wa (九岭山林蛙 in Chinese)

Diagnosis. *Rana jiulingensis* sp. nov. is distinguished by the following morphological characteristics: (1) body medium-sized, SVL = 48.3–57.8 (51.7 ± 4.3 , $n = 4$) mm in adult males, 48.2–57.5 (50.8 ± 4.4 , $n = 4$) mm in adult females; (2) head length significantly larger than head width; (3) supratympanic fold absent; (4) dorsolateral fold distinct and thin, extending straight from posterior margin of upper eyelid to above groin; (5) internarial distances larger than interorbital distances; (6) tympanum diameter significantly smaller than eye diameter, TD/ED = 0.63–0.87; (7) fingers without circummarginal grooves, unwebbed, relative finger lengths $I < II < IV < III$; (8) presence of supernumerary tubercles below the bases of each finger, presence of three separated metacarpal tubercles; (9) toes without circummarginal grooves, toe webbing formula: $I \frac{1}{3} - 2 \ II \frac{1}{3} - 2 \frac{1}{3} \ III \ 1 \ \frac{1}{2} - 2 \ \frac{2}{3} \ IV \ 3 - 1 \ \frac{1}{3} \ V$, relative toe lengths $I < II < III < V < IV$; (10) tibio-tarsal articulation reaching forward beyond tip of snout; (11) heels overlapping; (12) dorsal skin smooth, flanks smooth with few granules; (13) absence of vocal sacs in males; (14) breeding males possess creamy white nuptial pad with tiny hoar spines on the finger I, divided into three parts; (15) presence of reddish tubercles on loreal and temporal regions in breeding males.

Description of holotype. SYS a005519, adult male, SVL 57.8 mm. Head length significantly larger than head width (HW/HL = 0.85); snout pointed and projecting; nostril closer to tip of snout than eye; canthus rostralis distinct; internasal distance slightly larger than interorbital distance; tympanum rounded, smaller than eye (TD/ED = 0.72); tympanic rim prominent; pupil horizontal; loreal region concave, sloping outwards; vomerine teeth present; tongue deeply notched posteriorly; vocal sacs absent.

Forearms 0.19 of SVL and hand 0.26 of SVL; fingers slender, without web but with narrow fringe; tip of fingers rounded, not expanded, without circummarginal grooves; relative finger lengths $I < II < IV < III$; subarticular tubercles significantly prominent, rounded; distinct, small, rounded supernumerary tubercles below the bases of each finger; inner metacarpal tubercle indistinct, ovoid, partly covered by nuptial pad; two outer metacarpal tubercles distinctly separated, slightly larger, long elliptic; nuptial pad with tiny spines on the finger I, divided into three parts, the basal one around the inner metacarpal tubercle and partly covering it, the largest one from the edge of the basal one to the subarticular tubercle of finger I, the smallest one extending from the edge of the biggest one to the tip of finger I.

Tibia 0.63 of SVL and foot 0.88 of SVL; heels overlapping when hindlimbs flexed at right angles to axis of body; tibio-tarsal articulation reaching forward beyond the tip

of snout when hindlimb stretched along the side of the body; relative toe lengths $I < II < III < V < IV$; toes webbing formula: $I \frac{1}{3} - 2 \ II \frac{1}{3} - 2\frac{1}{3} \ III \ 1 \frac{1}{2} - 2\frac{2}{3} \ IV \ 3 - 1\frac{1}{3} \ V$; absence of lateral fringes on the lateral edges of toes I and V; subarticular tubercles oval and distinct; inner metatarsal tubercle large, ovoid, outer metatarsal tubercle small.

Dorsal skin smooth with sparse tiny granules; several small tubercles on flank; supratympanic fold absent; dorsolateral fold distinct and thin, extending straight from posterior margin of upper eyelid to above groin; several tiny granules on the skin of loreal and temporal regions; ventral surface smooth, large flattened tubercles densely arranged on the rear of thigh and around vent.

Coloration of holotype. In life, dorsal surface yellowish brown with few black spots; black speckles forming a linear stripe between eyelids; dorsolateral fold intermittently edged with black on two sides; loreal region yellowish; temporal region yellowish, slightly tinged with grey; tiny granules on loreal and temporal regions reddish; dorsal forelimbs and hindlimbs reddish with indistinct greenish grey transverse bars. Throat yellowish; chest and belly creamy white; ventral surface of forelimbs and hindlimbs flesh color; nuptial pad creamy white; tubercles around vent yellowish.

In preservative, dorsal surface turns grey with black spots and light grey patches; limbs taupe with brown transverse bars. Ventral surface white, with greyish mottling on throat and belly; ventral surface of limbs beige; hands and toe webs dark grey.

Variations. Measurements of type series specimens are given in Table 2. Coloration of dorsal skin varies from brown to yellowish brown (Fig. 4). Black edges on dorsolateral fold indistinct in all paratypes. SYS a006495 and 6496 with V-shaped mark. The number of transverse bars ranges from two to five on forearms, three or four on thigh, and three to six on tibia.

Distribution and ecology. Currently, *Rana jiulingensis* sp. nov. is known from Guanshan Nature Reserve in the Jiuling Mountains and Mount Wugong in the Wugong Mountains of northwestern Jiangxi, and Mount Mufu and Mount Dawei in the Mufu Mountains of northeastern Hunan. This suggests that its geographic distribu-

Table 2. Measurements (in mm) of the type series of *Rana jiulingensis* sp. nov. (* = holotype).

	SYS a005519*	SYS a005511	SYS a006494	SYS a006495	SYS a002584	SYS a002585	SYS a006451	SYS a006496
Sex	Male	Male	Male	Male	Female	Female	Female	Female
SVL	57.8	51.6	48.3	49.1	57.5	48.4	49.4	48.2
HL	21.6	19.3	18.4	17.7	22.3	18.9	18.2	19.4
HW	18.4	17.0	15.7	12.6	19.3	16.1	15.5	15.8
SL	7.8	7.5	7.3	7.5	8.1	7.1	7.2	7.4
IN	4.1	3.8	3.5	3.2	4.2	3.6	4.1	4.1
IO	3.4	2.7	3.0	3.1	3.6	3.4	3.3	3.3
ED	6.3	5.5	5.2	5.0	5.2	4.4	4.7	4.6
TD	4.6	3.5	3.3	3.9	4.5	3.7	3.2	3.4
TED	1.9	2.0	1.7	2.0	1.7	1.7	1.7	1.6
HND	15.0	14.0	14.0	12.5	15.3	13.9	13.2	12.5
RAD	11.2	11.2	9.0	9.3	10.5	10.4	10.9	10.1
FTL	50.7	44.2	41.7	42.8	47.6	43.8	44.3	41.1
TIB	36.6	30.4	29.5	30.7	36.1	31.8	31.8	29.0



Figure 4. Variations of *Rana jiulingensis* sp. nov. **A, B** dorsolateral view and ventral view of male paratype SYS a006495 **C** male paratype SYS a006496 **D** male paratype SYS a00511.

tion is the central and northern parts of the Luoxiao Range (Fig. 1). All individuals were found on the surface of paths or on the bush leaves beside paths in subtropical evergreen broadleaved forests. Males SYS a005511 and 5519, which were collected in mid-September, bear a well-developed nuptial pad, while males SYS a006494 and 6495, collected in early August, are without a nuptial pad. This suggests that the breeding season of this species might begin in September.

Discussion

All recognized species of the subgenus *Rana* from China (except for *R. chevronata*) are included in our work for morphological and molecular analyses. Four monophyletic clades are supported by high values (BPP = 1.00 and BS > 85, respectively) in the phylogenetic tree. Three of them correspond to the morphologically recognized *R. japonica* group, *R. chensinensis* group, and *R. amurensis* group. The fourth, unnamed monophyletic clade includes *R. johnsi*, *R. sangzhiensis*, and *R. zhengi*. Within this unnamed clade, *R. sangzhiensis* and *R. zhengi* cluster together with significant support (BPP = 1.00 and BS = 100) and little divergence (0.0–0.4% in COI and 0.0–0.4% in 16S), which is consistent with the original morphological identification by Zheng et al. (1997). Therefore *R. zhengi* is considered a synonym of *R. sangzhiensis*. Furthermore,

all species of this clade were morphologically previously assigned to *Pseudorana* (Fei et al. 2009). Thus, based on the phylogenetic relationships and morphological similarities, this monophyletic clade is proposed as a new species group, the *Rana johnsi* group. For the remaining species, their exact placements remain unresolved due to the insignificant support. Further study of these species is needed, and new species groups might be proposed for these outcast species.

Within the *Rana japonica* group, the genetic divergences among three species, *R. longicrus*, *R. zhenhaiensis*, and *R. culaiensis*, are relatively closer than other species. Additionally, the validations of these species have been supported by the morphological examinations (Li et al. 2008; Fei et al. 2009). Anuran frogs are suggested with conservative phenotypes (Cherry et al. 1978). Cryptic species, which are morphologically identical but genetically differentiated, are also common in most species complexes (e.g. Yan et al. 2011; Kuraishi et al. 2013; Xiong et al. 2015; Lyu et al. 2019, 2020). With remarkable morphological diversity, but relatively smaller genetic differentiation, *R. longicrus*, *R. zhenhaiensis*, and *R. culaiensis* show a special situation. This suggests that an integrative taxonomic approach is especially important in delimitation of anuran species, and that reliance solely on morphological or molecular evidence would be misleading.

The discovery of *Rana jiulingensis* sp. nov. increases the diversity of the genus *Rana* in the Luoxiao Range to five species (Fig. 1). This situation indicates that the Luoxiao Range has the greatest diversity of *Rana* species in southern China and may be key to speciation of the genus *Rana*.

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Appendix I

Specimens examined

- Rana chaochiaoensis* (3): **China: Sichuan:** Zhaojue County (type locality): SYS a001815–1816, 1831.
- Rana chensinensis* (2): **China: Henan:** Mt Yawu: SYS a002392–2393.
- Rana culaiensis* (4): **China: Jiangxi:** Mt wugong: SYS a002634; Shanggao County: SYS a002641; Mt Meiling: SYS a004239, 4241.
- Rana hanluica* (35): **China: Hunan:** Mt Yangming (type locality): SYS a001137–1147; Mt Bamian: SYS a004086; Mt Badagong: SYS a004298; Mt Yunshan: SYS a004358–4359; Mt Xuefeng: SYS a007216; Suining County: SYS a007250–7251; Mt Shunhuang: SYS a007259–7260; **Guizhou:** Mt Leigong: SYS a002233; Mt Fanjing: SYS a004346; **Jiangxi:** Mt Jinggang: SYS a004195–4196; Mt Qiyun: SYS a004087; **Guangxi:** Longsheng County: SYS a002284–2288; Mt Dupangling: SYS a005086–5088; **Guangdong:** Renhua County: SYS a007009–7100.
- Rana jiemuxiensis* (2): **China: Hunan:** Jiemuxi Nature Reserve (type locality): SYS a004318–4319.
- Rana kukunoris* (7): **China: Sichuan:** Hongyuan County: SYS a006652–6654; Maonian County: SYS a005381–5384.
- Rana longicrus* (18): **China: Fujian:** Mt Yashu: SYS a005892, 5905; **Guangdong:** Renhua County: SYS a000732–0735, 5624–5625; Mt Nankun: SYS a000754, 4589, 5579; Yingde City: SYS a007519; Pu'ning City: SYS a004605; Mt Tonggu: SYS a005808; **Jiangxi:** Mt Qiyun: SYS a002355; Mt Jiulian: SYS a004487; Mt Magu: SYS a007038; Suichuan County: SYS a007097.
- Rana omeimontis* (5): **China: Sichuan:** Mt Emei (type locality): SYS a005304–5305; Anzhou District: SYS a005393; **Guizhou:** Qixingguan District: SYS a007294–7295.
- Rana zhenhaiensis* (7): **China: Zhejiang:** Fenghua District: SYS a006208, 7506–7507; **Jiangxi:** Mt Tongbo: SYS a001951–1953; Guanshan Nature Reserve: SYS a007000.

Supplementary material I

Tables S1, S2

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Data type: Tables for genetic distances

Explanation note: **Table S1.** Pairwise distances based on COI gene among all sample used in this study. **Table S2.** Pairwise distances based on 16S gene among all sample used in this study.

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