Article

"Same-same, but different": an unusual new species of the *Limnonectes kuhlii* Complex from West Sumatra (Anura: Dicroglossidae)

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Abstract

A new species in the dicroglossine genus *Limnonectes* from West Sumatra, Indonesia, is described. Analysis of DNA sequence data from the mitochondrial 12S and 16S gene regions places the species within the *Limnonectes kuhlii* Complex and demonstrates it to be the sister taxon of *Limnonectes kuhlii* sensu stricto from Java. Both molecular and morphological data support the recognition of this lineage as a new species. Notably, the presence of a spinule-covered mental plate distinguishes *Limnonectes sisikdagu* **sp. nov.** from all other members of the *L. kuhlii* Complex. Additionally, pair-wise sequence divergence greater than 10% separate the new species from its sister taxon, *L. kuhlii* from Java.

Key words: dicroglossine, *Limnonectes sisikdagu* **sp. nov.**, mitochondrial DNA, morphology, species complex

Introduction

Limnonectes Fitzinger (1843) comprises 55 currently recognized species (AmphibiaWeb, 2010). The genus is characterized by the presence of odontoid processes (hence the colloquial name of "fanged frogs"), male-biased size dimorphism, and male parental care (Emerson *et al.* 2000). Frogs of the genus *Limnonectes* are distributed throughout east and Southeast Asia, most are tied to forest stream habitats, and it is not uncommon to observe two or more congeners occurring in syntopy. The generotype, *Limnonectes kuhlii* Tschudi (1838), historically considered to be a single, broadly distributed species, was the focus of two recent molecular phylogenetic studies (Matsui *et al.* 2010; McLeod 2010) that highlighted a considerable amount of diversity hidden within this species complex. McLeod (2010) presented a robust molecular phylogeny of the *L. kuhlii* Complex (hereafter, *kuhlii* Complex) using mtDNA sequences from individuals representing approximately 63 populations across its known distribution. The results corroborated previous phylogenetic treatments of the *kuhlii* Complex (Emerson *et al.* 2000; Evans *et al.* 2003; Matsui *et al.* 2010; J. Zhang *et al.* 2005). Furthermore, McLeod (2010) demonstrated that *L. kuhlii*, which historically had been recognized as a single species, is a complex of more than 22 well-supported evolutionary lineages (*viz.*, species), 16 of which are currently subsumed under the nominal *L. kuhlii*. Tschudi (1838) designated the island of Java as the type locality for *L. kuhlii*. McLeod (2010) followed this designation and restricted all individuals from Java to retain the name *L. kuhlii*. Additionally, the study also uncovered several cases of sympatric/syntopic lineages, and in no case were co-occurring lineages each other's closest relatives (McLeod, 2010).

In 2001, a series of anuran specimens was collected from three stream systems in West Sumatra by one of us (D.I.). These specimens were identified as *Limnonectes kuhlii* and deposited in the Field Museum of Natural History. In 2010, these specimens were examined by one of us (D.S.M) as part of a larger study on the *kuhlii* Complex. Tissue samples from these specimens were sequence for mitochondrial DNA data and mensural data were collected from whole specimens. We present these data herein and describe a new species that is part of the *kuhlii* Complex and is demonstrated to be the sister taxon to "true" *Limnonectes kuhlii* from Java.

Accepted by M. Vences: 15 Apr. 2011; published: 19 May 2011

Material and methods

Species concept and species delimitation. We employ the Evolutionary Species Concept (Simpson, 1961; Wiley, 1978) expanded to the General Lineage Concept of species (de Queiroz, 1998, 1999). We consider distinct species as populations for which the hypothesis of conspecificity can be confidently rejected by analyses of both morphological and genetic data. Whereas the utility of mtDNA in phylogenetic studies of amphibians has been debated (e.g., Hertwig *et al.* 2004) and it seems clear that sequence data alone should not be used as the sole criterion for delimiting species, uncorrected pair-wise divergences of the mtDNA may be useful to identify candidate species (Fouquet *et al.* 2007; Vences *et al.* 2005; Vences *et al.* 2005; Vieites *et al.* 2009).

Morphology. Measurements were made with digital calipers to the nearest 0.01 mm. Morphometric characters of post metamorphic individuals used here follow those of McLeod (2008). Abbreviations used are: ED = eye diameter; EN = eye–nostril distance; FEL = thigh (femur) length; FOL = foot length; HL = head length; HW = head width; IN = internarial distance; IO = interorbital width; LAL = lower arm length; MH = mandible height; MN = mandible–nostril distance; OH = odontoid height; PAL = palm length; RFL = relative finger length when digits are adpressed; RL = rostrum length; RTL = relative toe length when digits are adpressed; SVL = snout–vent length; TBL = shank (tibia) length; TD = tympanum diameter; UAW = upper arm length; UEW = upper eyelid width; NM = no measurement taken. Digital webbing formulae follows that of Guayasamin *et al.* (2006). Digits of the hand are numbered II–V based on presumed homology to the digits of non-anuran tetrapods (Alberch & Gale, 1985). Sex and life stage were determined by examination of gonads and by inspection of prominent secondary sexual characters (e.g., nuptial pads). All illustrations were prepared with the aid of a binocular dissecting microscope and camera lucida.

Molecular methods. Mitochondrial DNA data were selected for use in this study to take advantage of abundant comparative material already available from previous work (Che *et al.* 2007; Emerson *et al.* 2000; Evans *et al.* 2003; Frost *et al.* 2006; Jiang & Zhou, 2005; Matsui *et al.* 2010; Zhang *et al.* 2005; Zhang *et al.* 2009). The gene order of the ~2400 bp of mitochondrial DNA region analyzed (5'–3') is transfer RNA for phenylalanine, 12S ribosomal DNA (rDNA), transfer RNA for Valine, and16S ribosomal DNA. DNA extraction and sequencing are detailed in McLeod (2010).

Phylogenetic analysis. New sequence data from the West Sumatran specimens (Genbank accession numbers: JF836873-JF836881) and data from Matsui *et al.* (2010) available from GenBank were added to the data matrix of McLeod (2010). Sequences were aligned using MAFFT (Katoh *et al.* 2002) and then adjusted by eye in Se-Al Carbon ver 2.0a11(Rambaut, 2002). Models of molecular evolution were selected for each DNA region using Model Test (Posada & Crandall, 1998). During preliminary partitioned and unpartitioned phylogenetic analyses, changes in topology or support were not observed, so in all final analyses were conducted on non-partitioned dataset. Maximum likelihood analyses were performed using RAxML-HPC Blackbox ver, 7.2.6 on the CIPRES portal at the San Diego Supercomputing Center (Miller *et al.* 2009; Stamatakis, 2006) via 100 non-parametric rapid bootstrap replicates. Bayesian analyses were conducted using MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck, 2003) on the BioHPC installation at Cornell University. Two independent analyses were run with four Metropolis-coupled Markov chains each. All Markov chains were run for 7 million generations, sampling every 1000 generations. To assess convergence between chains, we verified that the average standard deviation of split frequencies approached zero, the potential scale reduction factor approached 1, and that the log likelihood scores had reached stationarity. The output files were examined in Tracer ver. 1.4 (Rambaut and Drummond, 2007) to determine the number of generations to exclude as burn-in and, as a final check for convergence by ensuring that all parameters and statistics had reached stationarity and sufficient (>100) ESS. Based on work by Evans *et al.* (2003) and others (Che *et al.* 2007; Emerson *et al.* 2000; Frost *et al.* 2006; Zhang *et al.* 2005), *Occidozyga laevis* and *Fejervarya limnocharis* were used to root the phylogenies. A simplified tree showing the results of the re-analyses is presented in order to demonstrate the placement of the West Sumatran specimens within the larger *kuhlii* Complex (Fig. 1). Clade labels correspond to those of McLeod (2010). Clades with labels E1–4 comprise 27 representative species of *Limnonectes* from Evans *et al.* (2003).

Results

Clade A comprises two Sunda Shelf lineages—*L. kuhlii* from Java and *L.* **sp. nov.** from West Sumatra. Clades B and C contain Indochinese members of the *kuhlii* Complex, and Clade D comprises all Bornean *kuhlii* Complex

taxa. All analyses, regardless of method, recovered the same topology with the new species from West Sumatra and *L. kuhlii* as sister taxa, though support for nodes deep in the tree differed between analyses. McLeod (2010) presented a tree with reasonably strong support for a single *kuhlii* Complex clade (89/90 for ML bootstrap and Bayesian posterior probability support values, respectively) being sister to a clade containing non-*kuhlii* Complex species from the study of Evans *et al.* (2003). Results from analyses including new data suggest that the members of Clade A (*L. kuhlii* from Java and *L.* **sp. nov.** From West Sumatara) are more closely related to Clade E1 (containing *L. kardasani, L. microdiscus, L. laticeps,* and *L. gyldenstolpei*) than to other members of the *kuhlii* Complex, as in McLeod 2010 (Fig. 1). This relationship, though strongly supported in Bayesian analyses, received weak maximum likelihood bootstrap support. In an attempt to present the most conservative results, the deepest (and most weakly supported) relationships have been depicted as an unresolved polytomy in this study. Whereas these results do not undermine the relationships in question, it is evident that additional work is needed. A more robust molecular approach to phylogenetic relationships (e.g., including the addition of unlinked nuclear loci) will be required to better understand the phylogenetic relationships among clades within the genus *Limnonectes*.

FIGURE 1. Simplified phylogram demonstrating the position of the West Sumatran samples within the context of the genus *Limnonectes* and the *Limnonectes kuhlii* Complex. Phylogram based on a Bayesian analysis of 12–16S mitochondrial DNA sequences. Numbers above branches are Bayesian posterior probabilities; numbers below branches are non-parametric bootstrap support values from maximum likelihood analysis.

Limnonectes sisikdagu **sp. nov.**

Holotype. Adult male (FMNH 266636; Fig. 2), from Batang Andaleh, Lubek Selasih near Solok at 01˚0'37"S 100˚37'55"E, 1204 m to 01˚0'33"S 100˚38'20"E, 1252 m, collected on 06 September 2001 by Djoko Iskandar.
    Paratypes. Paratypes collected by Djoko Iskandar and deposited at the Field Museum of Natural History. Four adult males (FMNH 266619, 266630–31, 266637), four adult females (FMNH 266608, 266610, 266615,

266618), two juveniles (FMNH 266619, 266621). FMNH 266608 and FMNH 266610 (females), collected from Batang Harau, near Harau Valley, Payakumbuh at 00°4'06"S 100°39'17"E, 536 m to 00°6'42"S 100°39'51"E, 566 m on 07–08 August 2001. FMNH 266615, 266618 (females), 266616 (male), 266619 and 266621 (juveniles) were all collected from Sarasah Bunta, near Harau Valley, Payakumbuh at 00°5'37"S 100°40'15"E, 523 m to 00°6'42"S 100°40'28"E, 530 m on 08–17 August 2001. FMNH 266630 and 266631 (males) were collected at Batang Tarusan, Lubuk Selasih, near Solok at 01°0'44"S 100°37'55"E, 1170 m to 01°54'42"S 100°28'19"E, 1166 m on 02–10 September 2001. FMNH 266637 (male) was collected with holotype.

Referred specimens. Collected by David Bickford and deposited at the Raffles Museum of Biodiversity Research (RMBR). RMBR 393 (HM067244) from Batu Layng 03°27'49"S 102°18'83"E, 410m on 19 July 2007; RMBR 515 (HM067245) from Batu Layng 03°27'57"S 102°19'10"E, 460m on 21 July 2007. Represented in this study and McLeod (2010) by sequence data derived from tissue samples. Whole, preserved voucher specimens unavailable for examination.
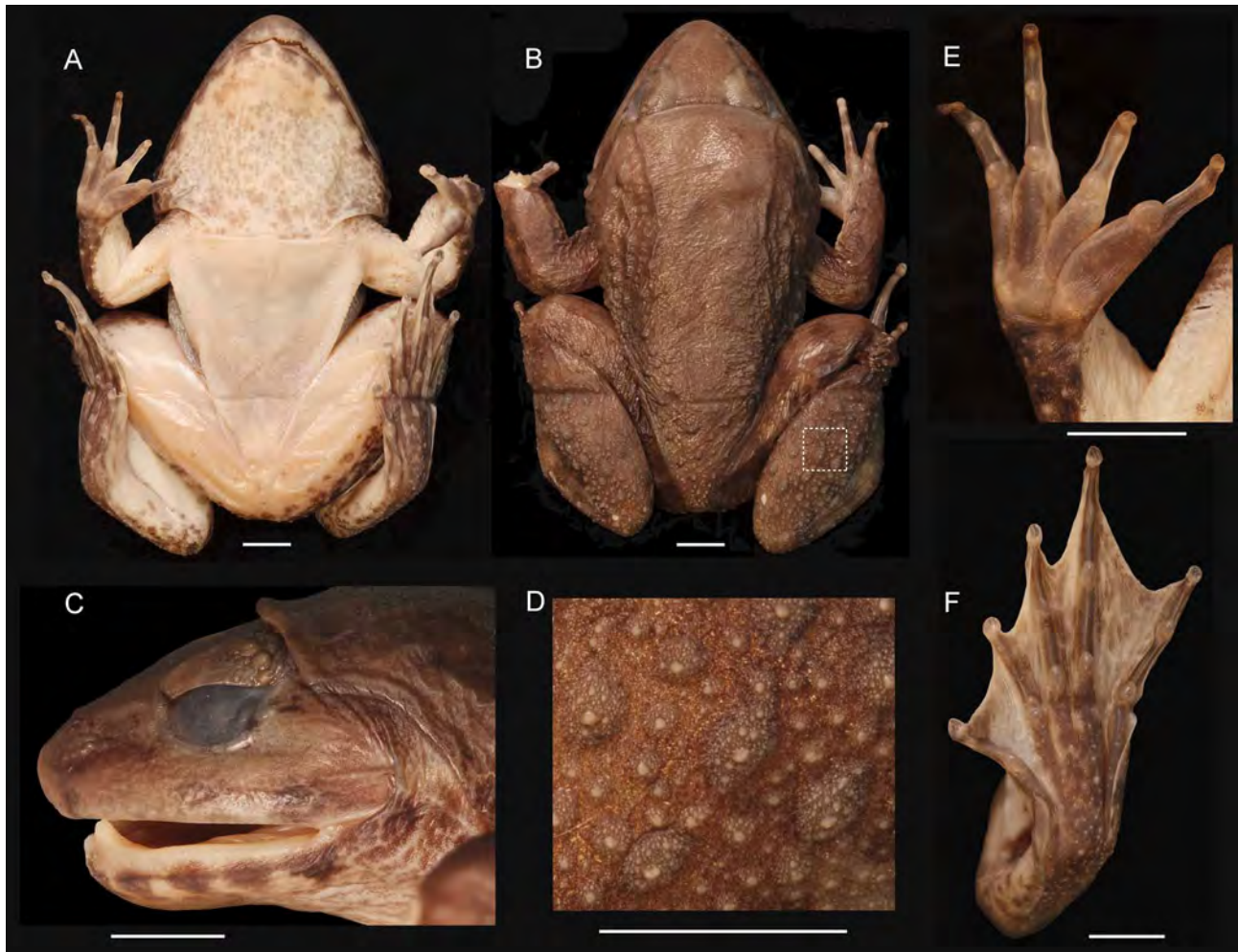

FIGURE 2. Holotype of *Limnonectes sisikdagu* in alcohol (FMNH 266636; adult male; SVL = 49.4 mm). (A) Ventral, (B) dorsal, and (C) lateral view of body and head; (D) magnified view of tubercules on leg (refence in image B); (E) palmar view of hand; (F) plantar view of foot. Scale bar = 5 mm.

Diagnosis. *Limnonectes sisikdagu* is considered a member of *Limnonectes* on the basis of molecular evidence and the following morphological diagnostic characters: presence of fanglike odontoid processes on the lower jaw (a sexually dimorphic feature; larger in males than females); male-biased size dimorphism (generally characterized by the hypertrophy of the head in males) (Emerson *et al.*, 2000). Additionally, these specimens are part of the *L. kuhlii* Complex on the basis of molecular data, and possession of an indistinct (or hidden) tympanum and fully webbed toes, both of which are characters traditionally used to diagnose *L. kuhlii* sensu lato from its congeners (Boulenger, 1920; Duméril & Bibron, 1841; Inger, 1966; Taylor, 1962; Tschudi, 1838).

Limnonectes sisikdagu can be distinguished from true *L. kuhlii* (Java), and from other members of the *kuhlii* Complex, by the following combination of characters: (1) males with spinule-covered mental patch (Fig. 3); (2)

adult male SVL 41.84–49.43 mm (mean = 45.36; SD ± 3.22; n = 5), adult female SVL 44.44–56.63 mm (mean = 49.10; SD ± 5.50; n = 4); (3) males with nuptial pads on first finger (Finger II; Fig. 4); (4) males with moderately enlarged heads (HL 43.84–47.97% of SVL; 39.43–42.76% in females); (5) males and females with heads that are longer than wide (HL 107–114% HW in males; 108–112% in females); (6) prominent post-orbital fold; (7) discontinuous dorsolateral fold; (8) throat lightly pigmented (mottled), moderate pigmentation on borders of thigh and leg, venter and ventral thigh immaculate; (9) moderately dense small, low glandular warts tipped with translucent spinules on flanks of body, around vent and on dorsum of thigh, shank, and foot; (10) dorsal skin and throat feebly crenulate; (11) relative finger length (longest to shortest) when adpressed: IV–V–II–III.

Description of holotype. Adult male (Figs. 2–4). Habitus robust with moderately enlarged head (HL 48% SVL); head longer than wide (HL 109% HW). Rostrum pointed in dorsal view, projecting beyond lower jaw, obtuse (sloping) in profile; nostril dorsolaterally oriented, closer to tip of snout than to eye; internarial distance greater than interorbital distance; canthus rounded; lores concave; upper lip distinctly swollen and flared, not reaching post-rictal tubercle; eye diameter 23% head length; upper eyelid width greater than interorbital distance. Supratympanic fold moderate, extending from eye to angle of jaw (insertion of arm); tympanic annulus not visible through skin; post-orbital skin fold intersects supratympanic fold just posterior to eye. Vomerine teeth on oblique ridges, separated from each other by much less than width of one ridge. Choanae oval, perpendicular to longitudinal axis of body. Odontoid processes robust with rounded tips, length nearly twice depth of mandible at base of processes. Symphysial knob at mandibular symphysis. Tongue oval, deeply notched posteriorly. Mental patch on anterior portion of chin covered with small translucent spinules, larger than, but resembling, those on nuptial pads.

Finger tips rounded, not expanded into discs, with rounded distal pad; decreasing lengths of fingers: IV–V–II–III; no webbing between fingers; distinct, movable fringe of skin on pre-and postaxial sides of Fingers III and IV; digits indicated by Roman numeral (tubercle count in parentheses): IV (2), III (2) II (1), I (1); proximal subarticular tubercles prominent, round, elevated on Fingers III and IV; distal subarticular tubercles low, flat and indistinct; thenar metacarpal tubercle large, oval, not elevated; inner metacarpal tubercle oval, smaller than thenar tubercle, not contacting outer or thenar tubercles; outer metacarpal tubercle smaller than inner tubercle, oval, elevated; prominent nuptial pad composed of minute spines on medial surface of Finger II from distal subarticular tubercle along penultimate distal phalanx and in a small patch on the thenar metacarpal tubercle. Tips of toes rounded, not expanded into discs, toe pads elevated; decreasing lengths of toes: IV–III–V–II–I; toes webbed to middle of terminal phalanx (webbing formula = **I**0$^+$–0$^+$**II**0$^+$–0$^+$**III**0$^+$–0$^+$**IV**0$^+$–0$^+$**V**); distinct, movable flap of skin on postaxial side of Toe V from middle of terminal phalanx to proximal end of metatarsus; distinct, movable flap of skin on preaxial side of Toe I from middle of terminal phalanx to level of inner metatarsal tubercle, continuing as weak fold on distal one third of tarsus; subarticular tubercles prominent, elevated, round; digits indicated by roman numeral (tubercle count in parentheses): V (2), IV (3) III (2), II (1), I (1); inner metatarsal tubercle oval, elongate with elevated post axial border.

Skin on top of head, throat, dorsal surfaces of forelimbs, and dorsum crenulate; skin on sides, around vent, and dorsal surfaces of thigh, shank and foot distinctly tuberculate, covered with moderately dense small, low glandular warts with translucent spinules; ventral skin smooth; pair of thin dorsolateral folds extend from post-orbital fold to mid-body, and although interrupted, on to the posterior third of body.

Color in preservative light brown dorsally and laterally; indistinct dark brown bars on upper lip, distinct and regularly spaced on lower lip; supratympanic fold dark brown; faint, dark brown crossbars on dorsal and lateral aspects of limbs, irregular dark brown spots on posterior thigh; throat mottled with light pigmentation, less pigmentation on mental patch; venter immaculate, ventral portions of limbs lightly dusted with melanophores; palmar and plantar surfaces dark brown, webbing between toes mottled.

Measurements. Morphometric data (in mm) for the holotype (male, FMNH 266636) are: SVL = 49.43; ED = 5.46; EN = 4.22; ES = 7.82; FEL = 25.48; FOL = 36.07; HL = 23.71; HW = 21.7; IN = 4.17; IO = 3.55; LAL = 10.27; MN = 21.54; PAL = 12.75; TBL = 25.54; UEW = 4.05; OL = 2.84; MD = 1.5.

Variation. Variation in body proportions given in Table 1. All adult males have nuptial pads and spinule-covered mental patches. Relative head length (HL/SVL) is significantly larger (p = 0.02) in males (0.44–0.48) than in females (0.39–0.44). Based on the available data, it seems that females may attain a greater SVL, a trend contrary to the generally accepted paradigm of male-biased size dimorphism for the *kuhlii* Complex. A female-biased size dimorphism has been observed in other members of the *kuhlii* Complex (McLeod, pers. obs.), but it is possible that the observed variation in *L. sisikdagu* is the result of insufficient sampling.

TABLE 1. Morphological measurements and ratios of specimens examined in this study. Ranges of values are presented for each character followed by the mean and standard deviation in parentheses.

| Character	*Limnonectes kuhlii* Male $n = 4$	Female $n = 13$	*Limnonectes sisikdagu* Male $n = 5$	Female $n = 4$
ED	4.71–9.17 (6.55 ± 1.92)	5.41–8.15 (6.87 ± 0.86)	4.32–5.46 (4.78 ± 0.45)	4.76–5.40 (5.02 ± 0.27)
EN	3.25–7.06 (5.26 ± 1.58)	3.35–5.71 (4.71 ± 0.71)	3.28–4.32 (3.96 ± 0.42)	3.54–4.80 (4.22 ± 0.55)
ES	5.14–12.14 (8.69 ± 2.87)	5.84–10.54 (8.29 ± 1.4)	5.92–7.82 (6.97 ± 0.73)	6.88–7.81 (7.24 ± 0.42)
FEL	19.10–41.91 (31.64 ± 9.92)	22.22–36.59 (30.41 ± 3.77)	21.75–25.48 (22.28 ± 2.00)	21.79–26.09 (23.89 ± 1.88)
FOL	27.19–48.85 (40.08 ± 9.86)	30.84–43.38 (38.07 ± 3.96)	28.91–36.07 (31.53 ± 2.89)	30.76–38.66 (33.82 ± 3.79)
HL	15.28–40.25 (28.91 ± 10.42)	17.55–31.28 (24.16 ± 3.71)	18.53–23.71 (20.53 ± 2.22)	17.99–22.33 (20.11 ± 1.83)
HW	15.24–40.8 (28.92 ± 10.62)	16.43–29.55 (23.34 ± 3.47)	16.25–21.70 (18.79 ± 2.28)	16.06–20.67 (18.29 ± 1.95)
IN	3.43–6.68 (5.13 ± 1.33)	3.81–5.80 (4.82 ± 0.56)	3.24–4.17 (3.71 ± 0.34)	3.30–4.05 (3.67 ± 0.31)
IO	2.76–7.97 (5.73 ± 2.2)	3.13–5.86 (4.45 ± 0.77)	2.31–3.55 (2.93 ± 0.57)	2.48–3.03 (2.77 ± 0.25)
LAL	7.31–14.91 (11.74 ± 3.36)	7.97–13.65 (10.52 ± 1.53)	7.82–10.27 (8.62 ± 0.97)	8.51–9.77 (9.15 ± 0.64)
MN	14.15–36.97 (26.08 ± 9.72)	16.00–25.83 (20.76 ± 2.52)	16.56–21.54 (18.32 ± 2.14)	15.41–19.83 (17.42 ± 1.86)
PAL	9.79–19.36 (15.59 ± 4.54)	11.45–17.98 (14.73 ± 1.65)	9.87–12.75 (11.22 ± 1.18)	11.14–12.46 (11.78 ± 0.59)
SVL	36.87–81.45 (62.39 ± 19.31)	41.11–74.02 (59.41 ± 8.91)	41.86–49.43 (45.36 ± 3.22)	44.44–56.63 (49.10 ± 5.50)
RFL	IV–V–II–III	IV–V–II–III	IV–V–III–II	IV–V–III–II
RTL	IV–III–V–II–I	IV–III–V–II–I	IV–III–V–II–I	IV–III–V–II–I
TBL	18.40–36.26 (28.3 ± 7.89)	20.49–31.52 (26.95 ± 2.87)	19.80–25.54 (22.18 ± 2.24)	21.03–26.34 (23.46 ± 2.44)
TYE	0–5.76 (3.17 ± 2.92)	3.63–5.50 (4.52 ± 0.58)	NA	NA
UEW	2.87–6.18 (4.52 ± 1.35)	2.97–6.23 (4.97 ± 0.85)	3.02–4.05 (3.55 ± 0.40)	3.05–4.03 (3.56 ± 0.45)
OL	1.49–7.65 (4.14 ± 2.6)	1.60–3.68 (2.62 ± 0.6)	1.52–3.16 (2.38 ± 0.64)	1.69–2.35 (1.91 ± 0.30)
MD	0.86–3.19 (1.87 ± 0.97)	1.05–1.86 (1.42 ± 0.25)	0.99–1.50 (1.21 ± 0.19)	0.99–1.19 (1.07 ± 0.09)
PAL/LAL	1.28–1.42	1.22–1.67	1.23–1.45	1.24–1.34
PAL/SVL	0.23–0.27	0.23–0.28	0.24–0.26	0.21–0.25
OL–MD/HL	0.04–0.11	0.02–0.08	0.03–0.09	0.03–0.05
ED/HL	0.2–0.31	0.24–0.33	0.22–0.24	0.23–0.27
HL/HW	0.99–1.01	0.96–1.08	1.07–1.14	1.08–1.12
HL/SVL	0.41–0.49	0.38–0.43	0.44–0.48	0.39–0.43
SVL–HL/ SVL	0.51–0.59	0.57–0.62	0.52–0.56	0.57–0.61
IN/IO	0.80–1.24	0.91–1.31	1.09–1.60	1.27–1.35
IN/SVL	0.07–0.09	0.06–0.09	0.08–0.08	0.07–0.08
EN/IN	0.95–1.10	0.84–1.21	0.89–1.33	0.99–1.38
UEW/IO	0.68–1.04	0.92–1.37	1.08–1.52	1.14–1.54
EN/ES	0.58–0.63	0.47–0.62	0.54–0.62	0.51–0.66
TBL/SVL	0.44–0.50	0.42–0.5	0.47–0.52	0.47–0.49
FEL/SVL	0.49–0.52	0.49–0.54	0.47–0.52	0.46–0.50
LAL/SVL	0.18–0.20	0.15–0.21	0.17–0.21	0.17–0.20
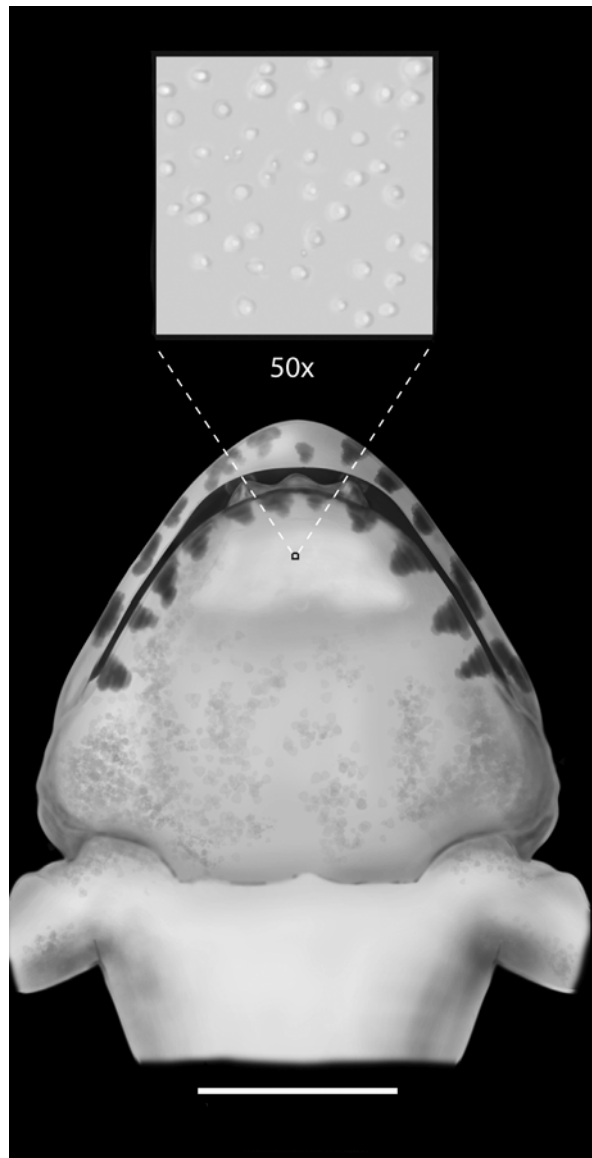
FIGURE 3. Illustration of spinule-covered mental patch on ventral surface of anterior chin in holotype (FMNH 266636). Scale bar = 10 mm.
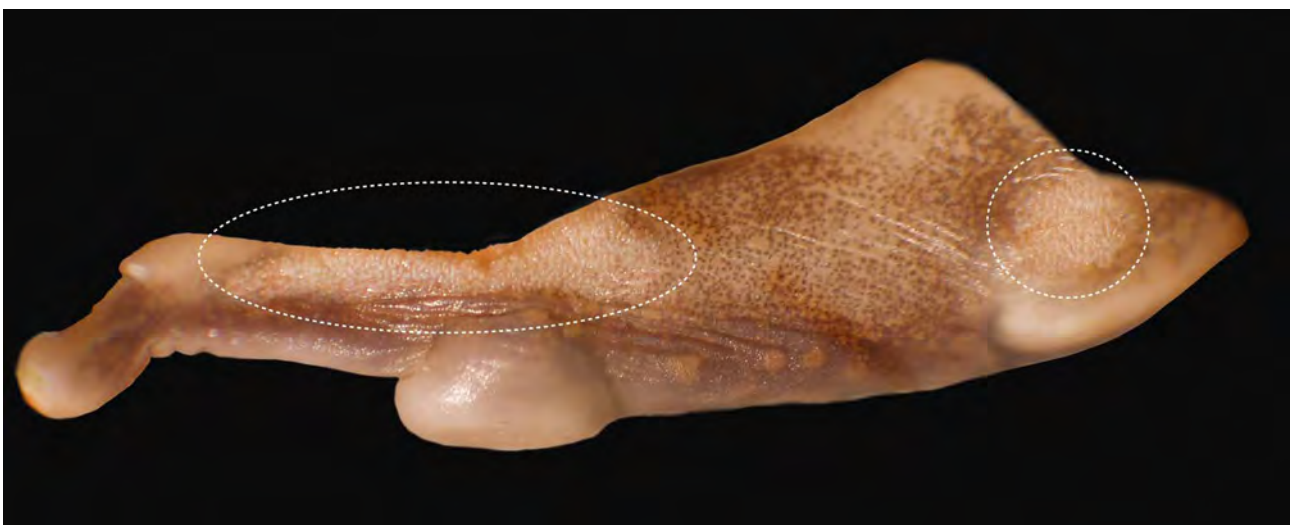

FIGURE 4. Nuptial pads on first finger (Digit II) of holotype (FMNH 266636).

FIGURE 5. Tuberculation of leg in *Limnonectes kuhlii* from Java (A, B) and *Limnonectes sisikdagu* from West Sumatra (C, D). Scale bar = 5 mm.

Etymology. The specific name, *sisikdagu*, is derived from the Bhasa Indonesian words for "chin" (dagu) and "shield" (sisik), referring to the distinctive spinule-covered mental patch in males, a character that makes this frog unique among all the other known members of the *kuhlii* Complex. The specific name is used as a noun in apposition.

Comparisons. There are few obvious characters that differentiate one member of the *kuhlii* Complex from another. Fortunately, *L. sisikdagu* has a unique character—the presence of a spinule-covered mental patch in males— that distinguishes it from all other members of the *kuhlii* complex and from all other known members of the genus.

Limnonectes sisikdagu is a small member of the *kuhlii* Complex, and though the range in snout–vent length overlaps that of *L. kuhlii* from Java, the mean and maximum sizes attained by both males and females of *L. sisikdagu* are considerably smaller than those of *L. kuhlii* (Table 1). Male *L. kuhlii* lack the nuptial pads that are present in *L. sisikdagu*. An externally visible tympanic annulus is present in some samples of *L. kuhlii*, but in none of the examples of *L. sisikdagu*. In dorsal view, the head of *L. sisikdagu* is more elongate and pointed than that of *L. kuhlii* (evidenced by the statisically significant difference in HL/HW ratios discussed below). All representatives of *L. kuhlii* examined here lack both the post-orbital fold and the dorsolateral fold present in *L. sisikdagu*. Though the tubercles do not extend as high onto the dorsum of the leg in *L. kuhlii* specimens, the tubercles are larger and more

densely arranged than in *L. sisikdagu* (Fig. 5). These two species can also be distinguished by color in preservative, *L. kuhlii* being uniformly darker brown than *L. sisikdagu*.

Student's t-tests return significant differences among several mensural morphometric characters between the two species (Table 2). Some characters differ significantly in only one sex, but when male/female data are considered together for the species, a significant difference between species can be observed (e.g., FEL/SVL ratio is different in females [$p = 0.04$] and when males and females are considered together [$p = 0.02$], but not when males are considered alone [$p = 0.38$]). Both sexes of *L. sisikdagu* have proportionately narrower heads (as determined by HL/HW) than either sex of *L. kuhlii*, in which head length and width are approximately equal. Additionally, the distance between eyes (as determined by IN/IO) is significantly narrower in *L. sisikdagu* than in either sex of *L. kuhlii*. Female *L. sisikdagu* have significantly smaller eyes (ED), larger eye–nares distances (EN), and shorter femurs (FEL) than do female *L. kuhlii*. Male *L. sisikdagu* have a greater upper eyelid width (UEW) relative to interorbital distance (IO) than do male *L. kuhlii*.

TABLE 2. Selected results from inter-specific comparisons of morphological characters using 2-tailed Student's t-tests (*p* values).

Student T-tests (*L. kuhlii: L. sisikdagu*)			
Character	Male	Female	Male+Female
ED/HL	0.836	0.030	0.018
HW/HL	0.000	0.005	0.000
IN/IO	0.037	0.002	0.001
EN/IN	0.518	0.016	0.019
UEW/IO	0.010	0.075	0.014
TBL/SVL	0.132	0.132	0.011
FEL/SVL	0.148	0.026	0.003

Distribution and ecology. Based on the specimens examined, *Limnonectes sisikdagu* occurs in central West Sumatra (Fig. 6). As with other members of the *kuhii* Complex, *L. sisikdagu* is found along forested streams. The new species seems to occur at elevations approximately between 520 and 1204 m. It is known to occur along streams in disturbed areas where forest has been cleared for agricultural exploitation.

The type locality, Batang Andaleh, is a tributary of Batang Tarsan, which is the main river from Solok and Lubuk Selasih to the coast. Main crops in this area are rice and tea, and the forest has been cleared for agriculture, mainly rice. Specimens from Batang Harau (near Harau Valley, Payakumbuh) were found at a river with protected forest border that varies from 50–200 m. The lower story of forest is disturbed and includes bamboo, cinnamon, *Melastoma, Dillenia, Dicranopteris, Hevea,* and *Uncaria*. The river has clear water and a gravel bed with few boulders. Collections from Sarasah Bunta (near Harau Valley, Payakumbuh) were made along streams that flow from waterfalls. Batang Sarahah is a tributary of Batang Tarusan. The tree/shrub genera in this location include *Gracinia, Elatostema, Rhinostaea, Eugenia, Zingiber, Calamus,* and *Areca*.

Discussion

Emerson *et al.* (2000) and Evans *et al.* (2003) were among the first to suggest, using molecular methods, that *L. kuhlii* sensu lato was, in fact, a species complex. McLeod (2010) corroborated these findings and suggested that the diversity within the *kuhlii* Complex was much greater than previously suspected. The recognition of *L. sisikdagu* furthers our understanding of the complexity of this group and invites the question: How much more *kuhlii* Complex diversity awaits discovery?

FIGURE 6. Distribution of samples of *Limnonectes siskdagu* used in this study. Holotype indicated with star. Open square represents specimens collected from Batu Layang and identified as "Lineage 2" in McLeod (2010).

Evidence from uncorrected pair-wise distance data suggests that there may be multiple species of the *kuhlii* Complex in Sumatra (Table 3). Specimens from Batu Layang (Bengkulu district, south west Sumatra), identified as "Lineage 2" in McLeod (2010), are 5.8–6.2% divergent from those of *L. sisikdagu* (West Sumatra). Collections made at Batu Layang are geographically separated from the known localities for *L. sisikdagu* by > 300 km and may represent a unique species (Figure 6). Unfortunately, voucher specimens from Batu Layang were unavailable for examination. To be taxonomically conservative we assign "Lineage 2" of McLeod (2010) to *L. sisikdagu* until such a time as whole specimens can be examined to determine if the genetic distance can be corroborated by identifiable morphological differences allowing for the recognition of additional species from Sumatra. In a pattern similar to that of other lineages of the *kuhlii* Complex, the intraspecific sequence divergence for West Sumatran specimens is 0.0–3.8% and the interspecific sequence divergence is 10.9–16.9% (McLeod, 2010).

Given the rapid loss of forests in tropical Southeast Asia (e.g., Sodhi *et al.* 2009), and the threat that this poses to forest biota, it is critical that we attempt to identify the true biodiversity of this region before it is lost. Recognizing diversity is only the first step towards developing informed conservation and management strategies. To evaluate the status and conservation needs of endemic species such as *L. sisikdagu*, and Javan *L. kuhlii,* it is essential that we understand the natural history and ecology of these species.

Table 3. Uncorrected mean pair-wise divergence values (proportion of sites differing between haplotypes) within and between species.

SPECIES	ID NUMBER	#	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17
L. sisikdagu **sp. nov.** (W. Sumatra)	HM067244	1	—															
	HM067245	2	0.00595	—														
	FMNH 266610	3	0.06060	0.06183	—													
	FMNH 266615	4	0.05968	0.05955	0.00139	—												
	FMNH 266618	5	0.05888	0.05876	0.00139	0.00000	—											
	FMNH 266621	6	0.05881	0.05869	0.00140	0.00000	0.00000	—										
	FMNH 266619	7	0.05930	0.05918	0.00140	0.00000	0.00000	0.00000	—									
	FMNH 266616	8	0.05830	0.05819	0.00275	0.00373	0.00373	0.00373	0.00374	—								
	FMNH 266637	9	0.05861	0.05849	0.00280	0.00377	0.00377	0.00377	0.00377	0.00323	—							
L. kuhlii (Java)	AB526316	10	0.10864	0.10895	0.11451	0.11293	0.11416	0.11453	0.11345	0.11402	0.11462	—						
	AY313687	11	0.12907	0.12753	0.13733	0.12551	0.12697	0.12750	0.12661	0.12333	0.12356	0.09932	—					
	AF183137.183138	12	0.09658	0.09837	0.10097	0.10172	0.10435	0.10436	0.10345	0.10175	0.09879	0.06729	0.04588	—				
L. gyldenstolpei	AF183123.183124	13	0.13994	0.13796	0.15699	0.13993	0.13983	0.13984	0.14017	0.13710	0.13823	0.13948	0.16873	0.14560	—			
L. laticeps	AF183125.183126	15	0.13135	0.13031	0.14522	0.13357	0.13257	0.13416	0.13295	0.13450	0.13571	0.14064	0.16544	0.14323	0.13863	—		
L. microdiscus	AY313688	16	0.16245	0.16373	0.16886	0.15843	0.16063	0.16104	0.15961	0.15714	0.15988	0.16944	0.19082	0.15779	0.15214	0.14942	—	
L. kardasani	AY313693	17	0.13841	0.14031	0.14764	0.14760	0.14692	0.14727	0.14797	0.14690	0.14948	0.14206	0.16565	0.13213	0.13112	0.13096	0.14216	—

Acknowledgments

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APPENDIX 1. Specimens examined, all from Indonesia. Comparative material was examined from the holdings of the Field Museum of Natural History (FMNH), the Louisiana State University Museum of Natural Science (LSUMNS), the Museum National d'Histoire Naturalle (MNHN), Rijksmuseum van Natuurlijke Historie (RMNH), and the Texas Natural History Collection of the Texas Memorial Museum (TNHC).

Limnonectes kuhlii: JAVA IS.: MNHN 4469 (lectotype), RZB 4297 (2 specimens: paralectotypes); Jawa Barat Province: Kecamatan Kadudampit: TNHC 59826, 59829, LSU 81895; BSI-FS 0026–0032, 0067– 69, 0081–92 (uncataloged specimens, property of MZB).

Limnonectes sisikdagu: SUMATRA IS.: West Sumatra: FMNH 266608, FMNH 266610, FMNH 266615, FMNH 266616, FMNH 266618, FMNH 266619, FMNH 266621, FMNH 266630, FMNH 266631, FMNH 266636 (holotype), FMNH 266637.