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A New Species of *Pelophryne* from Malay Peninsula (Anura, Bufonidae)

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Abstract: A small, semi-arboreal toad of the genus *Pelophryne* from Peninsular Malaysia has been treated as *P. brevipes* or *P. signata*. The peninsular toad and Bornean *P. signata* are very similar to each other morphologically, although slightly different in relative forelimb length, dorsal coloration, and tuberculation. However, in partial mtDNA sequence, the peninsular toad is substantially distinct from *P. signata* from Borneo and *P. brevipes* from the Philippines, although it is close to a congener from Sumatra. Thus, the peninsular toad is described as a new species based on specimens from Genting Highlands, state of Pahang, central Peninsular Malaysia. Of the two morphotypes recognized in the genus, the new species belongs to the one with the tips of the fingers expanded into truncate discs, in which the new species is the smallest in body size. The new species also occurs in Singapore and possibly in Sumatra.

Key words: Body size; Cryptic species; Malaysia; MtDNA phylogeny; *Pelophryne ingeri* sp. nov.

INTRODUCTION

The small-sized, semi-arboreal bufonid genus *Pelophryne* Barbour, 1938 now contains 12 species (Matsui et al., 2017). Of these, *P. signata* (Boulenger, 1895) was initially described as a member of *Nectophryne* Buchholz and Peters, 1875 based on specimens from Mount Rabong, Kalimantan, Indonesian Borneo (Boulenger, 1895). The species is now thought to be widely distributed in Borneo Island, including East Malaysia (Inger et al., 2017). However, Bornean *P.*

signata was in the past treated as *P. brevipes* (Peters, 1867), because Inger (1966) relegated the species, together with *Nectophryne exigua* Boettger, 1901 from Sarawak, to a junior subjective synonym of *P. brevipes*, which was originally described from Zamboanga, Mindanao Island, in the Philippines. Inger subsequently changed his idea and first resurrected *P. exigua* from *P. brevipes* without notes (Inger and Tan, 1996). He (Inger and Stuebing, 2009) finally resurrected *P. signata* from *P. brevipes* based mainly on different coloration. Instead, Inger and Stuebing (2009) synonymized *P. exigua* with *P. signata*. Thus, *P. signata* is now considered to occur in Borneo (Inger et al., 2017). Along with these taxonomic changes, the toad from the Malay Peninsula (West

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Malaysia) once treated as *P. signata* (e.g., Hendrickson, 1966) was called *P. brevipes* (e.g., Grandison, 1972; Berry, 1975). Similar taxonomic treatments were done for the toad from Singapore. It has been called *P. brevipes* (Lim, 1990; Lim and Lim, 1992; Teo and Rajathurai, 1997), but is now changed to *P. signata* (Leong and Chou, 1999; Leong and Teo, 2009). Nevertheless, taxonomic relationships of Bornean and non-Bornean toads are still controversial, and in the taxonomy section of IUCN (Diesmos et al., 2004), it is mentioned that specimens from the Malay Peninsula and the Natuna Islands are still assigned to *P. brevipes*, being split from Bornean *P. signata* without reasons.

In my amphibian survey in Peninsular Malaysia nearly 30 years ago, I encountered specimens of a *Pelophryne* that were identified as *P. brevipes* by Inger's (1966) key. However, later DNA analyses revealed large genetic difference of the specimens not only from *P. brevipes* from the Philippines but also from the samples of Bornean *P. signata*. Although morphological differences of the populations from the peninsula and Borneo are only very slight, their genetic differences are as large as those observed among congeneric species. The type locality of *P. signata* is on Borneo as shown above. Hence I describe the *Pelophryne* from the Malay Peninsula as a new species.

MATERIALS AND METHODS

Phylogenetic relationships were estimated from the alignment matrix with ca. 900–2600 nucleotide sites of 12S and 16S rRNA, and tRNA^{val} genes using maximum likelihood (ML) and Bayesian inference (BI). I used GenBank data of *Pelophryne* and outgroup species, with the addition of three new samples (Table 1). Methods for DNA extraction, and amplification and sequencing of the mtDNA fragments, tree construction, and calculation of genetic distances (uncorrected p-distance) are the same as those reported by Matsui et al. (2010). Voucher specimens are

stored at the Graduate School of Human and Environmental Studies, Kyoto University (KUHE), Japan.

I recorded frog calls in the room using a stereo cassette recorder (Sony TC-D5M) with a microphone (Sony ECM-23F). The recordings were subsequently digitized at 44.1 kHz/16 bits. We analyzed recordings with Raven Lite 1.0 for Mac OS X (<http://www.birds.cornell.edu/raven>) on a Macintosh computer. Temporal data were obtained from the oscillogram and frequency information was obtained from the audiospectrograms using Fast Fourier Transformation (1024 point Hanning window). Definitions of acoustic parameters follow Matsui (1997) and Matsui and Dehling (2012).

For specimens preserved in 70% ethanol, and stored at the Graduate School of Human and Environmental Studies, Kyoto University (KUHE), we took body measurements mainly following Matsui (1984): 1) snout-vent length (SVL); 2) head length (HL); 3) nostril-eyelid length (N-EL); 4) snout length (SL); 5) eye length (EL, including eyelid); 6) tympanum-eye length (T-EL); 7) tympanum diameter (TD); 8) head width (HW); 9) internarial distance (IND); 10) interorbital distance (IOD); 11) upper eyelid width (UEW); 12) forelimb length (FLL); 13) lower arm and hand length (LAL); 14) 1st finger length (1FL); 15) outer palmar tubercle length (OPTL); 16) inner palmar tubercle length (IPTL); 17) hand length (HAL); 18) hindlimb length (HLL); 19) thigh length (THIGH), from vent to tip of knee; 20) tibia length (TL); 21) foot length (FL); 22) 1st toe length (1TOEL); 23) outer metatarsal tubercle length (OMTL); 24) inner metatarsal tubercle length (IMTL); 25) third finger disk diameter (3FDW); 26) fourth finger disk diameter (4FDW); 27) fourth toe disk diameter (4TDW); and 28) fifth toe disk diameter (5TDW).

All measurements were made to the nearest 0.1 mm with dial calipers under a binocular dissecting microscope. The system for description of toe-webbing states, the system

TABLE 1. Sample of *Pelophryne* and allied species used for DNA analysis in this study together with the information on voucher, collection locality, and GenBank accession numbers. Voucher abbreviations: KUHE=Graduate School of Human and Environmental Studies, Kyoto University; UTA=University of Texas Arlington; CMNHH=Cincinnati Museum of Natural History; BORN=Institute for Tropical Biology and Conservation, University Malaysia Sabah; UL=unnumbered larva.

Species	Voucher	Locality	GenBank	Reference
1 <i>Pelophryne</i> sp.	KUHE 35585	Malaysia, Peninsula, Genting	AB331720	Matsui et al. (2007)
2 <i>Pelophryne brevipes</i>	UTA 63762	Indonesia, Sumatra	KX192080, KX192088	Smart et al. (2017)
3 <i>Pelophryne signata</i>	KUHE 53200	Malaysia, Sarawak, Ana Rais	AB746456	Matsui et al. (2012)
4 <i>Pelophryne signata</i>	KUHE 53890	Malaysia, Sarawak, Penrissen	LC485457	This study
5 <i>Pelophryne guentheri</i>	KUHE 53168	Malaysia, Sarawak, Kubah	LC208829	Matsui et al. (2017)
6 <i>Pelophryne penrissensis</i>	KUHE 54474	Malaysia, Sarawak, Penrissen	LC208826	Matsui et al. (2017)
7 <i>Pelophryne penrissensis</i>	KUHE 55496	Malaysia, Sarawak, Penrissen	LC208827	Matsui et al. (2017)
8 <i>Pelophryne penrissensis</i>	KUHE 55644	Malaysia, Sarawak, Penrissen	LC208828	Matsui et al. (2017)
9 <i>Pelophryne brevipes</i>	CMNHH 1617	Philippines	AF375503, AF375530	Van Boclaer et al. (2010)
10 <i>Pelophryne api</i>	KUHE 53624	Malaysia, Sarawak, Mulu	LC208831	Matsui et al. (2017)
11 <i>Pelophryne api</i>	KUHE 53645	Malaysia, Sarawak, Mulu	LC485458	This study
12 <i>Pelophryne misera</i>	KUHE 37191	Malaysia, Sabah, Kinabalu	AB331721	Matsui et al. (2007)
13 <i>Pelophryne misera</i>	KUHE 37192	Malaysia, Sabah, Kinabalu	LC208830	Matsui et al. (2017)
14 <i>Pelophryne misera</i>	KUHE 37193	Malaysia, Sabah, Kinabalu	LC485456	This study
15 <i>Ansonia penangensis</i>	KUHE UNL	Malaysia, Peninsula, Penang	AB435262, AB435263	Matsui et al. (2010)
16 <i>Ingerophrynus divergens</i>	KUHE 54484	Malaysia, Sarawak, Mulu	LC208832	Matsui et al. (2017)
17 <i>Sabahphrynus maculatus</i>	BORN 08425	Malaysia, Sabah, Crocker	AB331718	Matsui et al. (2007)
18 <i>Leptophryne borbonica</i>	KUHE 53887	Malaysia, Sarawak, Penrissen	AB746458	Matsui et al. (2012)

proposed by Savage and Heyer (1997) was followed. For comparisons, I examined specimens of *Pelophryne* stored at KUHE, BORNEENSIS collection in the Institute for Tropical Biology and Conservation, University Malaysia Sabah (BORNEENSIS), Sarawak Research Collection, Sarawak Forest Department (SRC), and Lee Kong Chian Natural History Museum (LKCNCM,

formerly ZRC). I also examined the holotype of *Nectophryne exigua* Boettger, 1900 (Senckenberg Museum, Frankfurt am Main [SMF] 3737) and a syntype of *Hylaplesia brevipes* (Naturhistorisches Museum Wien [NHMW 16554]).

RESULTS

Of the maximum 2649 nucleotides generated, 662 were variable, and 368 were parsimony-informative. The best substitution model for ML derived from Kakusan4 (Tanabe, 2011) was the general time reversible model (GTR: Tavaré, 1986) with a gamma shape parameter (estimated gamma values were 0.245), and the likelihood value of $\ln L$ -11045.940, while for Bayesian analysis was also GTR with a gamma shape parameter (0.270), and the likelihood value of $\ln L$ -11064.789.

Phylogenetic analyses employing two different optimality criteria yielded different topologies only in the outgroup genera, and topologies between species of *Pelophryne* were completely identical as shown in Fig. 1 by the ML tree. The specimens of *Pelophryne* formed a clade (BS/BPP=100%/1.00), in which two sister clades were recognized; one (BS/BPP=100%/1.00) including *P. api* Dring, 1983 and *P. misera* (Mocquard, 1890), and another (BS/BPP=99%/1.00) including *P. brevipes* from the Philippines, *P. penrisse-nensis* Matsui, Nishikawa, Eto, and Hossman (2017), *P. guentheri* (Boulenger, 1882), and a clade (BS/BPP=97%/1.00) of *P. signata*, *P. brevipes* from Sumatra, and *Pelophryne* sp. from Peninsular Malaysia. The latter two species formed a clade (BS/BPP=100%/1.00) with the genetic distance (uncorrected p-distance in 471 bp of 16S rRNA) of 2.4% (Table 2). The distance of *Pelophryne* sp. from Peninsular Malaysia and Bornean *P. signata* was 2.4–2.8%, and of *P. brevipes* from Sumatra from *P. signata* was 3.2–3.6%, which were same as or larger than those observed between *P. signata* and *P. guentheri* (3.2%). Genetic distance of 6.3% between *P. brevipes* from the Philippines and Sumatra is thought to be too large to regard them conspecific.

Unfortunately, I could not examine Sumatran specimens to compare morphology with the peninsular ones. On the other hand, the types of *Nectophryne exigua* (SMF 3737)

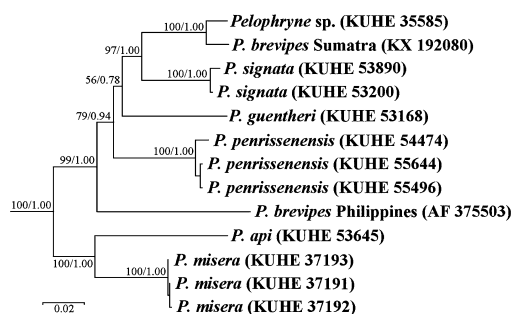


FIG. 1. ML tree from a 900–2649 bp sequence of mitochondrial 12S and 16S rRNA, and tRNA^{val} genes for samples of *Pelophryne*. Numbers above or below branches represent bootstrap supports for ML inference and Bayesian posterior probability (ML-BS/BPP).

and *Hylaplesia brevipes* (NHMW 16554) gave little information because of greatly faded coloration, although relatively large body size and short hindlimb parts were found to characterize *H. brevipes* (Table 3). Meanwhile, two males of *Pelophryne* sp. from Peninsular Malaysia were morphologically nearly uniform, and could be separated from *P. signata* from Borneo, although not so sharply as genetic separation, hence I describe them as a new species.

SYSTEMATICS

Pelophryne ingeri sp. nov.
(Figs. 2–4)

Pelophryne signata: Hendrickson, 1966, p. 73 (part).

Pelophryne brevipes: Grandison, 1972, p. 54 (part); Berry, 1975, p. 55 (part).

Diagnosis

The new species is placed in the genus *Pelophryne* by having the fingers and toes with a peculiar, fleshy web. A small *Pelophryne*, adult males 16–17 mm; tips of fingers expanded into truncate discs; disk of third finger slightly smaller than tympanum; tibio-tarsal articulation of adpressed limb reaching to posterior corner of eye; dorsum clay

TABLE 2. Uncorrected p-distances (%) among samples of *Pelophryne* and other bufonids for 471 bp fragments of 16S rRNA.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 <i>Pelophryne</i> sp. Malay Peninsula																
2 <i>P. brevipes</i> Sumatra	2.4															
3 <i>P. signata</i> Sarawak	2.4	3.2														
4 <i>P. signata</i> Sarawak	2.8	3.6	0.8													
5 <i>P. guentheri</i>	4.8	5.2	3.2	3.2												
6 <i>P. penrissensis</i>	4.8	5.2	5.2	5.2	6.0											
7 <i>P. penrissensis</i>	4.4	4.8	4.8	4.8	5.6	0.4										
8 <i>P. penrissensis</i>	4.4	4.8	4.8	4.8	5.6	0.4	0.0									
9 <i>P. brevipes</i> Philippines	6.3	6.3	4.4	4.4	4.4	6.7	6.3	6.3								
10 <i>P. api</i>	4.4	5.2	4.8	4.8	5.6	5.2	4.8	4.8	6.3							
11 <i>P. misera</i>	6.7	6.3	4.8	4.8	5.2	7.9	7.5	7.5	6.3	6.0						
12 <i>P. misera</i>	6.7	6.3	4.8	4.8	5.2	7.9	7.5	7.5	6.3	6.0	0.0					
13 <i>P. misera</i>	6.7	6.3	4.8	4.8	5.2	7.9	7.5	7.5	6.3	6.0	0.0	0.0				
14 <i>Ansonia penangensis</i>	10.3	9.5	10.3	10.7	11.1	11.9	11.5	11.5	10.7	10.7	11.1	11.1	11.1			
15 <i>Leptophryne borbonica</i>	9.9	10.7	10.7	11.1	10.7	11.1	10.7	10.7	12.7	11.5	13.1	13.1	13.1	13.1		
16 <i>Sabahphrynus maculatus</i>	10.3	8.7	9.5	9.9	10.3	10.3	9.9	9.9	11.5	11.9	11.1	11.1	11.1	11.9	9.5	
17 <i>Ingerophrynus divergens</i>	11.5	11.9	10.7	10.7	10.7	11.5	11.1	11.1	12.7	11.5	12.7	12.7	12.7	11.5	12.7	9.1

brown, with a cruciform pattern outlined by darker bands; creamy band extending from below eye to groin, merging on flank with creamy abdomen with small dark spots; males with mandibular spines and nuptial pad.

Etymology

The specific name is dedicated to the late Dr. Robert F. Inger, Emeritus Curator of the Field Museum of Natural History, Chicago, for his great contributions to Southeast Asian herpetology, including taxonomy of *Pelophryne*.

Holotype

KUHE 15647, an adult male collected near a pond in Genting Highlands, State of Pahang, Malaysia (03°24'N, 101°46'E, 850 m a.s.l.) by M. Matsui and K. Araya on 17 January 1993.

Paratype

KUHE 35585, an adult male, data same as the holotype.

Description of holotype (measurements in mm)

Snout-vent length (SVL) 16.3; habitus slender (Figs. 2, 3); head depressed, slightly longer (HL 5.9, 36.2%SVL) than broad (HW 5.6, 34.0%SVL); snout truncate with median projection, oblique in profile, projecting beyond lower jaw; eye length (EL 2.3, 14.2%SVL) subequaling snout length (SL 2.4, 14.6%SVL); canthus slightly rounded, constricted; lore vertical, not concave; nostril lateral, below canthus, much nearer to tip of snout than to eye (N-EL 1.5, 9.0%SVL); internarial distance (IND 1.5, 9.3%SVL) much narrower than interorbital distance (IOD 2.5, 15.1%SVL), latter wider than upper eyelid (UEW 1.6, 9.9%SVL); pineal spot absent; tympanum conspicuous, oval, less than half length of eye (TD 0.9, 5.6%SVL); vomerine teeth absent; tongue oval, without papillae; slit-like vocal openings on floor of mouth well anterior to jaw commissure on both sides.

Forelimb thin, long (12.3, 75.5%SVL);

TABLE 3. Measurements in types of *Pelophryne ingeri* sp. nov., male *P. signata* from Sarawak, holotype of *Nectophryne exigua* (SMF 3737), and a syntype of *Hylaplesia brevipes* (NHMW 16554). SVL (in mm) and ratios (R) of other characters to SVL. See text for character abbreviations.

	<i>P. ingeri</i> sp. nov.		<i>P. signata</i>		<i>N. exigua</i>	<i>H. brevipes</i>
	2 males		7 males			
SVL	16.7	(16.3–17.0)	16.2±1.0	(15.4–17.3)	14.7	18.0
RHL	33.7	(31.2–36.2)	33.0	(31.7–37.6)	31.6	28.3
RN-EL	8.0	(7.0–9.0)	7.9	(6.9–9.7)	—	6.1
RSL	13.9	(13.2–14.6)	13.8	(11.4–15.0)	15.6	11.7
REL	13.3	(12.4–14.2)	14.1	(12.6–15.5)	13.6	15.0
RT-EL	1.7	(1.5–1.9)	1.3	(0.8–2.4)	—	1.1
RTD	5.6	(5.5–5.6)	5.8	(4.3–7.3)	4.8	4.4
RHW	32.6	(31.2–34.0)	32.6	(31.4–37.5)	29.9	30.6
RIND	9.1	(9.0–9.3)	8.8	(8.2–10.2)	9.5	10.0
RIOD	13.8	(12.5–15.1)	13.4	(12.9–14.0)	12.9	12.8
RUEW	9.5	(9.0–9.9)	9.0	(8.4–9.3)	8.2	8.9
RFL	73.6	(71.8–75.5)	80.4	(76.0–93.1)	71.4	73.3
RLAL	52.7	(47.8–57.7)	54.4	(51.7–58.4)	51.0	50.6
RIFL	6.1	(5.5–6.7)	5.8	(3.9–7.4)	—	—
ROPTL	4.0	(4.0–4.1)	3.8	(3.0–4.6)	—	—
RIPTL	2.9	(2.6–3.1)	3.2	(2.3–3.7)	—	—
RHAL	22.5	(20.8–24.3)	26.1	(23.9–27.2)	23.8	23.3
RHLL	143.7	(142.3–145.0)	155.3	(138.3–177.5)	148.3	126.1
RTHIGH	44.8	(39.7–49.9)	46.2	(41.6–55.7)	44.9	37.8
RTL	46.5	(42.3–50.6)	45.7	(43.1–48.6)	45.6	39.4
RFL	33.3	(33.1–33.5)	37.0	(31.0–37.2)	34.0	30.6
R1TOEL	5.5	(5.2–5.8)	6.5	(5.4–7.1)	5.4	—
ROMTL	3.7	(3.0–4.3)	3.5	(2.6–5.2)	—	—
RIMTL	3.5	(3.1–3.9)	3.6	(2.6–5.5)	4.8	—
R3FDW	5.0	(4.7–5.3)	5.7	(4.9–6.1)	—	—
R4FDW	4.4	(4.2–4.6)	4.6	(4.1–5.6)	—	—
R4TDW	3.7	(3.5–4.0)	4.5	(3.5–4.9)	—	—
R5TDW	3.5	(3.1–3.9)	4.1	(2.9–4.4)	—	—

fingers with fleshy web; finger length formula: $I < II < IV < III$ (Fig. 4A); length of first, measured from distal edge of inner palmar tubercle (1FL 1.1, 6.7%SVL) much shorter than length of eye; tips of fingers expanded, truncate, forming small pads without circummarginal grooves; disc of third finger (3FDW 0.9, 5.5%SVL) equal to tympanum; one phalanx of first finger projecting, other fingers webbed

at bases only; inner palmar tubercle small (IPTL 0.4, 2.6%SVL), oval, smaller than outer palmar tubercle (OPTL 0.7, 4.0%SVL); subarticular tubercles obscure; no supernumerary metacarpal tubercles.

Hindlimb slender, moderately long (HLL 23.2, 142.3%SVL) less than two times length of forelimb; tibia moderate (TL 8.3, 50.6%SVL), heels meeting when limbs are



FIG. 2. Dorsolateral view of male holotype of *Pelophryne ingeri* sp. nov. (KUHE 15647).

held at right angles to body; tibiotarsal articulation of adpressed limb reaching to posterior corner of eye; foot (FL 5.4, 33.1%SVL) much shorter than tibia; toe length formula $I < II < III < V < IV$; fifth toe much longer than third; tips of toes truncate, not expanded (5TDW 0.6, 4.0%SVL); webbing fleshy, formula: I 0–0 II 0–2 III 1–3 IV 3–2 V (Fig. 4B); subarticular tubercles present, but indistinct; an oval inner metatarsal, length (IMTL 0.6, 3.9%SVL), shorter than first toe (1TOEL 0.8, 5.2%SVL) but longer than outer metatarsal tubercle (OMTL 0.5, 3.0%SVL).



FIG. 4. Ventral view of left hand (A) and foot (B) of male holotype of *Pelophryne ingeri* sp. nov. (KUHE 15647) Scale bar=3 mm.

Dorsum scattered with small and large, round tubercles, large ones especially clear on outline of cruciform pattern (Fig. 5A); no temporal fold from eye to above axilla, but weak ridge from posterior corner of eye to above tympanum; side of trunk with scattered larger tubercles; dorsal surface of limbs scattered with small, low warts; tarsus without indistinct dermal ridge; throat, chest, and abdomen finely granular; skin of gular region modified, scattered with a row of translucent mandibular spines; indistinct yellowish tinge, but without asperities, forming a nuptial pad

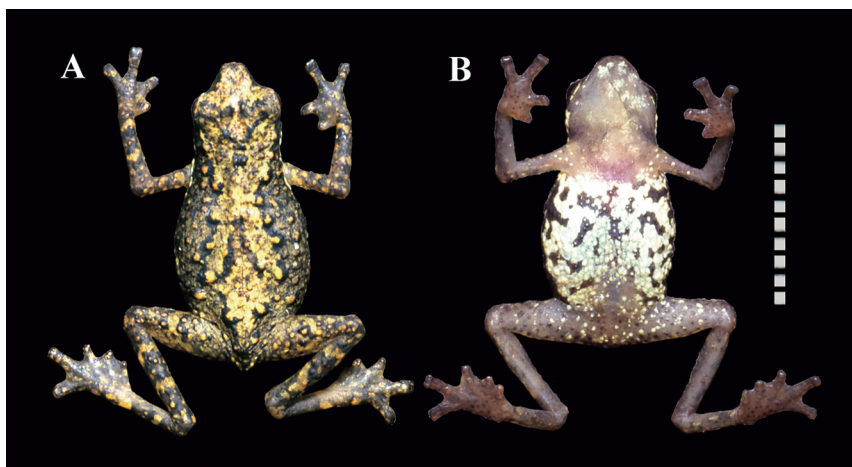


FIG. 3. Dorsal (A) and ventral (B) views of male holotype of *Pelophryne ingeri* sp. nov. (KUHE 15647). Scale bar=10 mm.

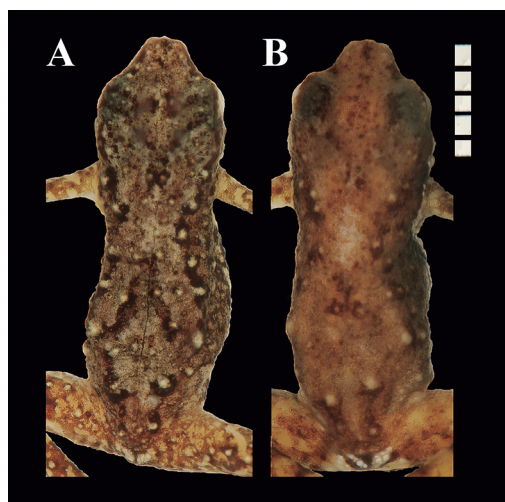


FIG. 5. Dorsal view of male *Pelophryne ingeri* sp. nov. (A) and *P. signata* (B) showing differences in development of tubercles. Scale bar=5 mm.

covering medial surface of first finger from its base to level of subarticular tubercle.

Color

In life, head with dark brown interorbital bar; dorsally clay brown with light cruciform pattern outlined in dark brown (Fig. 3A); another clay brown diamond from mid-trunk to above cloaca, also outlined in dark brown; ventrally to dark brown outline of cruciform pattern, a wide blackish brown band from eye and surrounding tympanum and above arm insertion to groin; brown band bordered below by a creamy yellow streak from below eye and tympanum, extending backwards to groin and merging on flank with creamy venter; irregular dark crossbars on dorsal surface of limbs; throat and chest creamy brown lightly mottled with brown; abdomen cream irregularly marked with dark brown (Fig. 3B); ventral side of limbs light brown; iris orange-red. In alcohol, color pattern slightly faded.

Variation

Two males of the type series are generally similar to each other in morphology. Varia-

tion in size and body proportions is given in Table 3. In the paratype, a dark brown reverse triangle marking is evident on head in front of cruciform pattern. Grandison (1972) reported the body size of 16.3 mm in a male and 18.8 mm in a female from Gunung (=Mt.) Benom, Pahang.

Range

Peninsular Malaysia: Genting Highlands, 850 m, Pahang (type locality); Ulu Chempoh, Janda Baik, Pahang (Berry, 1975); Gunung Benom, 800 and 1600 feet, Pahang (Grandison, 1972); Tioman Island at 900 to 2900 feet (Hendrickson, 1966; Berry, 1975, Lim and Lim, 1999). Singapore: Bukit Timah Nature Reserve (Lim and Lim, 1992). Indonesia: Lampung, Sumatra (Smart et al., 2017), ? Natuna Islands, ?Mentawai Islands (Inger, 1966).

Natural history

The type specimens were found in a narrow area in primary forest, on fern leaves less than 1 m above ground. Grandison (1972) also noted that her specimens were found 0.6 m from the forest floor on a sodden tree stump or on the rough bark of a tree, about 1.5 m from the ground. I could not find breeding place near the type locality, but in Singapore, Leong and Teo (2009) found endotrophic tadpoles in primary rain forest on the forest floor and on vegetation 0.6–1.5 m above ground.

Other frog species observed in the type locality immediately near the habitat were *Limnonectes blythii* (Boulenger, 1920), *Chalcophryne labialis* (Boulenger, 1887), *Odorrana hosii* (Boulenger, 1891), *Abavorana luctuosa* (Peters, 1871), *Rhacophorus bipunctatus* Ahl, 1927, *Zhangixalus prominans* (Smith, 1924), *Polypedates leucomystax* (Gravenhorst, 1829), and *Philautus larutensis* (Boulenger, 1900).

Calls

Calls are ticking notes in continuous or very long series. I failed to record the first type of

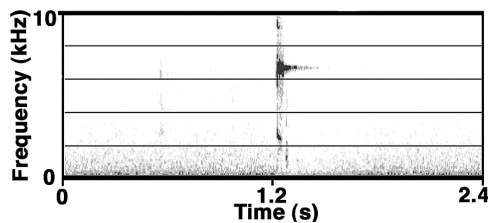


FIG. 6. Sonogram showing advertisement call of *Pelophryne ingeri* sp. nov., holotype (KUHE 15647) from Genting Highlands, Pahang, recorded at an air temperature of 23.6°C.

calls in the field, but could record the second type of calls of the male holotype on 18 January 1993 in the room at the air temperature of 23.6°C. The call consists of a series of notes each lasting 0.37–0.42 (mean \pm SD = 0.38 ± 0.02 , $n=7$) s and emitted at an interval of 5.27–6.05 (mean \pm SD = 5.61 ± 0.24 , $n=7$) s. Each note is vaguely pulsed. The dominant frequency is at 6.6–6.75 (6.63 ± 0.05 , $n=6$) kHz and ill-defined harmonics are at about 9.0–9.3 (9.23 ± 0.08 , $n=6$) kHz (Fig. 6).

Comparisons

In the genus *Pelophryne*, *P. ingeri* sp. nov. is in a group with the species having the tips of the fingers expanded into truncate discs. In this group, *P. ingeri* sp. nov., with the male SVL of 16.3 and 17.0 mm (this study), is a small species, and has so far been treated as *P. brevipes* or *P. signata*. Both of them are also small in body size, 13.5–15.9 mm in males and 15.3–17.6 mm in females in *P. signata* (Inger and Stuebing, 2009), and 16.0–17.7 mm and 16.6–17.4 mm, respectively, in *P. brevipes* (Inger, 1954).

Pelophryne signata, including *Nectophryne exigua* Boettger, 1900 as a synonym (Inger and Stuebing, 2009), occurs on Borneo and is morphologically very similar to *P. ingeri* sp. nov. They have similar body color, with the cream or yellow venter with small, isolated black spots, and with a cream or yellow streak on the side of the head running from below the eye under the tympanum and

over the axilla. However, *P. ingeri* sp. nov. has more large warts, especially clearer cruciform dorsal pattern than *P. signata* (Fig. 5). Moreover, *P. ingeri* sp. nov. has a shorter hindlimb with the tibiotarsal articulation of adpressed limb reaching only to posterior corner of eye, contrasting to *P. signata*, whose articulation reaches to center of eye.

Pelophryne brevipes occurs on Mindanao and Basilan islands of the Philippines, and has brown or black pigment covering most of the belly and underside of the hind limb and lacks the uninterrupted light lateral streak (Inger and Stuebing, 2009) unlike *P. ingeri* sp. nov.

Another small Philippine species *P. lighti* (Taylor, 1920), occurring on Mindanao and other islands, is 16.3–18.5 mm (Inger, 1960), and differs from *P. ingeri* sp. nov. by having dark upper lip without light stripes or row of light spots (Inger, 1966).

Other species of the group have larger body size than *P. ingeri* sp. nov.: *P. guentheri* (mature male 29.9 mm and females 28.3–31.1 mm [Inger and Stuebing, 2009]; *P. penrissenensis* (male SVL 24.9–28.0 mm and female SVL 30.8 mm [Matsui et al., 2017]; *P. rhopophilia* Inger and Stuebing, 1996 (males 21.6–24.8 mm) [Inger and Stuebing, 1996, 2009]; *P. murudensis* Das, 2008 (males 21.9–25.6 mm [Das, 2008]), *P. saravacensis* Inger and Stuebing, 2009 (adult males 17–20 mm, an adult female 22 mm [Inger and Stuebing, 2009]).

The remaining *Pelophryne* species form another group by having the tips of the fingers rounded and not expanded, and can be differentiated from *P. ingeri* sp. nov., although all of them are rather small in body size like *P. ingeri* sp. nov.: *P. api* (males 17.8–22.3 mm and females 22.0–24.3 mm [Dring, 1983]), *P. alboteniata* Barbour, 1938 (males 18.2–19.9 mm and females 19.2–22.8 mm [Inger, 1966]), *P. misera* (adult males 16–21 mm, adult females 17–21 mm [Inger, 1966]), and *P. linanitensis* Das, 2008 (adult males to 18.6 mm [Das, 2008]).

DISCUSSION

Taxonomic status of *Pelophryne* from Peninsular Malaysia has been unclear probably because not a large number of specimens have been secured and compared with Bornean *P. signata* and Philippine *P. brevipes*. As stated in Grandison (1972), the species assignment of the specimens has been made simply on the basis of classification of Bornean species. In contrast, results of the present study clarified that the *Pelophryne* from Peninsular Malaysia (*P. ingeri* sp. nov.) actually represents a cryptic species of Bornean *P. signata*, from which it differs slightly morphologically, but fairly greatly genetically.

Our phylogenetic analysis also indicated that the *Pelophryne* from Sumatra is much closer to Peninsular Malaysian population than to Bornean *P. signata*. Inger (1966) listed the range of *P. brevipes* as Malay Peninsula, Singapore, Aor Island, Natuna Islands, Mentawai Islands, Sumatra, Borneo, and Mindanao. As mentioned above, Inger (1966) mistakenly synonymized *P. signata* with *P. brevipes*; only Mindanao can be regarded as exact range of *P. brevipes*, and Borneo is thought to be the range of *P. signata*. Of the remaining localities, Malay Peninsula is considered to be that of *P. ingeri* sp. nov. from the present genetic evidence, and Singapore is also regarded so from its geographic location. Sumatra is occupied not by *P. brevipes*, but by *P. ingeri* sp. nov. from their close genetic relationship. The lack of samples, however, prohibits morphological examination and concrete taxonomic conclusion.

I have no data about specimens from the Natuna and Mentawai (=Mentawai) islands, but as to Aor (=Aur) Island, assigning *P. signata* or *P. brevipes* to this island population seems incorrect. In describing geographic variation of *P. brevipes* (including *P. signata*), Inger (1966) mentioned that specimens from Aur Island were larger than those from Mindanao and had less extensive

webbing in hand and foot. Toads of the island were reported to have male SVL of 20.2–21.7 mm and female SVL of 22.6–25.2 mm, significantly larger than both *P. brevipes* and *P. signata* (see above for the size).

It is possible that the Aur Island population actually represents an undescribed species. Aur Island is situated off the east coast of the peninsula like Tioman Island, but is about 75 km from the peninsula, more than twice distant than Tioman Island (32 km). Unfortunately, more recent herpetological survey of the Aur Island did not record specimens of *Pelophryne* (Grismer et al., 2001). Additionally, *Pelophryne* from the Tioman Island seems not sufficiently studied. Because the island harbors some endemic species like *Ansonia tiomanica* Hendrickson, 1966 and *Kalophrynus tiomanensis* Chan, Grismer, and Grismer, 2011, *Pelophryne* from the island should be reexamined more closely, especially molecularly.

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LITERATURE CITED

- BERRY, P. Y. 1975. *The Amphibian Fauna of Peninsular Malaysia*. Tropical Press, Kuala

- Lumpur.
- BOULENGER, G. A. 1895 "1894". Third report on additions to the batrachian collection in the Natural-History Museum. *Proceedings of the Zoological Society of London* 1894: 640–646.
- DAS, I. 2008. Two new species of *Pelophryne* (Anura: Bufonidae) from Gunung Murud, Sarawak (Northwestern Borneo). *Raffles Bulletin of Zoology* 56: 435–443.
- DRING, J. 1983. Some new frogs from Sarawak. *Amphibia-Reptilia* 4: 103–115.
- GRANDISON, A. G. C. 1972. The Gunong Benom Expedition 1967. 5. Reptiles and amphibians of Gunong Benom with a description of a new species of *Macrocalamus*. *Bulletin of British Museum of Natural History (Zoology)*, London 23: 45–101.
- GRISMER, L. L., NORSHAM, S. Y., LIM, B. L., LEONG, T. M., DAS, I., ROLAND, A. S., GRISMER, J. L., CRANE, K. M., DIAZ, R. E., FIGUEROA, S. V., LEDBETTER, C. A., NEWBOLD, S. C., NEWBOLD, S. R., PATEL, C. P., CASTRO, J., ESCOBAR III, R. A., GUERRERO, S., PINEDO, J. W., TRUJILLO, J. D., AND KAISER, H. 2001. First report on the herpetofauna of Pulau Aur, Johor, West Malaysia. *Hamadryad* 26: 350–353.
- HENDRICKSON, J. R. 1966. Observations on the fauna of Pulau Tioman and Pulau Tulai. 6. The amphibians. *Bulletin of the National Museum of Singapore* 34: 72–84.
- DIESMOS, A., ALCALA, A., BROWN, R., AFUANG, L., GEE, G., SUKUMARAN, J., YAAKOB, N., LEONG, T.-M., CHUAYNKERN, Y., THIRAKHUPT, K., DAS, I., ISKANDAR, D., MUMPUNI, INGER, R., STUEBING, R., YAMBUN, P., AND LAKIM, M. 2004. *Pelophryne brevipes*. In: The IUCN Red List of Threatened Species 2004: e.T54866A11217332.
- INGER, R. F. 1954. Systematics and zoogeography of Philippine Amphibia. *Fieldiana: Zoology* 33: 183–531.
- INGER, R. F. 1960. Notes on toads of the genus *Pelophryne*. *Fieldiana: Zoology* 39: 415–418.
- INGER, R. F. 1966. The systematics and zoogeography of the Amphibia of Borneo. *Fieldiana: Zoology* 52: 1–402.
- INGER, R. F. AND STUEBING, R. B. 1996. Two new species of frogs from Southeastern Sarawak. *Raffles Bulletin of Zoology* 44: 543–549.
- INGER, R. F. AND STUEBING, R. B. 2009. New species and new records of Bornean frogs (Amphibia: Anura). *Raffles Bulletin of Zoology* 57: 527–535.
- INGER, R. F., STUEBING, R. B., GRAFE, T. U., AND DEHLING, J. M. 2017. *A Field Guide to the Frogs of Borneo. Third Edition*. Natural History Publications (Borneo), Kota Kinabalu.
- INGER, R. F. AND TAN, F.-L. 1996. Checklist of the frogs of Borneo. *Raffles Bulletin of Zoology* 44: 551–574.
- LEONG, T. M. AND CHOU, L. M. 1999. Larval diversity and development in the Singapore Anura (Amphibia). *Raffles Bulletin of Zoology* 47: 81–137.
- LEONG, T. M. AND TEO, S. C. 2009. Endotrophic tadpoles of the Saint Andrew's Cross Toadlet, *Pelophryne signata* (Amphibia: Anura: Bufonidae) in Singapore. *Nature in Singapore* 2: 21–25.
- LIM, K. K. P. 1990. Two recent records of the toad, *Pelophryne brevipes* (Peters, 1867) (Anura: Bufonidae) from Singapore. *Raffles Bulletin of Zoology* 38: 25–26.
- LIM, K. K. P. AND LIM, F. L. K. 1992. *A Guide to the Amphibians and Reptiles of Singapore*. Singapore Science Centre, Singapore.
- LIM, K. K. P. AND LIM, F. L. K. 1999. The terrestrial herpetofauna of Pulau Tioman, Peninsular Malaysia. *Raffles Bulletin of Zoology, Supplement* (6): 131–155.
- MATSUI, M. 1984. Morphometric variation analyses and revision of the Japanese toads (Genus *Bufo*, Bufonidae). *Contributions from the Biological Laboratory of the Kyoto University* 26: 209–428.
- MATSUI, M. 1997. Call characteristics of Malaysian *Leptolalax* with a description of two new species (Anura: Pelobatidae). *Copeia* 1997: 158–165.
- MATSUI, M., TOMINAGA, A., LIU, W.-Z., KHONSUE, W., GRISMER, L. L., DIESMOS, A. C., DAS, I., SUDIN, A., YAMBUN, P., YONG, H.-S., SUKUMARAN, J., AND BROWN, R. M. 2010. Phylogenetic relationships of *Ansonia* from South-east Asia inferred from mitochondrial DNA sequences: Systematic and biogeographic implications (Anura: Bufonidae). *Molecular Phylo-*

- genetics and Evolution* 54: 561–570.
- MATSUI, M. AND DEHLING, J. M. 2012. Notes on an enigmatic Bornean megophryid, *Leptolalax dringi* Dubois, 1987 (Amphibia: Anura). *Zootaxa* 3317: 49–58.
- MATSUI, M., NISHIKAWA, K., ETO, K., AND HOSSMAN, M. Y. B. 2017. A new species of *Pelophryne* from Western Sarawak, Malaysian Borneo (Anura, Bufonidae). *Zoological Science* 34: 345–350.
- MATSUI, M., NISHIKAWA, K., YEO, S. T., AND ETO, K. 2012. Notes on a rare Bornean bufonid *Ansonia latidisca* Inger, 1966, with special reference to its phylogenetic position. *Current Herpetology* 31: 87–96.
- MATSUI, M., YAMBUN, P., AND SUDIN, A. 2007. Taxonomic relationships of *Ansonia anotis* Inger, Tan, and Yambun, 2001 and *Pedostibes maculatus* (Mocquard, 1890), with a description of a new genus (Amphibia, Bufonidae). *Zoological Science* 24: 1159–1166.
- SAVAGE, J. M. AND HEYER, W. R. 1997. Digital webbing formulae for anurans: A refinement. *Herpetological Review* 28: 131.
- SMART, U., SARKER, G. C., ARIFIN, U., HARVEY, M. B., SIDIK, I., HAMIDY, A., KURNIAWAN, N., AND SMITH, E. N. 2017. A new genus and two new species of arboreal toads from the highlands of Sumatra with a phylogeny of Sundaland toad genera. *Herpetologica* 73: 63–75.
- TANABE, A. S. 2011. Kakusan4 and Aminosan: two programs for comparing nonpartitioned, proportional, and separate models for combined molecular phylogenetic analyses of multilocus sequence data. *Molecular Ecology Research* 11: 914–921.
- TAVARÉ, S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. p. 57–86. In: R. M. Miura (ed.), *Some Mathematical Questions in Biology—DNA Sequence Analysis*. American Mathematical Society, Providence, Rhode Island.
- TEO, R. C. H. AND RAJATHURAI, S. 1997. Mammals, reptiles and amphibians in the Nature Reserves of Singapore—diversity, abundance and distribution. *Gardens' Bulletin Singapore* 49: 353–425.
- VAN BOCKLAER, I., LOADER, S. P., ROELANTS, K., BIJU, S. D., MENEGON, M., AND BOSSUYT, F. 2010. Gradual adaptation toward a range-expansion phenotype initiated the global radiation of toads. *Science* 327: 679–682.

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