**Reappraisal of the Javanese Bullfrog complex, *Kaloula baleata* (Müller, 1836) (Anura: Microhylidae) reveals a new species from northwestern Peninsular Malaysia**

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**Abstract**

A new species of *Kaloula* from the *K. baleata* complex is described based on genetic and morphological divergence. The new species shares morphological similarities and phylogenetic affinity to *K. indochinensis* from eastern Indochina and *K. baleata* from Java, Indonesia and can be distinguished from other congeners by the following combination of characters: (1) male SVL 49.2–56.2 mm; (2) finger tips expanded into large, transversely expanded discs (2.8–3.1 mm); (3) inner metatarsal tubercle large, oval, distinctly raised, slightly shorter than first toe (3.3–3.5 mm); (4) three subarticular tubercles on fourth toe; (5) yellow to orange, irregularly shaped patch on the axillary, inguinal and posterior region of thigh.

Key words

**Introduction**

Recent re-evaluations of wide-ranging species complexes in Sundaland have revealed a staggering amount of hidden diversity. Many of these previously widespread species have been shown to comprise distinct lineages with more restricted distribution ranges (Chan *et al.* 2013; Wood *et al*. 2009; Chan & Grismer 2010; Mcleod 2010; Matsui *et al*. 2010; Chan *et al.* 2011; Grismer *et al.* 2012a,b; Johnson *et al*. 2012; Sumontha *et al.* 2012). The *Kaloula baleata* complex is one such group that includes multiple species masquerading under a single name. This species has been reported to occur in Vietnam (Orlov *et al.* 2002; Nguyen 2009), Laos (Teynié *et al.* 2004), Thailand (Pauwels *et al.* 2000), Peninsular Malaysia (Berry 1975; Chan *et al.* 2010), Borneo (Inger & Stuebing 2005; Das & Kraus 2007), Indonesia (Iskandar 1998), and Palawan Island in the Philippines (Taylor 1920; Brown 2007; Diesmos and Brown 2011), with a subspecies *K. baleata goshi* reported from Little and South Andaman Islands, India (Das & Dutta 1998). We assess the taxonomic validity of *K. baleata* in Peninsular Malaysia using multivariate statistics to demonstrate that a population from northwestern Peninsular Malaysia are not only genetically (Blackburn et al. 2013) but also morphologically distinct from the true *K. baleata* from Java and therefore deserve specific taxonomic recognition.

**Materials And Methods**

*Sampling and morphology*.—Specimens examined include the true *Kaloula baleata* from Java, Indonesia (N=7), *K. indochinensis* from Vietnam and Laos (N=31), and a sampling population from Gubir, Kedah in northwestern Peninsular Malaysia that was hitherto considered to be *K. baleata* (N=4). Only male specimens were included in morphological analyses to eliminate the effect of sex from the results. The following morphological characters were assessed to the nearest 0.1 mm: snout–vent length (SVL), from tip of snout to vent; head length (HL), from posterior margin of mandible to tip of snout; head width (HW), measured at the level of the jaw articulation; snout length (SNL), from anterior corner of eye to tip of snout; eye diameter (ED), length between anterior and posterior corners of eye; interorbital diameter (IOD), distance between medial margins of palpebrae at its closest point; internarial distance (IND), measured from medial, inner margins of nostrils; third finger disc width (Fin3DW), widest horizontal diameter of third finger disc; femur length (FL), measured from the midventral line to the knee articulation in line with femur; tibia length (TBL), from knee inflection to tarsal inflection; inner metatarsal tubercle length (MTTL), from base to tip of inner metatarsal tubercle. Toe webbing formula follows Savage and Heyer (1997). The type series was deposited at the La Sierra University Herpetological Collection (LSUHC), La Sierra University, Riverside, California, USA.

*Multivariate analyses.—*Multivariate analyses were performed and visualized in the statistical software environment R (version 2.15.1). Operational taxonomic units (OTU’s) were assigned according to geography and phylogeny (Blackburn *et al*. 2013) into the following groups: 1) *baleata* (Java); 2) *indochinensis* (eastern Indochina); and 3) PM (northwestern Peninsular Malaysia). Data were first tested for normality using the Shapiro-Wilk Normality Test in the package *mvnormtest*. Variables were analyzed with ANCOVA using SVL as a covariate for body size correction (McCoy et al. 2006; García-Berthou 2001) to determine whether individual characters differed between OTU’s. To test the significance of body size itself, a Mann-Whitney U test was performed on SVL. Characters that were insignificant across all OTU’s were considered as noise and excluded from subsequent analyses. A Principal Component Analysis (PCA) was then performed on the covariance matrix to find the best low-dimensional representation of morphological variation in the data and to further determine whether the variation could form the basis of distinct, detectable group structure (Leps & Smilauer 2003). To eliminate the effect of body size, each measurement was transformed to size-independent values prior to inclusion in the PCA using the following allometric equation: Madj = logM – β(logSVL – logSVLmean) where Madj is the adjusted value of the character; M is the original value; SVL is the snout-vent-length of the individual under consideration; SVLmean is the overall mean snout-vent-length of all individuals in the dataset (all OTU’s combined); β is the unstandardized coefficient of the within-OTU linear regression of logM against logSVL (Thorpe 1983; Turan 1999; van Rooijen & Vogel 2008). Variables were transformed using base-10 logarithms. Results of the PCA were visualized using the packages *ggplot2* and *rgl*. A linear discriminant analysis (LDA) was then performed on the PCA scores to further optimize the separation between OTU’s by maximizing between-OTU variance while minimizing within-OTU variance. This was done using the dapcfunction in the package *adegenet*.

**Results**

The Shapiro-Wilk Normality Test showed that the data deviated significantly from normality (W = 0.8195; *p* < 0.000). Compared to *baleata*, the Mann-Whitney U test and ANCOVA showed that PM was significantly different for all characters except SVL, ED and IOD and all characters except SVL, IOD and Fin3DW when compared to *indochinensis*. Between PM and *baleata,* HW, IND, TBL, and Fin3DW showed the highest statistical significance (*p* < 0.01) whereas compared to *indochinensis*, HL, HW, SNL, TBL and MTTL were the most significant (*p* < 0.001). The character IOD showed no significance across all OTU’s and was therefore excluded from the PCA and LDA (Table 1). Scatterplots of these characters show the separation between OTU’s in morphospace (Fig. 1).

We discuss the first three principal components that accounted for 83.7% of the total variance. Remaining components contributed less than 6% each to the overall variance. Loadings for the first principal component (PC 1) were negative for all characters except IND, TBL, and Fin3DW and loaded heaviest on Fin3DW, showing that Fin3DW accounted for most of the variability along this axis. Loadings for PC 2 were all positive and significantly heavy on Fin3DW, ED, and MTTL whereas PC 3 had the heaviest loading on MTTL. These results show that Fin3DW and MTTL are responsible for most of the variance along the first three principal components axes (Table 2). Ordinations of the first three principal components scores show a clear separation between PM and the other OTU’s in two-dimensional (Fig. 2A,B) and three-dimensional morphospace (Fig. 2C). Results of the LDA show similar separations (Fig 2D) and recovered high membership probabilities for the assignment of every individual to its predefined cluster (Fig 3).

**Systematics**

The molecular phylogeny from Blackburn *et al.* (2013) and our morphological analyses indicate that the *Kaloula* from northwestern Peninsular Malaysia is not conspecific with *K. baleata* and represents a genetically and morphologically divergent lineage that can be diagnosed from all other known congeners. Under the framework of a Unified Concept of Species (de Queiroz 2005), we consider this separately evolving lineage as a new species that is thus described herein.

**Species description**

*Kaloula kedahensis* **sp nov.**

Kedah Narrow-mouthed Frog

Fig. 4

**Holotype.—** Adult male (ZRC XXX/LSUHC 5077), SVL 56.2 mm, collected by XXX on XXX at Gubir, Kedah, Peninsular Malaysia (GPS; elevation).

**Paratypes.—**Adult males LSUHC 5074–76 have the same collection data as the holotype.

**Diagnosis.—**A large sized *Kaloula*, male SVL 49.2–56.2 mm (x̅ = 53.5 ± 3.0); finger tips expanded into large, transversely expanded discs, Fin3DW 2.8–3.1 mm (x̅ = 3.0 ± 0.1); inner metatarsal tubercle large, oval, distinctly raised, slightly shorter than first toe, MTTL 3.3–3.5 mm (x̅=3.4 ± 0.1); three subarticular tubercles on fourth toe; yellow to orange, irregularly shaped patch on the axillary, inguinal and posterior region of thigh.

**Comparisons**.— All comparisons are based on male specimens only. *Kaloula kedahensis* **sp. nov.** can be distinguished from *K.* *baleata* by having larger HL (13.7–15.5 mm vs. 11.9–13.4 mm), HW (17.7–19.9 mm vs. 15.0–16.8 mm), SNL (5.9–6.3 mm vs. 4.4–5.4 mm), IND (3.8–4.1 mm vs. 2.7–3.5 mm), FL (20.6–23.2 mm vs. 15.9–19.6 mm), TBL (18.6–19.6 mm vs. 14.6–17.3 mm), Fin3DW (2.8–3.1 mm vs. 1.4–2.2 mm), and MTTL (3.3–3.5 mm vs. 2.6–3.3 mm). From *K. indochinensis*, it differs by having larger SVL (49.2–56.2 mm vs. 44.1–53.7 mm), HL (13.7–15.5 mm vs. 10.1–12.9 mm), HW (17.7–19.9 mm vs. 13.0–17.4 mm), SNL (5.9–6.3 mm vs. 4.3–5.6 mm), IND (3.8–4.1 mm vs. 2.4–3.6 mm), FL (20.6–23.2 mm vs. 13.8–19.3 mm), (18.6–19.6 mm vs. 14.8–18.3 mm), MTTL (3.3–3.5 mm vs. 2.1–2.9 mm), and three subarticular tubercles on fourth toe as opposed to two.

*Kaloula kedahensis* differs from other congeners by the following opposing characters; distribution ranges are given in parenthesis: *K. assamensis* (northeastern India), SVL up to 38.0 mm, dorsum pale brown with bright yellow vertebral stripe and broad dark brown lateral stripes, axillary and inguinal spots absent, stratified coloration on flanks and thighs; *K. aureata* (Nakhon Si Thammarat Province, southern Thailand), SVL up to 65.0 mm, dorsum golden with dark brown reticulations and dorsolateral stripe; *K. borealis* (eastern China and Korea), finger tips slightly dilated but not forming wide discs, inner and outer metatarsal tubercle distinctly large and raised, inner metatarsal tubercle longer than first toe, dorsum gray-brown with dark blotches forming a distinct network on the flanks; *K. conjuncta* (widespread throughout the Philippines), SVL up to 47.0 mm, web reaching disc of fourth toe, stratified lateral flank coloration, weak outer metatarsal tubercle; *K. kalingensis* (Luzon, Polillo and Palaui Islands, Philippines), SVL up to 39.3 mm, dorsal tubercles absent, weak outer metatarsal tubercle, axillary and inguinal patch usually absent, small and red when present, light pericloacal ring present; *K*. *kokacii* (Catanduanes Island, Bicol Peninsula of Luzon Island, Philippines), SVL up to 44.3 mm; dorsal tubercles, axillary and inguinal spots absent, light pericloacal ring present; *K. mediolineata* (mainland Thailand and Laos), SVL up to 63.0 mm, finger tips slightly dilated but not forming wide discs, web reaching disc of first toe and disc of postaxial side of second and third toe, inner and outer metatarsal tubercle extremely large and raised, inner metatarsal tubercle longer than first toe, distinct dorsolateral and sacral stripes; *K. nongganensis* (Guangxi province, China), osseous tubercles on dorsal surface of finger tips in males, nearly fully webbed toes in males, dorsum olive green with dark moss-green marbling, chest beige with lemon-colored spots; *K. picta* (widespread throughout the Philippines), SVL up to 56.1 mm, finger tips slightly dilated but not expanded into wide discs, dorsolateral stripes distinct, stratified lateral flank coloration; *K. pulchra* (northeastern India, southern China, and Indo-Malaya), SVL up to 75.0 mm, inner metatarsal tubercle large, raised, and longer than first toe, dorsolateral stripes present, axillary and inguinal spots absent; *K. rigida* (Luzon Island, Philippines), SVL up to 56.1 mm, finger tips slightly dilated but not expanded into wide discs, stratified lateral flank coloration, dorsolateral stripes distinct, axillary and inguinal spots absent; *K. rugifera* (central and south-central China), osseous tubercles on dorsal surface of finger tips in males, dorsum olive brown with yellowish-olive chin and throat, axillary and inguinal spots absent; *K. verrucosa* (southeastern China) SVL up to 61.0 mm, finger tips slightly dilated but not expanded into wide discs, osseous tubercles on dorsal surface of finger tips in males, dorsum without distinct markings, axillary and inguinal spots absent; *K. walteri* (Bicol Peninsula of Luzon and Polillo Island, Philippines), SVL up to 31.5 mm, finger tips slightly dilated but not expanded into wide discs; outer metatarsal tubercle absent or indistinct, stratified lateral flank coloration, axillary and inguinal spots absent.

**Description of Holotype.—**Fig. 4. Adult male, SVL 56.2 mm; habitus robust; head wider than long (HW/HL=1.26; HW/SVL=0.35); snout slightly projecting beyond lower jaw, truncated in dorsal view, rounded and caudoventrally sloping in lateral view; eyes large, not protruding beyond labials in dorsal view, diameter less than snout length (ED/SNL=0.85) and interorbital distance (ED/IOD=0.86); canthus rostralis rounded, not visible; lores oblique, slightly concave; nares open laterally, slightly below canthus, nearly terminal on snout (IND/SNL=0.66); labial region not swollen; inter-orbital region flat; dorsal rostrum slightly convex; tympanum covered with skin, not visible; supratympanic fold present, extending from posterior edge if eyelid to just above and anterior of forelimb insertion and continuous with a distinct supraaxillary fold; choanae transversely oriented, tapered at both ends, separated by distance almost equal to their horizontal length; vomerine teeth absent; tongue wide, spatulate with a small posterior notch, posterior two thirds free; vocal slits posterior to rictus.

Forelimbs relatively long and robust; order of digits from shortest to longest: I≤II<IV<III; no webbing between digits; distal ends of fingers expanded into wide, transversely expanded discs lacking circummarginal grooves; subarticular tubercles distinct, large, round, numbering one on digits I and II, two on digits III and IV, proximal subarticular tubercles slightly larger than distal ones; inner metacarpal tubercle oval, larger than subarticular tubercles; outer metacarpal tubercle oval, subequal in size to inner, divided to form smaller tubercle medially.

Hindlimbs robust, relatively short (/SVL=0.34); order of digits from shortest to longest: I<II<V<III<IV; tip of toes slightly expanded into small, round discs; subarticular tubercles distinct, numbering one on digits I and II, two beneath digits III and V, and three on digit V, oval on digits I and II, elongate and connected on digits III–V; toe webbing formula: I 1–2 II 1–3 III 2–3.5 IV 4–2 V; inner metatarsal tubercle large, raised, oval, slightly shorter than first toe; outer metatarsal tubercle round, slightly raised, half the size of inner.

Skin on dorsal surfaces smooth, infused with low, well-spaced, broad, flat, tubercles that are most prominent on the temporal region; ventral surfaces slightly granular; throat distinctly granular; loose skin overlying median subgular vocal sac forming a sternal fold; nuptial pad absent.

**Color in preservative.—**Dorsal surfaces brown with a pale, narrow interorbital bar. Light brown, symmetrical, broad, wavy stripes from posterior end of upper eyelid, extending dorsolaterally to the scapular region and fading posteriorly. Back with small, irregular dark patches and even smaller white spots. Faint, thin, light colored band around the wrist. White axillary and inguinal patch and creamy, irregular patches on posterior region of thigh. Venter creamy, heavily stippled with brown. Gular blackish brown covered with small whitish spots.

**Variation.—**The paratypes LSUHC 5075–76 have a broader, more prominent interorbital bar between the anterior portion of the upper eyelids. LSUHC 5074 has an indistinct interorbital bar and faded post palpebrae stripes. Dorsal surfaces of limbs in LSUHC 5074 lighter in color with irregular, dark bordered light bands across the wrist, ankle, and hindlimbs. Measurements of the type series are presented in Table 3.

**Distribution.—***Kaloula kedahensis* is currently known from Gubir, Kedah but is likely to occur in other parts of Peninsular Malaysia and southern Thailand (Fig. 5).

**Etymology.—**The specific epithet “*kedahensis*” is in reference to the type locality of the new species in the northwestern state of Kedah.

**Natural History.—**

**Discussion**

The phylogeny presented in Blackburn *et al.* (2013) showed that the *baleata* clade consisted of at least five divergent lineages. Peninsular Malaysian populations were separated into two distinct lineages, the first was “LSUHC 5074 Peninsula” (described here as *K. kedahensis*), while the other comprised populations from southern Peninsular Malaysia and Borneo. The latter lineage was recovered as sister to the clade that includes *K. baleata* (Java and Bali) and undescribed populations from Palawan and Sulawesi, indicating that southern Peninsular Malaysian and Bornean populations of *Kaloula* may not be conspecific with either *K. baleata* or *K. kedahensis*. The taxonomic resolution of these populations is pending acquisition of additional specimens from other parts of Peninsular Malaysia and Borneo.

**Acknowledgements**

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**Tables and Figures**

Table 1. Summary statistics for compared OTU's. Ranges follow mean ± standard deviation. Significance codes: \*\*\* = 0; \*\* = 0.001; \* = 0.05

Table 2. Summary statistics and loadings for the first three principal components (PC 1 to PC 3) and the first two discriminant functions (LD1 and LD2). Values in bold print indicate the heaviest loadings for the respective principal components.

Table 3. Measurements for the type series. See Materials & Methods for abbreviations.

Fig. 1. Scatterplots of significant morphometric characters with accompanying regression lines; blue=PM; pink=*baleata*; green=*indochinensis*

Fig. 2A,B**–** Scatterplots of PC 1/PC 2 and PC 2/PC 3 with corresponding 95% confidence ellipses; C**–**Ordination of the first three principal components in three-dimensional space; D**–**Scatterplot of the first two discriminant functions resulting from the LDA on the first three principal components scores. Inset represents PCA eigenvalues and clusters are summarized by inertia ellipses.

Fig. 3. Membership probabilities and proportion of successful reassignments of individuals to their original clusters based on retained discriminant functions. Heat colors represent probabilities (red = 1.0, white = 0.0); blue crosses represent the prior cluster provided to the LDA; blue crosses on red rectangles indicate that LDA classification is consistent with the predefined clusters.

Fig. 4 Holotype

Fig. 5. Distribution of the *Kaloula baleata* complex in Peninsular Malaysia. Star denotes the type locality of *K. kedahensis* **sp.** nov; circles denote localities at which *K. baleata* have been reported.