



A new *Leptobrachium* (Anura: Megophryidae) from the highlands of southeastern Laos

BRYAN L. STUART¹, SOMPHOUTHONE PHIMMACHAK^{2,3},
SENGVILAY SEATEUN^{2,4} & NIANE SIVONGXAY²

¹North Carolina Museum of Natural Sciences, 11 West Jones Street, Raleigh NC 27601, USA

²National University of Laos, Faculty of Science, Department of Biology, P.O. Box 2273, Dong Dok Campus, Vientiane, Laos

³Kasetsart University, Faculty of Science, Department of Zoology, Ngam Wong Wan Road, Chatuchak, Bangkok, 10900, Thailand

⁴Wildlife Conservation Society, P.O. Box 6712, Vientiane, Laos

Abstract

Twelve species of *Leptobrachium* are known from Vietnam, Laos and Cambodia, most of which have been described to science in the past twelve years. Our fieldwork on the Dakchung Plateau of southeastern Laos discovered a small *Leptobrachium* with a yellow upper iris that resembled *L. buchardi*, a species known only by the female holotype from the Bolaven Plateau of southern Laos. We use our new collections to expand the original description of *L. buchardi*, and describe the Dakchung species as new. *Leptobrachium xanthops* **sp. nov.** is distinguished from its congeners by having small body size (males with SVL 44.7, females with SVL 38.8–45.2), the upper one-half of iris and scleral arc pale yellow, a dark venter, and sexually active males without spines on the upper lip.

Key words: Bolaven Plateau; Dakchung Plateau; Laos; *Leptobrachium buchardi*

Introduction

Twelve species of the megophryid frog genus *Leptobrachium* are known from Indochina, consisting of the countries of Vietnam, Laos, and Cambodia (Ohler *et al.* 2004; Stuart 2005; Bain *et al.* 2009; Nguyen *et al.* 2009; Stuart *et al.* 2011), of which nine have been described since 1998. Most *Leptobrachium* have conspicuously colored eyes, with a colored upper iris and scleral arc, and these prominent features are often used to diagnose species (e.g., Dubois & Ohler 1998; Lathrop *et al.* 1998; Matsui *et al.* 1999; Ohler *et al.* 2004; Brown *et al.* 2009; Matsui *et al.* 2010).

Our fieldwork in 2011 on the Dakchung Plateau of southeastern Laos revealed a small *Leptobrachium* species with a yellow upper iris and scleral arc that was similar in size to *L. buchardi*, a species known only by the single female holotype from the nearby Bolaven Plateau of southern Laos and reported to have a green upper iris (Ohler *et al.* 2004). We collected *L. buchardi* at its type locality in 1999 and 2010, and we use those collections to expand its original description and show that it is distinct from the Dakchung species, which is described here as new.

Material and methods

Specimens were collected on the Bolaven Plateau, Pakxong District, Champasak Province, Laos, by Bryan L. Stuart and Harold Heatwole in September 1999 and Bryan L. Stuart, Niane Sivongxay, and Sengvilay Seateun in July 2010, and on the Dakchung Plateau, Phou Ajol Mountain, Dakchung District, Xe Kong Province, Laos, by Bryan L. Stuart, Somphouthone Phimmachak, and Sengvilay Seateun in May 2011 (Figure 1). Specimens were fixed in 10% buffered formalin after preserving liver in 20% DMSO-salt saturated storage buffer and RNAlater (Ambion). Specimens were later transferred to 70% ethanol. Specimens and tissue samples were deposited at the Field

Museum of Natural History (FMNH), North Carolina Museum of Natural Sciences (NCSM), and National University of Laos, Faculty of Science, Department of Biology (NUOL).

Comparative material examined in the holdings of the Australian Museum (AMS), FMNH, Natural History Museum, London (BMNH), NCSM, and University of Science-Ho Chi Minh City (UNS) is listed in the Appendix. Data were also taken from the literature (Bourret 1937; Lathrop *et al.* 1998; Matsui *et al.* 1999; Orlov 2005; Rao *et al.* 2006).

Measurements were taken to the nearest 0.1 mm with dial calipers: snout-vent length (SVL); head length from tip of snout to rear of jaws (HDL); maximum head width (HDW); snout length from tip of snout to anterior corner of eye (SNT); eye diameter (EYE); interorbital distance (IOD); internasal distance (IND); shank length (SHK); thigh length (TGH); forearm length, from elbow to base of outer palmar tubercle (LAL); manus length from tip of third digit to base of outer palmar tubercle (HND); pes length from tip of fourth toe to base of inner metatarsal tubercle (FTL); inner metatarsal tubercle length (IML); and inner metatarsal tubercle width (IMW).

Total genomic DNA was extracted from liver using PureGene Animal Tissue DNA Isolation Protocol (Gentra Systems, Inc.). A 555 nucleotide basepair fragment of mitochondrial (mt) DNA that encodes part of the 16S rRNA gene was amplified by PCR (the polymerase chain reaction; one cycle of 94°C 5 min, 35 cycles of 94°C 45 s, 60°C 30 s, 72°C 1 min, one cycle of 72°C 10 min) using the primers 16Sar-5' and 16Sbr-3' (Palumbi 1996). PCR products were cleaned using GELase (Epicentre Technologies) or ExoSAP-IT (USB). Cycle sequencing products were sequenced in both directions on a 3730 DNA Analyzer (Applied Biosystems) using the amplifying primers and Big Dye version 3 chemistry (Perkin Elmer). Sequences were edited with Sequencher v. 4.1 (Genecodes) and deposited in GenBank under accession numbers JN711501-JN711503. Homologous fragments of 16S obtained by Rao & Wilkinson (2008), Zheng *et al.* (2008), Matsui *et al.* (2010), and Stuart *et al.* (2011) were downloaded from GenBank so that types or topotypes of every species of *Leptobrachium* reported from Vietnam, Cambodia, or Laos were included in the analysis (Table 1). Downloaded sequences were trimmed to match the length of the fragment obtained here. The data set was aligned using MUSCLE (Edgar 2004), and pairwise distances were calculated using PAUP* 4.0b10 (Swofford 2002).

***Leptobrachium buchardi* Ohler, Teynié & David, 2004**

Expanded description. Three adult males (FMNH 258082, 258084, 258087) and seven adult females (FMNH 258083, 258085–86, 258089–90, NCSM 77762–63) from the Bolaven Plateau of southern Laos have habitus moderately stocky; body tapering to groin. Head broad and depressed; head length and width subequal. Snout rounded in dorsal view, sharply sloping in profile, barely projecting beyond lower jaw in profile; nostril slightly closer to tip of snout than eye, below canthus, internarial shorter than interorbital distance; canthus rostralis distinct; lores oblique, moderately concave; eye large, slightly projecting from side of head, diameter shorter than snout length, interorbital distance longer than upper eyelid width; no pineal ocellus; tympanum round or oval, annulus weakly visible, tympanum diameter about 50% eye diameter in males, 40% in females, and greater than distance between tympanum and eye; tongue heart-shaped, notched posteriorly; large, slit-like vocal sac openings on floor of mouth near lateral margin of tongue in males, absent in females; vomerine teeth absent.

Forelimb slender, more robust in males. Fingers moderately slender, without webbing. Tip of fingers blunt, slightly swollen; relative finger lengths II = IV < I < III; two oval palmar tubercles in contact, subequal in size, low callous bumps on ventral surface of fingers; nuptial pad absent.

Hindlimb slender and relatively short. Toes moderately slender; webbing on toes I and II to level of distal margin of subarticular tubercle and continuing as a fringe to base of tip, on preaxial side of toe III to level of distal margin of proximal subarticular tubercle continuing as a fringe to base of tip, on postaxial side of toe III to level of proximal margin of distal subarticular tubercle continuing as a fringe to base of tip, on toe IV to level of proximal subarticular tubercle continuing as a fringe to base of tip, and on toe V to midway between base and tip. Tips of all toes blunt, slightly swollen; relative toe lengths I < II < V < III < IV; distinct, oval, inner metatarsal tubercle, length about 80% distance between tip of toe I and tubercle; no outer metatarsal tubercle.

Skin above smooth with fine network of ridges, some scattered small tubercles posteriorly; no spines on upper lip; low supratympanic ridge from posterior edge of eye to axilla; ventrally granular, skin smooth on ventral surfaces of limbs; oval axillary gland on ventrolateral surface slightly posterior to insertion of forelimb with body; oval femoral gland on posteroventral surface of thigh, slightly closer to knee than vent.

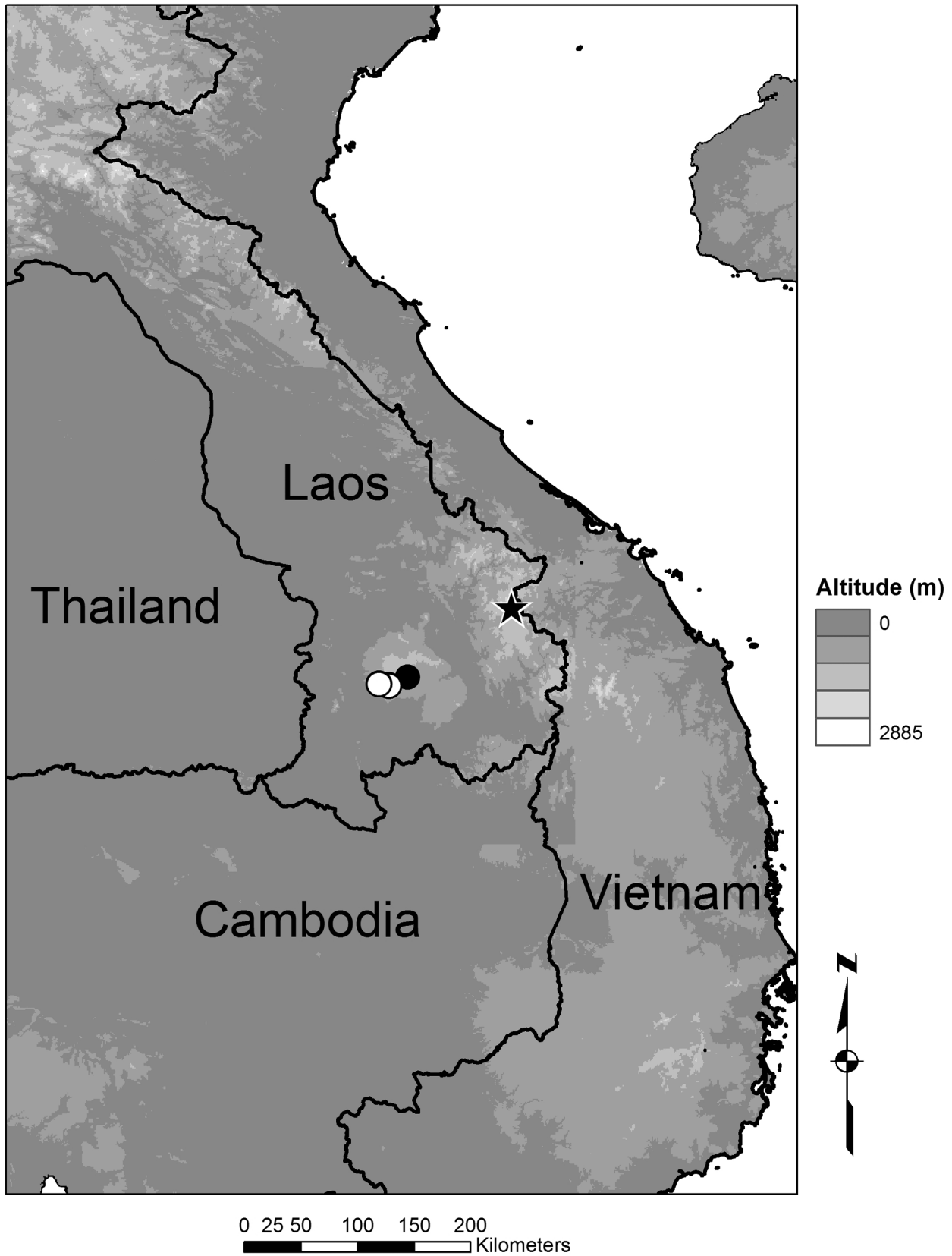


FIGURE 1. Map illustrating the type locality (star) of *Leptobrachium xanthops* sp. nov. on the Dakchung Plateau, Dakchung District, Xe Kong Province, Laos, and the type locality (black circle) and new localities (white circles) of *L. buchardi* on the Bolaven Plateau, Pakxong District, Champasak Province, Laos.

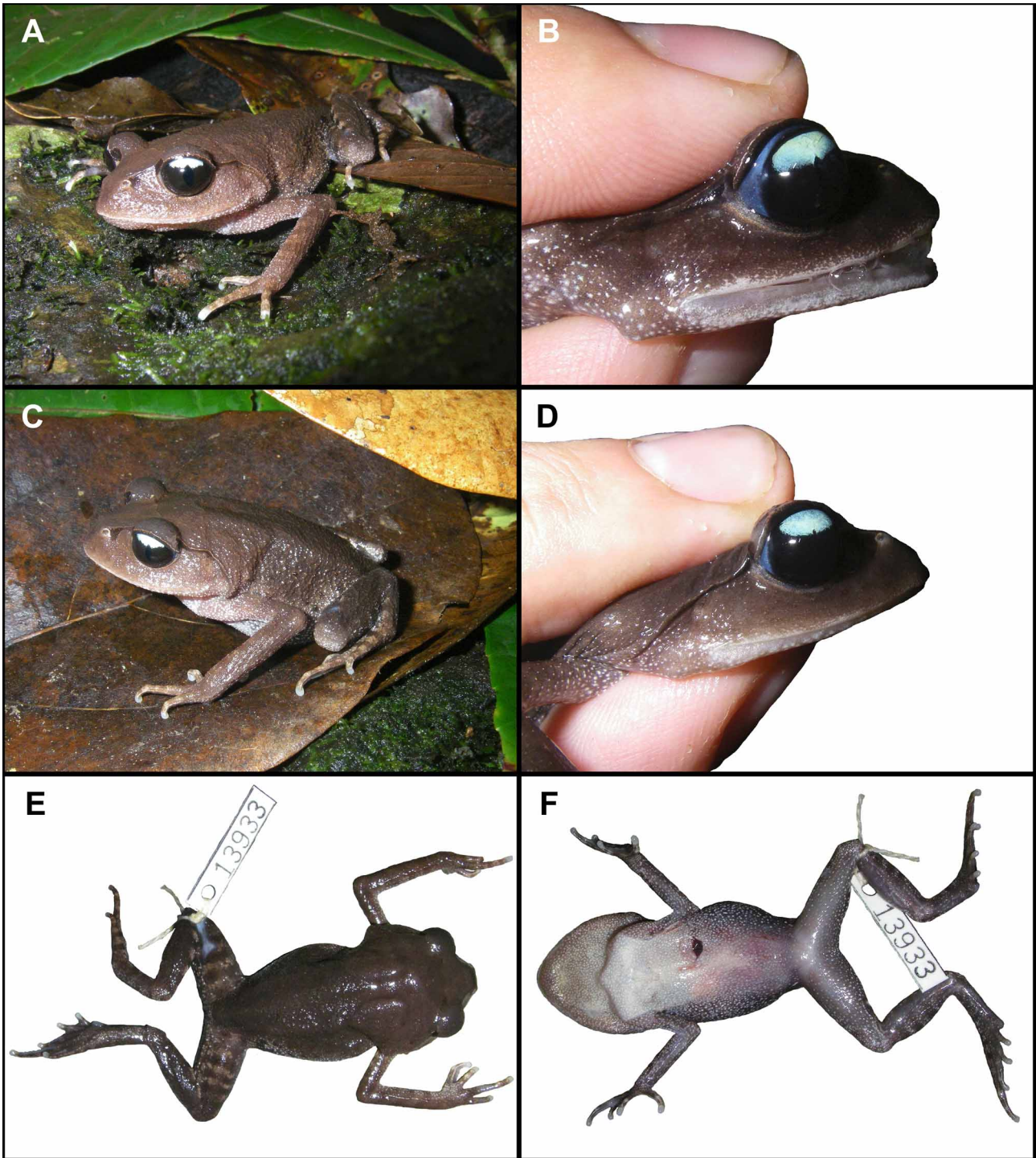


FIGURE 2. *Leptobrachium buchari* in life. (A) Lateral view and (B) eye with palpebrum retracted of NCSM 77762. (C) Lateral view, (D) eye with palpebrum retracted, (E) dorsal view, and (F) ventral view of NCSM 77763.

In life (based on NCSM 77762–63; Figure 2), dorsum dark gray-brown, without distinct markings, few small black spots posteriorly; flank gray with black spots and white tubercles; upper surface of forelimb as dorsum, with indistinct darker bands, upper surface of hindlimb with black bands and bronze bands, some gray bands on dorsal surface of tibiotarsus and foot; eye dark brown with upper one-third of iris white with light green wash (NCSM 77762) or white with light blue wash (NCSM 77763), scleral arc bright blue (visible in the posterior corner of the eye and when the palpebrum is retracted); narrow black streak under canthus and supratympanic fold; chin white with light brown markings, chest white, belly and ventral surface of limbs gray, minute white spots on tubercles on chin, chest, belly, and ventral surface of limbs; axillary and femoral glands white. In preservative (based on all ten

specimens), bronze limb bands fade to tan or gray. Other specimens closely resemble NCSM 77762–62 in preservative, except limb bands more distinct in some specimens (e.g. FMNH 258084) and more small black spots visible on posterior dorsum in some specimens (e.g. FMNH 258083).

Measurements are summarized in Table 1.

TABLE 1. Measurements (mm) of *Leptobrachium* from southern Laos. Abbreviations defined in the text.

Measurement	<i>L. xanthops</i> sp. nov.			<i>L. buchardi</i>		
	Male <i>n</i> =1	Females <i>n</i> =2 Range	Immature female <i>n</i> =1	Topotype males <i>n</i> =3 Range; Mean ± SD	Topotype females <i>n</i> =7 Range; Mean ± SD	Holotype female ¹ <i>n</i> =1
SVL	44.7	50.3–56.9	47.1	45.1–48.0; 46.1 ± 1.7	49.2–56.5; 54.1 ± 2.6	49.5
HDL	22.9	23.7–28.1	22.7	21.2–22.2; 21.7 ± 0.5	23.8–25.8; 24.8 ± 0.9	21.7
HDW	20.8	22.0–25.5	20.6	20.3–22.0; 21.4 ± 1.0	22.9–24.5; 23.9 ± 0.6	20.6
SNT	8.7	8.3–10.5	7.9	8.3–8.7; 8.5 ± 0.2	9.0–10.2; 9.8 ± 0.4	9.6
EYE	7.1	7.9–8.6	7.2	6.6–7.2; 6.9 ± 0.3	7.7–8.7; 8.2 ± 0.3	7.2
IOD	6.1	6.4–6.5	6.6	6.1–7.0; 6.5 ± 0.5	6.6–7.8; 7.3 ± 0.4	6.6
IND	4.6	4.9–5.3	4.3	3.9–4.5; 4.2 ± 0.3	4.7–5.6; 5.2 ± 0.3	4.3
SHK	16.7	19.1–21.3	18.2	17.0–18.3; 17.6 ± 0.7	18.0–20.8; 19.6 ± 1.1	17.1
TGH	19.5	22.7–26.5	21.4	17.8–18.9; 18.3 ± 0.6	19.3–24.6; 22.0 ± 2.0	18.8
LAL	14.9	18.2–18.8	16.6	14.8–15.4; 15.2 ± 0.3	17.3–19.7; 18.6 ± 0.9	14.8
HND	10.3	12.7–13.6	12.4	10.3–12.2; 11.3 ± 1.0	10.3–13.7; 12.2 ± 1.2	12.5
FTL	17.0	21.3–21.8	19.6	17.7–18.7; 18.2 ± 0.5	18.8–21.5; 20.4 ± 1.1	18.3
IML	2.4	2.7–3.0	2.5	2.3–2.7; 2.4 ± 0.2	2.3–3.6; 2.8 ± 0.4	2.6
IMW	1.4	1.5–1.9	1.6	1.0–1.6; 1.3 ± 0.3	1.3–1.7; 1.5 ± 0.2	-

¹Data from Ohler *et al.* (2004)

Distribution and natural history: *Leptobrachium buchardi* is known only from the Bolaven Plateau in Pakxong District, Champasak Province, Laos (Figure 1). The holotype was obtained at 1,200–1,250 m elevation “under a stone in a disturbed area” (Ohler *et al.* 2004). The new specimens reported here were collected at 1,000–1,240 m elevation in wet evergreen forest and in coffee plantations near the edge of wet evergreen forest, during the day under leaf litter and at night on leaf litter or bare soil, at least 10 m from water.

Leptobrachium xanthops sp. nov.

Holotype. NCSM 78468 (field tag BLS 14376), adult male (Figure 3), Laos, Dakchung Plateau, Phou Ajol Mountain, Xe Kong Province, Dakchung District, 15.68239°N 107.19424°E, 1,475 m elev., coll. 18 May 2011 by Bryan L. Stuart, Somphouthone Phimmachak, and Sengvilay Seateun.

Paratypes. Two adult females: NCSM 78466, same data as holotype except coll. 17 May 2011; NCSM 78467, same data as holotype except coll. 15.68516°N 107.19814°E, 1,450 m elev., 17 May 2011. One subadult female: NUOL 00001, same data as holotype except coll. 15.68444°N 107.19689°E, 1,500 m elev., 19 May 2011.

Referred material. One metamorph: NCSM 78469, same data as holotype except coll. 15.68477°N 107.19407°E, 1,490 m elev.

Etymology. The specific epithet taken from *xanthos* Gr. for yellow and *ops* Gr. for eye, in reference to the iris color of the new species.

Diagnosis. Assigned to the genus *Leptobrachium* on the basis of having head width longer than shank length; skin above with a network of ridges; large axillary glands; extremities of digits rounded; and upper part of iris colored differently from lower part (Dubois & Ohler 1998). A small-sized *Leptobrachium* having males with SVL

44.7, females with SVL 38.8–45.2; lower one-half of iris dark brown, upper one-half of iris pale yellow, scleral arc pale yellow; dark venter (purplish-gray in preservative) with minute white spots on tubercles; and sexually active males without spines on the upper lip.

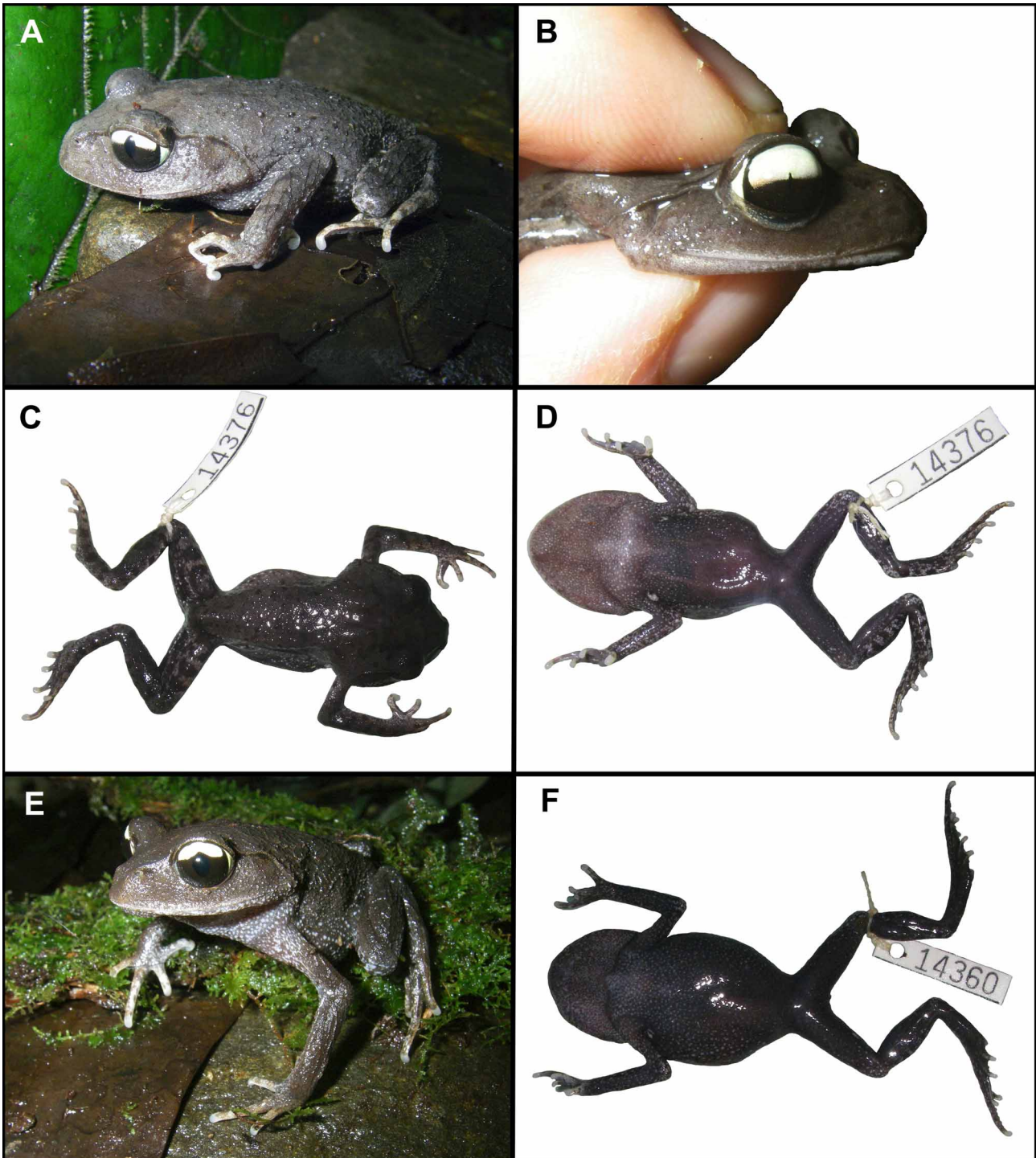


FIGURE 3. *Leptobrachium xanthops* sp. nov. in life. (A) Lateral view, (B) eye with palpebrum retracted, (C) dorsal view, and (D) ventral view of the male holotype NCSM 78468. (E) Lateral view and (F) ventral view of the female paratype NCSM 78467. The upper iris and scleral arc are pale yellow in life, but appear near to white under camera flash.

Description of holotype. Habitus moderately stocky; body tapering to groin. Head broad and depressed; head length slightly longer than head width. Snout rounded in dorsal view, sharply sloping in profile, barely projecting beyond lower jaw in profile; nostril slightly closer to tip of snout than to eye, below canthus, internarial shorter

than interorbital distance; canthus rostralis distinct; lores oblique, moderately concave; eye large, slightly projecting from side of head, diameter shorter than snout length, interorbital distance subequal to upper eyelid width; no pineal ocellus; tympanum round, slightly elevated from side of head, annulus weakly visible, tympanum diameter about 40% eye diameter and greater than distance between tympanum and eye; tongue heart-shaped, notched posteriorly; large, slit-like vocal sac openings on floor of mouth near lateral margin of tongue; vomerine teeth absent; testes mature.

Forelimb slender. Fingers moderately slender, without webbing. Tip of fingers blunt, that of fingers I slightly swollen; relative finger lengths $II < IV < I < III$; two oval palmar tubercles in contact, inner larger than outer, low callous bumps on ventral surface of fingers; nuptial pad absent.

Hindlimb slender and relatively short. Toes moderately slender; webbing on toe I and preaxial side of toe II to level of distal margin of subarticular tubercle, on postaxial side of toe II to base of tip, on preaxial side of toe III to level of proximal subarticular tubercle continuing as a fringe to base of tip, on postaxial side of toe III to midway between proximal subarticular tubercle and tip continuing as a fringe to base of tip, on preaxial and postaxial sides of toe IV to same level as postaxial side of toe III and continuing as a fringe to base of tip, and on toe V to subarticular tubercle continuing as a fringe to base of tip. Tips of all toes blunt, slightly swollen; relative toe lengths $I < II < V < III < IV$; distinct, oval, inner metatarsal tubercle, length about 75% distance between tip of toe I and tubercle; no outer metatarsal tubercle.

Skin above smooth with fine network of ridges, with small tubercles above sacrum; no spines on upper lip; low supratympanic ridge from posterior edge of eye to axilla; ventrally granular, skin smooth on ventral surfaces of limbs; large, round axillary gland on ventrolateral surface slightly posterior to insertion of forelimb with body; indistinct femoral gland on posteroventral surface of thigh, closer to knee than vent.

Color of holotype in life. Dorsum dark gray, with indistinct, black spots on tubercles, white spots on tubercles near vent; upper flank like dorsum, lower flank like belly, with small white spots at interface; upper surface of forelimb with narrow, indistinct black bands; upper surface of hindlimb with black bands and indistinct bronze bands on dorsal surface of thigh, black and light gray bands on dorsal surface of tibiotarsus and foot; lower one-half of iris dark brown, upper one-half of iris pale yellow, scleral arc pale yellow (visible in the posterior corner of the eye and when the palpebrum is retracted); irregular, black streak under canthus and supratympanic fold; ventral surface of body and limbs dark purplish-gray, chin and chest lighter than belly, minute white spots on tubercles on chin, chest, and belly; axillary and femoral glands white.

Color of holotype in preservative: Color in preservative almost uniformly dark gray-brown, with only faint traces of banding on hindlimb visible. Upper part of iris and scleral arc faded to white.

Variation: Paratypes closely resemble the holotype, except NCSM 78466 had indistinct bronze bands on forelimb in life. Females with thinner forelimbs than males. Females NCSM 78466–67 with large, brown ova in preservative, female NUOL 00001 with developing creamy-white ova in preservative. Measurements are summarized in Table 1.

Molecules. The holotype and two paratypes (NCSM 78466, NUOL 00001) are identical in the 16S gene fragment, but are divergent from topotypes of other *Leptobrachium* species known from southern Laos and adjacent central Vietnam. Specifically, the new species has an uncorrected pairwise divergence of 8.01% from *L. banae*, 4.01% from *L. buchardi*, 11.66% from *L. chapaense*, 3.06% from *L. ngoclinhense*, 3.09% from *L. pullum*, and 6.57% from *L. xanthospilum* (Table 2).

Distribution and natural history. *Leptobrachium xanthops* is known with certainty only from wet evergreen forest at 1,450–1,500 m elevation on the Dakchung Plateau, Phou Ajol Mountain in Xe Kong Province, Dakchung District, Laos. The species probably occurs elsewhere in the highlands of southeastern Laos and adjacent central Vietnam, and may be hidden in existing natural history collections under previously named species of *Leptobrachium*. The adults were found at night (1905–2215 h) on leaf litter within 15 m of 3–5 m wide swift, rocky streams. Calling was not heard. The subadult was taken at night (2000 h) on leaf litter away from water. The metamorph was found at night (2110 h) on a stone in shallow water at the bank of a 5 m wide swift, rocky, cascading stream.

Comparisons. *Leptobrachium xanthops* differs from all other species of *Leptobrachium* by having a pale yellow upper one-half of the iris and scleral arc. It further differs from all other species of *Leptobrachium* that occur in Laos, Vietnam, or Cambodia (Table 2) in size, body coloration, and/or male secondary sexual characters. Specifically, *L. ailaonicum*, *L. echinatum*, and *L. ngoclinhense* have males with $SVL > 60$ (44.7 in *L. xanthops*) and spines

on the upper lip (absent in *L. xanthops*). *Leptobrachium banae* and *L. xanthospilum* have males with SVL > 57 (44.7 in *L. xanthops*) and females with SVL > 80 (50.3–56.9 in *L. xanthops*). In addition, *L. banae* has red bands on the limbs (absent in *L. xanthops*) and *L. xanthospilum* has large, yellow, glandular spots on the flank (absent in *L. xanthops*). *Leptobrachium buchari*, *L. pullum*, and *L. smithi* have light venters with dark spots (dark purplish-gray venter in *L. xanthops*), and *L. leucops* has distinct dark markings on the dorsum and banding on the limbs (absent in *L. xanthops*). *Leptobrachium chapaense*, *L. mouhoti*, and *L. promustache* have males with SVL > 51 (44.7 in *L. xanthops*) and females with SVL > 58 (50.3–56.9 in *L. xanthops*), and *L. promustache* has males with spines on the upper lip (absent in *L. xanthops*).

Acknowledgements

Fieldwork was performed as part of the Biodiversity Conservation Project, a cooperative program between the National University of Laos and the Wildlife Conservation Society Laos Program. Research permits were provided by the Department of Forestry, Ministry of Agriculture and Forestry, Vientiane, and the Provincial and District Agricultural and Forestry Offices in Champasak Province (Pakxong District) and Xe Kong Province (Dakchung District). Specimen export permits to the Field Museum of Natural History and North Carolina Museum of Natural Sciences were provided by the Department of Forestry, Ministry of Agriculture and Forestry, Vientiane. Troy Hansel and the Wildlife Conservation Society Laos Program provided invaluable logistical assistance. Harold Heatwole assisted with fieldwork. Alan Resetar (FMNH) loaned specimens in his care, Barry Clarke (BMNH) facilitated a collection visit, and Jodi Rowley (AMS) provided unpublished data on *L. ngoclinhense* and improved the manuscript. Jonathan Raine constructed the map. This research was supported by The John D. and Catherine T. MacArthur Foundation and the National Geographic Society.

References

- Bain, R. H., Nguyen, T. Q. & Doan, K. V. (2009) First record of *Leptobrachium promustache* from Vietnam. *Herpetology Notes*, 2, 27–29.
- Bouret, R. (1937) Notes herpétologiques sur l'Indochine française. XIV. Les batraciens de la collection du Laboratoire des Sciences Naturelles de l'Université. Descriptions de quinze espèces ou variétés nouvelles. *Annexe au Bulletin Général de l'Instruction Publique*, 1937, 5–56.
- Brown, R. M., Siler, C. D., Diesmos, A. C. & Alcalá, A. C. (2009) Philippine frogs of the genus *Leptobrachium* (Anura; Megophryidae): phylogeny-based species delimitation, taxonomic review, and descriptions of three new species. *Herpetological Monographs*, 23, 1–44.
- Dubois, A. & Ohler, A. (1998) A new species of *Leptobrachium* (*Vibrissaphora*) from northern Vietnam, with a review of the taxonomy of the genus *Leptobrachium* (Pelobatidae, Megophryinae). *Dumerilia*, 4, 1–32.
- Edgar, R. C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792–1797.
- Lathrop, A., Murphy, R. W., Orlov, N. L. & Ho, C. T. (1998) Two new species of *Leptobrachium* (Anura: Megophryidae) from the Central Highlands of Vietnam with a redescription of *Leptobrachium chapaense*. *Russian Journal of Herpetology*, 5, 51–60.
- Matsui, M., Hamidy, A., Murphy, R. W., Khonsue, W., Yambun, P., Shimada, T., Ahmad, N., Belabut, D. M. & Jiang, J.-P. (2010) Phylogenetic relationships of megophryid frogs of the genus *Leptobrachium* (Amphibia, Anura) as revealed by mtDNA gene sequences. *Molecular Phylogenetics and Evolution*, 56, 259–272.
- Matsui, M., Nabhitabhata, J. & Panha, S. (1999) On *Leptobrachium* from Thailand with a description of a new species (Anura: Pelobatidae). *Japanese Journal of Herpetology*, 18, 19–29.
- Nguyen, V. S., Ho, C. T. & Nguyen, T. Q. (2009) Herpetofauna of Vietnam. Edition Chimaira, Frankfurt am Main.
- Ohler, A., Teynié, A. & David, P. (2004) A green-eyed *Leptobrachium* (Anura: Megophryidae) from southern Laos. *The Raffles Bulletin of Zoology*, 52, 695–700.
- Orlov, N. L. (2005) A new species of the genus *Vibrissaphora* Liu, 1945 (Anura: Megophryidae) from Mount Ngoc Linh (Kon Tum Province) and analysis of the extent of species overlap in the fauna of amphibians and reptiles of the north-west of Vietnam and Central Highlands. *Russian Journal of Herpetology*, 12, 17–38.
- Palumbi, S. R. (1996) Nucleic acids II: the polymerase chain reaction. pp. 205–247. In: D. M. Hillis, C. Moritz & B. K. Mable (eds.), *Molecular Systematics*. Second edition. Sinauer Associates, Inc., Sunderland, Massachusetts.
- Rao, D.-Q. & Wilkinson, J. A. (2008) Phylogenetic relationships of the mustache toads inferred from mtDNA sequences. *Molecular Phylogenetics and Evolution*, 46, 61–73.

- Rao, D.-Q., Wilkinson, J. A. & Zhang, M.-W. (2006) A new species of the genus *Vibrissaphora* (Anura: Megophryidae) from Yunnan Province, China. *Herpetologica*, 62, 90–95.
- Stuart, B. L. (2005) New frog records from Laos. *Herpetological Review*, 36, 473–479.
- Stuart, B. L., Rowley, J. J. L., Tran, D. T. A., Le, D. T. T. & Hoang, H. D. (2011) The *Leptobrachium* (Anura: Megophryidae) of the Langbian Plateau, southern Vietnam, with description of a new species. *Zootaxa*, 2804, 25–40.
- Swofford, D. L. (2002) PAUP*: Phylogenetic Analysis Using Parsimony *(and other methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- Zheng, Y., Li, S. & Fu, J. (2008) A phylogenetic analysis of the frog genera *Vibrissaphora* and *Leptobrachium*, and the correlated evolution of nuptial spine and reversed sexual size dimorphism. *Molecular Phylogenetics and Evolution*, 46, 695–707.

Appendix. Comparative specimens examined.

- Leptobrachium bucharidi*: FMNH 258082, FMNH 258084, FMNH 258087 (males), FMNH 258083, FMNH 258085–86, FMNH 258089–90, NCSM 77762–63 (females), Laos, Bolaven Plateau, Champasak Province, Pakxong District.
- Leptobrachium leucops*: NCSM 77465 (holotype male), AMS R173163, FMNH 280396, NCSM 77463–64, NCSM 77467, UNS 00121/AMS R173159, UNS 00122/AMS R173160, UNS 00123/AMS R173161, AMS R173162, AMS R173165, UNS 00124/AMS R173166, AMS R173168 (paratype males), UNS 00125/AMS R173167, AMS R173158 (paratype females), Vietnam, Langbian Plateau, Lam Dong Province, Lac Duong District. NCSM 77466, AMS R173164 (paratype males), Vietnam, Langbian Plateau, Khanh Hoa Province, Khanh Vinh District.
- Leptobrachium mouhoti*: FMNH 262756 (holotype male), FMNH 262753–55, FMNH 262757 (paratype males), Cambodia, Mondolkiri Province, O'Rang District. FMNH 261757–60, FMNH 261762–65 (paratype males), FMNH 261761 (paratype female), Cambodia, Mondolkiri Province, Pichrada District.
- Leptobrachium ngoclinhense*: AMS R173834, UNS 00416/AMS R173837 (males), Vietnam, Kon Tum Province, Dak Glei District, Mount Ngoc Linh.
- Leptobrachium pullum*: BMNH 1921.5.5.36 (lectotype male), BMNH 1921.5.5.32–35, BMNH 1972.1459–64 (paralectotype males), Vietnam, Langbian Plateau, Arbre Broyé. BMNH 1921.5.5.31 (paralectotype female), BMNH 1921.5.5.29–30, BMNH 1972.1465–66 (paralectotype males), Vietnam, Langbian Plateau, Camly. FMNH 280397, NCSM 77458–62, AMS R173138–39, UNS 00112/AMS R173140, UNS 00113/AMS R173141, UNS 00114/AMS R173142, AMS R173143–44 (males), AMS R173147 (female), Vietnam, Langbian Plateau, Lam Dong Province, Lac Duong District, Bi Doup-Nui Ba National Park. AMS R173145 (male), Vietnam, Langbian Plateau, Khanh Hoa Province, Khanh Vinh District.
- Leptobrachium xanthospilum*: FMNH 252919–20 (males), Vietnam, Gia-Lai Province, Ankhe District.