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A New Species of Large Flying Frog (Rhacophoridae: *Rhacophorus*) from Lowland Forests in Southern Vietnam

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ABSTRACT.—We describe a new species of *Rhacophorus* from lowland forests in southern Vietnam. The new species is most similar to *Rhacophorus kio*, but differs from this and all other *Rhacophorus* from Southeast Asia by a combination of its large body size (males 72.3–85.5 mm; females 89.4–90.7 mm snout–vent length); green dorsum; white venter; black patch at axilla; bluish-green posterior surface of thighs with pale yellow marbling; low, single-lobed supracolocal dermal ridge, and white sclera. In contrast to the widely distributed *R. kio*, the new species is known only from two fragments of disturbed, low-elevation forest approximately 30 km apart in a highly modified agricultural landscape. The continued survival of the new species is threatened by ongoing habitat loss and degradation.

A significant proportion of amphibian and reptile diversity in Southeast Asia remains hidden within morphologically cryptic species groups currently treated as a single species (e.g., Bain et al., 2003; Stuart et al., 2006; Che et al., 2009). This is true even for the charismatic “flying” frogs in the genus *Rhacophorus* (Kuhl and Van Hasselt, 1822).

The large “flying” frog *Rhacophorus reinwardtii* (Schlegel, 1840) was one of the first amphibians discovered in Asia (Kuhl and Van Hasselt, 1822). Described from Java, Indonesia this species was until recently thought to occur throughout Asia. In 2006, populations referred to previously as *R. reinwardtii* from China, Laos, Thailand, and Vietnam were described as *Rhacophorus kio* (Ohler and Delorme, 2006), and more recently, *R. “reinwardtii”* from southern Thailand and Peninsular Malaysia were described as *Rhacophorus norhayatii* (Chan and Grismer, 2010).

During recent surveys in lowland forest fragments in southern Vietnam, we discovered a large species of “flying” frog resembling *R. kio* but distinct from this species and all other congeners. Here we describe this species as new.

MATERIALS AND METHODS

Specimens were deposited at the Australian Museum (AMS), the University of Science, Ho Chi Minh City (UNS), and the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK). We recorded morphological data from specimens fixed in 10% formalin and then stored in 70% ethanol. Morphometric data were taken (to the nearest 0.1 mm) with digital calipers. Measurements include snout–vent length (SVL); head length from tip of snout to rear of jaws (HDL); head width at the commissure of the jaws (HDW); snout length from tip of snout to the anterior corner of eye (SNT); diameter of the exposed portion of the eyeball (EYE); interorbital distance (IOD); horizontal diameter of tympanum (TMP); distance from anterior edge of tympanum to posterior corner of the eye (TEY); internarial space (IN); distance from nostril to tip of snout (NS); distance from front of eye to nostril (EN); tibia length with the hindlimb flexed (TIB); manus length from tip of third digit to base of tubercle on prepollex (ML); pes length from tip of fourth toe to base of the inner metatarsal tubercle (PL); and length of inner metatarsal tubercle (IML). We used a traditional formula for finger numbering rather than one based on

homology (e.g., Alberch and Gale, 1985). Sex was determined by the presence of vocal sacs and gonadal inspection. Mass was recorded in life (to the nearest 0.1 g) using Pesola scales. Radiographs of the holotype were also prepared to examine osteological features including the presence of intercalary discs and the shape of the distal end of the terminal phalanges. Vocal sac terminology follows Liu (1935). Terminology for describing eye coloration in life follows Glaw and Vences (1997). Webbing formula follows that proposed by Savage and Heyer (1967) and modified by Myers and Duellman (1982) and Savage and Heyer (1997). We obtained comparative morphological data from museum specimens of *R. kio* throughout its range, using the same methodology, so as to confirm the distribution of the new species (Fig. 1; Appendix 1). We also examined morphological data from museum specimens of other *Rhacophorus* species, photographs of these specimens in life (Appendix 1), and from the literature (Günther, 1858; Blanford, 1881; Boulenger, 1893; Ahl, 1931; Andersson, 1939 “1938”; Ohler et al., 2000; Ohler and Delorme, 2006; Bordoloi et al., 2007; Ohler, 2009; Chan and Grismer, 2010; Fei et al., 2010).

We analyzed ~550 base pairs of mitochondrial DNA for the 16S ribosomal RNA gene from five adults of the new species and six *R. kio* specimens (Fig. 1; Table 1). DNA was extracted using DNeasy tissue extraction kits (Qiagen). We used the primers 16SAR and 16SBR of Palumbi et al. (1991) to amplify the 16S rRNA gene. Standard PCR protocols were used and PCR products were purified using ExoSap-IT (USB Corporation, OH, USA). Purified templates were sequenced directly by Macrogen (Seoul, Korea). Sequences were edited with Sequencher v. 4.10 (Gene Codes, Ann Arbor, MI) and deposited in GenBank under accession numbers JQ288087–JQ288097. Homologous fragments of 16S for *R. kio* were downloaded from GenBank or obtained from previous publications (Table 1). These sequences were trimmed to match the length of the fragment obtained here. The data set was aligned using the Clustal option in MEGA 5. Uncorrected pairwise sequence divergence was calculated using MEGA 5.

Rhacophorus helenae sp. nov.

Figures 2, 4

Holotype.—AMS R 173230, adult female, on a fallen tree 0.2 m above ground next to a trail in disturbed mixed evergreen, deciduous, and bamboo forest in Nui Ong Nature Reserve, Binh

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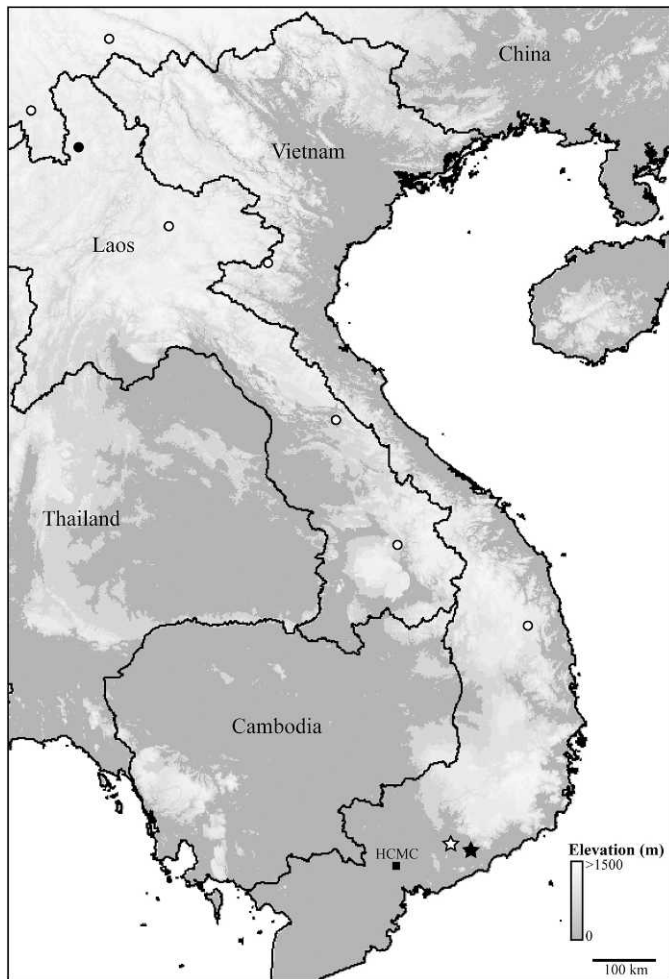


FIG. 1. Collection site of holotype (closed star) and paratypes (open star) of *Rhacophorus helenae* and sites from which *Rhacophorus kio* specimens or molecular data were examined (type locality closed circle, other sites are open circles). Ho Chi Minh City (HCMC) is located <120 km from known sites of the new species.

Thuan Province, Vietnam (11.0175°N, 107.7241°E, 158 m). Collected at 2100 h on 21 May 2009 by J. J. L. Rowley, D. H. Hoang, T. T. D. Le, T. H. Phung, and C. Minshew.

Paratypes.—AMS R 176399, UNS 00451, two adult males, at a small pond, collected at 2230 h on 10 September 2008. ZFMK

92544, adult male, and UNS 00450, adult female, collected from tall tree at side of dirt road at 2204 h on 12 September 2008. All paratypes were collected by T. A. D. Tran, T. T. N. Le, B. P. Dinh, and H. D. Dinh in disturbed mixed evergreen and deciduous forest in Tan Phu Forest, Dong Nai Province, Vietnam (11.1107°N, 107.4528°E, 85 m).

Diagnosis.—The new species is assigned to the genus *Rhacophorus* by the presence of intercalary cartilage between the terminal and penultimate phalanges of digits, Y-shaped distal end of terminal phalanx, tips of digits expanded into large disks bearing circummarginal grooves, webbed fingers, dermal fringes, vomerine teeth, and horizontal pupil (Liem, 1970; Duellman and Trueb, 1986; Brown and Alcalá, 1994). *Rhacophorus helenae* is distinguished from its Southeast Asian congeners by a combination of its large body size (males 72.3–85.5 mm; females 89.4–90.7 mm snout–vent length [SVL]), green dorsum, white venter, black patch at axilla, bluish-green posterior surface of thighs with pale yellow marbling, low, single-lobed supraclacal dermal ridge, and white sclera.

Comparisons.—Other large (maximum male SVL >60 mm) *Rhacophorus* species with an immaculate or predominantly green dorsum from Southeast Asia are *Rhacophorus burmanus*, *Rhacophorus dennysi*, *Rhacophorus duboisi*, *Rhacophorus feae*, *R. kio*, *Rhacophorus georgii*, *Rhacophorus maximus*, *Rhacophorus nigropalmatus*, and *Rhacophorus norhayatii*. From *R. burmanus*, *R. helenae* is distinguished by having a white venter (versus chocolate brown with white markings), black axillary patch, and tibiotarsal dermal projection present (versus both absent). From *R. dennysi*, *R. helenae* is distinguished by having a white venter (versus dirty white with darker mottling), black axillary patch present (versus absent), and supraclacal dermal ridge and tibiotarsal dermal projection present (versus both absent). From *R. duboisi*, *R. helenae* is distinguished by having a near-immaculate green dorsum (versus mottled green and brown), white venter (versus marbled black and white), bluish-green posterior surface of thigh with pale yellow marbling (versus marbled black and white), black axillary patch present (versus absent), and supraclacal dermal ridge and tibiotarsal dermal projection present (versus both absent). From *R. feae*, *R. helenae* is distinguished by having a black axillary patch present (versus absent) and supraclacal dermal ridge and tibiotarsal dermal projection present (versus both absent). From *R. georgii*, *R. helenae* differs by having a near-immaculate green dorsum (versus a dark green dorsum densely spotted with white) and occipital knobs absent (versus four, bony occipital knobs

TABLE 1. Samples and sequences of *Rhacophorus helenae* and *Rhacophorus kio* used in molecular comparisons. TL = type locality.

Species	Locality	Voucher no.	GenBank no.
<i>Rhacophorus helenae</i>	Vietnam, Binh Thuan Province (TL)	AMS R 173230	JQ288087
	Vietnam, Dong Nai Province	UNS 00450, AMS R 176399, UNS 00451, ZFMK 92544	JQ288088–288091
<i>Rhacophorus kio</i>	China, Yunnan Province	KIZ060821199, 060821287; SCUM 37941C	EF564570–564571 (as <i>Rhacophorus reinwardtii</i>); EU215532
	Laos, Phongsaly Province (TL)	MNHN 2004.0411 (holotype)	Not applicable (Ohler and Delorme, 2006)
	Laos, Bokeo Province	MNHN 1997.4092	AF215359 (as <i>Rhacophorus nigropalmatus</i>)
	Laos, Khammouane Province	FMNH 255304	JQ288092
	Laos, Houaphan Province	FMNH 255305	JQ288093
	Vietnam, Gia Lai Province	FMNH 252382–252383	JQ288094–288095
Vietnam, Nghe An Province	AMS R 173451, 173453	JQ288096–288097	

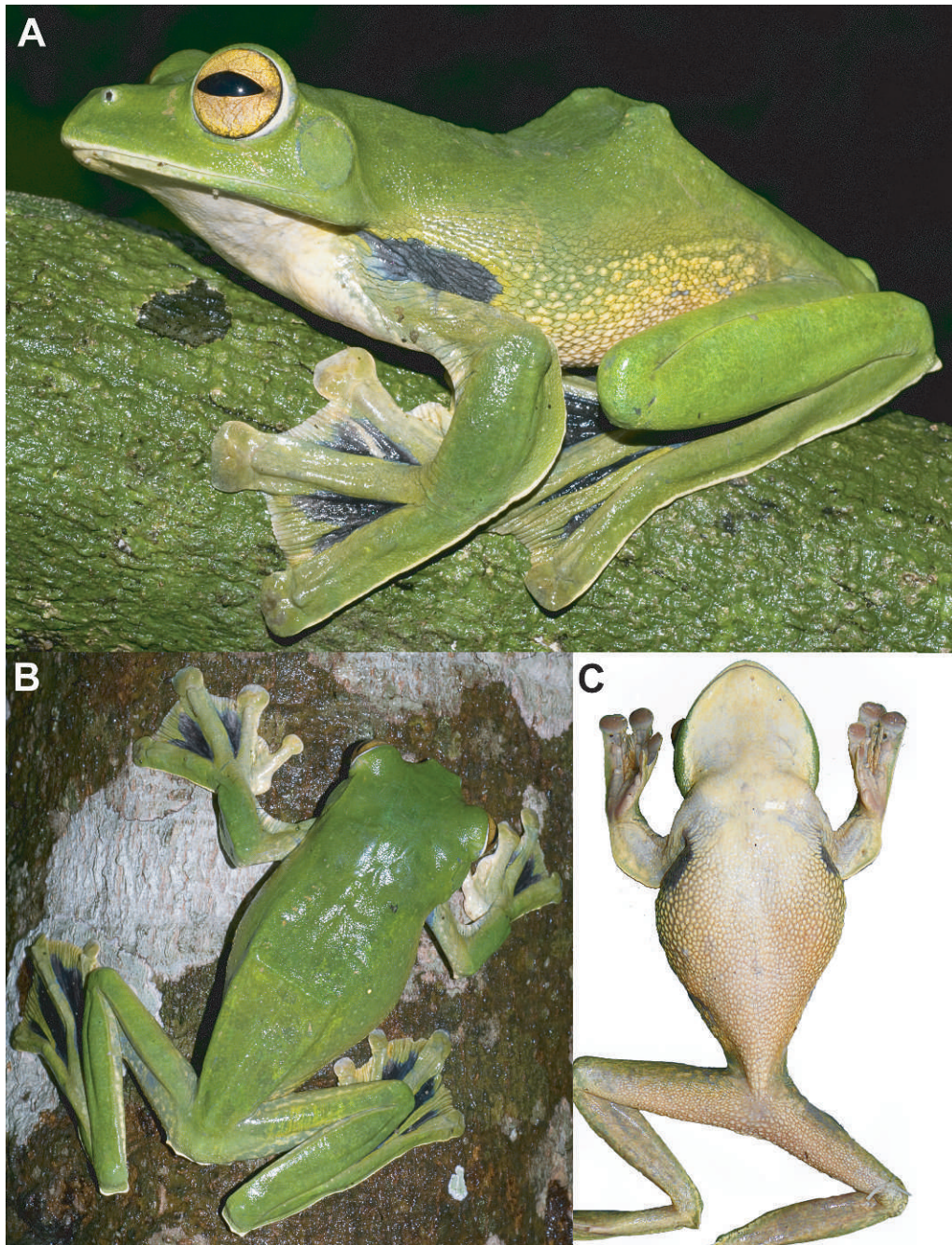


FIG. 2. Lateral (A), dorsal (B), and ventral (C) views of female holotype of *Rhacophorus helenae* (AMS R 173230) in life. Color reproduction supported by the Thomas Beauvais Fund.

present). From *R. maximus*, *R. helenae* is distinguished by having a bluish-green posterior surface of thigh with pale yellow marbling (versus grayish), black axillary patch present (versus absent), and a supraclacal dermal ridge and tibiotarsal dermal projection present (versus both absent). From *R. nigropalmatus*, *R. helenae* differs by having a near-immaculate green dorsum (versus green spotted with black), white venter (versus white with yellow mottling), black axillary patch present (versus absent), and posterior surface of thigh bluish-green with pale yellow marbling (versus solid yellow). From *R. norhayatii*, *R. helenae* is distinguished by having a larger body size (male SVL 72.3–85.5 mm vs. 41.7–64.7 mm), a white venter (versus white with black marbling and blue mottling),

posterior surface of thigh bluish-green with pale yellow marbling (versus black, studded with sky blue spots), and a low, single-lobed supraclacal dermal ridge (versus well-developed, double-lobed supraclacal dermal ridge). From the most similar species, *R. kio*, *R. helenae* is distinguished by having a white venter (versus bright yellow or lemon yellow venter; Fig. 3), bluish-green posterior surface of thigh with pale yellow marbling (versus solid yellowish orange posterior surface of thigh; Fig. 2–3 [in life] and Fig. 4C–D [in preservative]), a low, single-lobed supraclacal dermal ridge (versus a well-developed, double-lobed supraclacal dermal ridge; Fig. 4D), eye with white sclera (versus yellow sclera; Fig. 3A), and margins of webbing pale green or yellow (versus

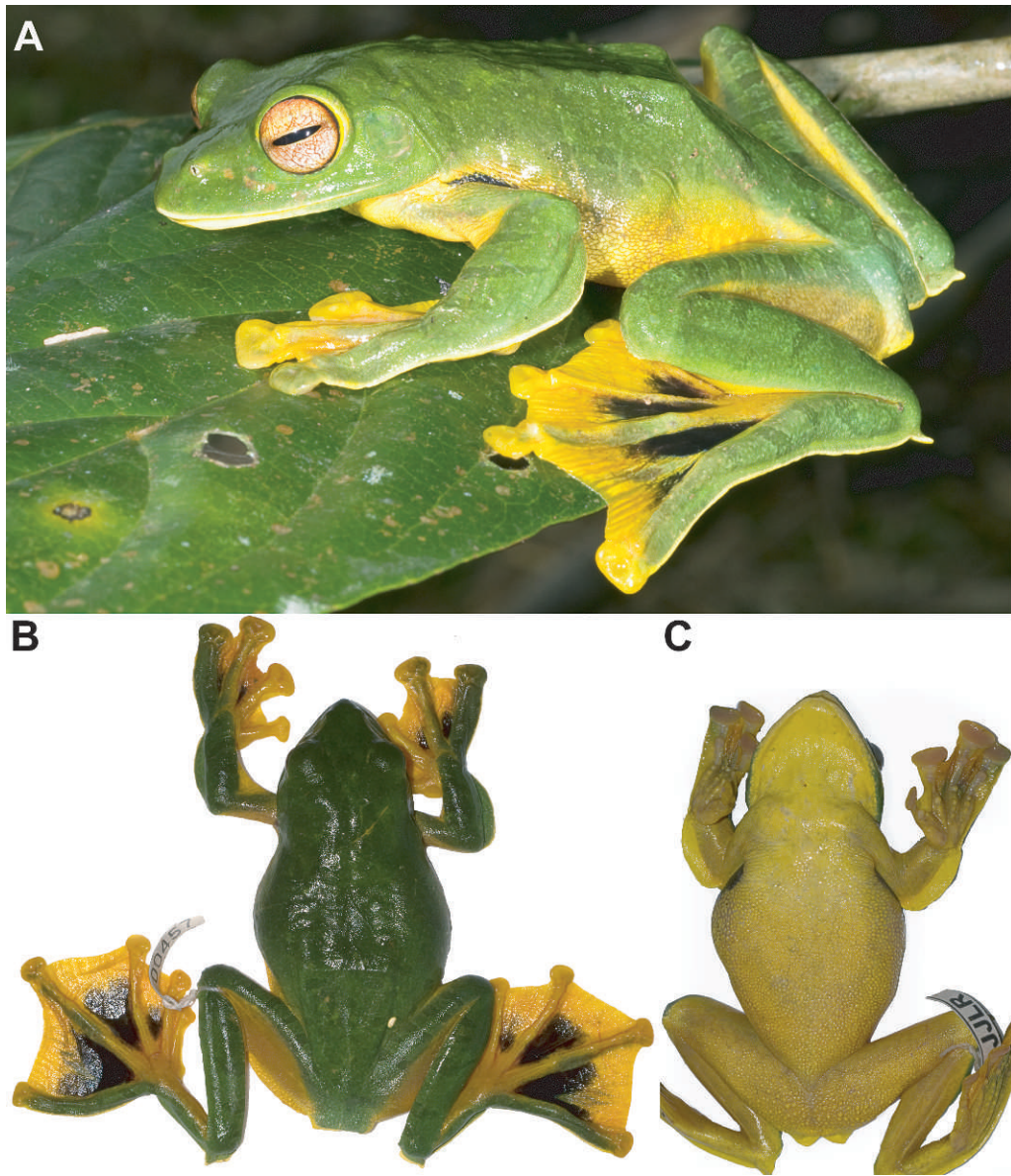


FIG. 3. Lateral (A), dorsal (B), and ventral (C) views of adult female *Rhacophorus kio* (AMS R 173453) from Nghe An Province, Vietnam, in life. Color reproduction supported by the Thomas Beauvais Fund.

extensive bright yellow–orange on the dorsal surfaces of hands and feet, particularly on webbing; Fig. 3). On the basis of the type series ($N = 5$) and *R. kio* specimens examined ($N = 15$), *R. helenae* (male SVL 72.3–85.5 mm; female SVL 89.4–90.7 mm) is also larger than *R. kio* (male SVL 66.6–69.5 mm, female SVL 82.6–88.9 mm).

The new species would have historically been assigned to *Rhacophorus reinwardtii*, but *R. helenae* is much larger (male SVL 72.3–85.5 mm compared to male SVL ~46–55 mm), has a near-immaculate green dorsum (versus darkly spotted), white venter (versus yellow), webbing proximally black and distally greenish (versus black with blue veins), and a low, single-lobed supraclacal dermal ridge (versus well-developed, double-lobed supraclacal dermal ridge).

Uncorrected pairwise 16S distance between *R. helenae* and *R. kio* from throughout its range was 4.38–4.74%. There was no intraspecific variation in *R. helenae* and very low intraspecific variation (0.00–0.36 %) in *R. kio* from throughout its range (from

China to central Vietnam and including sequences from the type locality) in the same gene fragment.

Description of Holotype.—Adult female; body dorsoventrally compressed; head length 102% of head width; snout truncate in dorsal view, sloping in profile, with slight point on tip of snout visible in ventral view; canthus rostralis distinct, rounded; loreal region sloping, slightly concave; interorbital region very slightly convex; nostrils oval, slightly protuberant, without flap of skin laterally, much closer to tip of snout than eye; pupil horizontal, tympanum prominent externally, tympanic rim elevated relative to skin of temporal region, 82% of eye diameter; pineal ocellus absent; skin not co-ossified to forehead; vomerine teeth present in oblique groups, separated by a distance less than length of each group, touching anterior edge of choanae; choanae oval, at margins of roof of mouth; tongue attached anteriorly, deeply notched posteriorly; very weak supratympanic fold extending to just beyond level of axilla. Forelimbs relatively robust, relative length of fingers I < II < IV < III; tips of all fingers with well-developed disks with distinct circummarginal grooves, disks

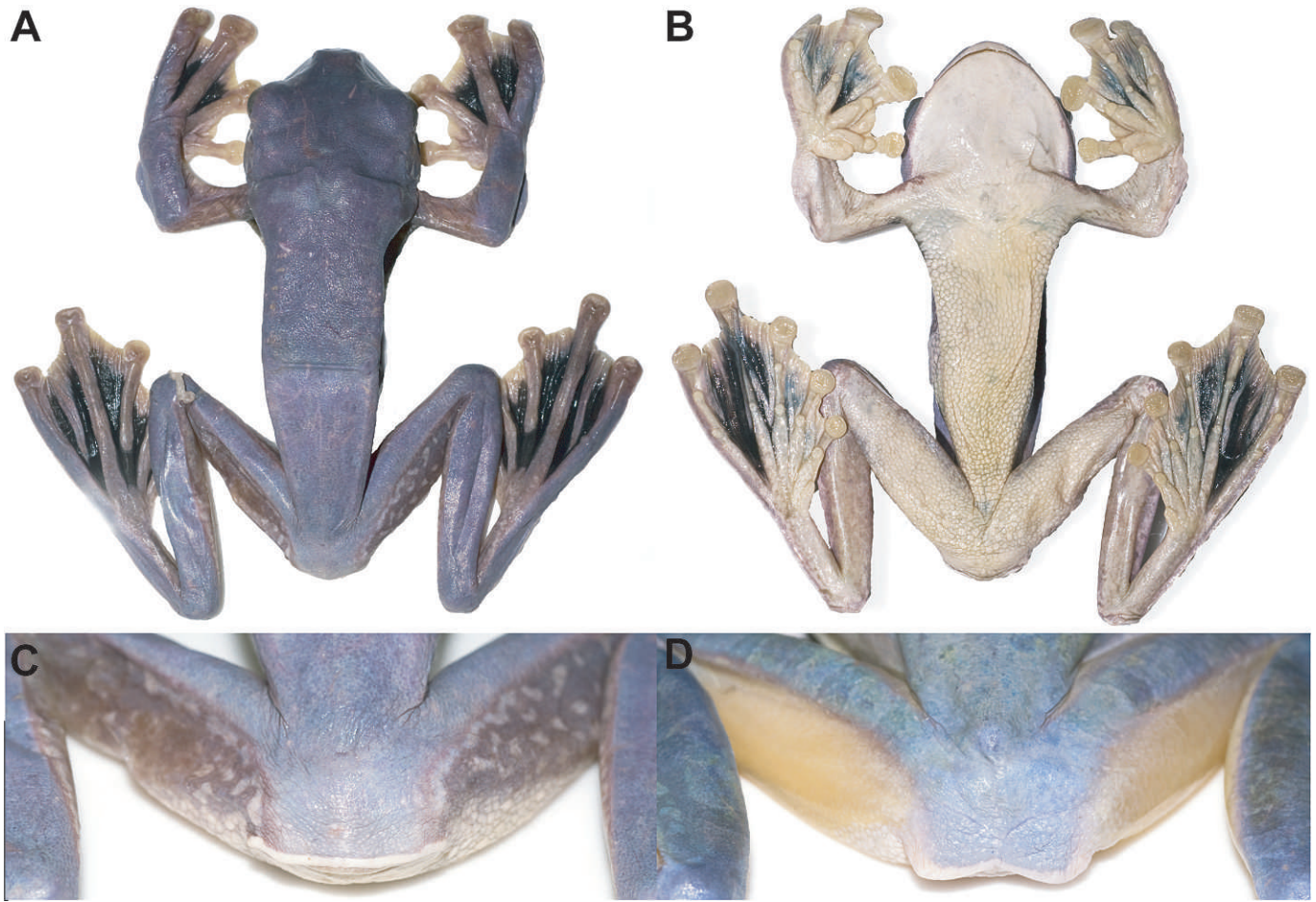


FIG. 4. Preserved adult female *Rhacophorus helenae* (holotype AMS R 173230) in dorsal (A) and ventral (B) views and a comparison of the outer thigh coloration and supraclacal dermal ridge in (C) *Rhacophorus helenae* (holotype AMS R 173230) and (D) *Rhacophorus kio* (AMS R 173453) in preservative. Color reproduction supported by the Thomas Beauvais Fund.

relatively wide compared to finger width (third finger disk 150% third finger width), third finger disk width subequal (99%) to tympanum diameter; webbing formula I $1\frac{1}{2}$ – $1\frac{1}{2}$ II 0 – 0 III 0 – 0 IV; subarticular tubercles prominent, rounded, formula 1, 1, 2, 2; palmar tubercle absent; accessory palmar tubercles absent; thenar tubercle absent; prepollex prominent, oval. Relative length of toes I < II < III < V < IV; tips of toes with well-developed disks with distinct circummarginal grooves; disks smaller than those of fingers; webbing complete, I 0 – 0 II 0 – 0 III 0 – 0 IV – 0 V; subarticular tubercles distinct, rounded, formula 1, 1, 2, 3, 2; inner metatarsal tubercle low, oval, 2.4 mm long; outer metatarsal tubercle and supernumerary tubercles absent. Dorsal skin smooth, ventral surface of thighs and belly coarsely granular, chest and throat smooth. Wide, smooth-edged dermal fringe along outer edge of forearm, narrower along tarsus; squarish projection at tibiotarsal articulation; low, single-lobed transverse supraclacal dermal ridge.

Measurements of Holotype.—SVL 90.7, HDL 30.3, HDW 29.6, SNT 6.1, EYE 8.1, IOD 10.3, TMP 6.7, TEY 1.6, IN 6.8, NS 6.1, EN 8.7, TIB 41.1, ML 26.4, PL 40.6, IML 2.4.

Coloration of Holotype in Life.—Dorsal surface uniformly green; flanks flecked with pale yellow; large (12.6 mm long) inky black patch in the axilla; dermal appendages on arms and legs including tibiotarsal projection and supraclacal dermal ridge lined with white. Dorsal surface of webbing between fingers I

and II pale green and between fingers II and IV black with pale green distally; pale yellow at very distal edges of webbing between fingers I and III. Dorsal surfaces of webbing between all toes proximally black and distally greenish; slight pale yellow at very distal edges of webbing between toes I and III. Ventral surface of throat, chest, and belly immaculate white, slight pinkish tinge posteriolaterally; ventral surfaces of hands and feet pinkish. Flanks, upper arms, ventral surface of lower arms, and anterior and posterior surface of thighs bluish-green speckled-marbled with pale yellow. Iris yellowish-gold with a sparse network of faint, dark-gold reticulations; iris periphery black; sclera white.

Coloration of Holotype in Preservative.—As in life, but with green fading to violet and pale yellow speckling/marbling fading to white.

Variation.—Based upon the type series, the new species is sexually dimorphic with females being larger than males (Table 2). Small white spots are present on the dorsum in all paratypes; UNS 00450 has sparsely scattered faint white specks (<0.5 mm) on entire dorsum and a single white spot on ~1.5 mm of toe V on the right foot; UNS 00451 has single white spot ~1.5 mm diameter on right side of mid-body; AMS R 176399 has four white spots <2 mm diameter on the body, thigh, and shank; ZFMK 92544 has five white spots on thigh and shank, <4 mm diameter. The extent of black markings varies slightly among

TABLE 2. Morphometric measurements (mm) of *Rhacophorus helena* holotype (HT) and paratypes (PT) and *Rhacophorus kio* specimens examined in this study.^a

Voucher number	Sex	SVL	HDL	HDW	SNT	EYE	IOD	TMP	TEY	IN	NS	EN	TIB	ML	PL	IML	HDW: HDL	HDL: HDW	TIB: SVL	HDL: SVL	TMP: EYE	Weight (g)
<i>Rhacophorus helena</i>																						
AMS R 173230	F	90.7	30.3	29.6	14.8	8.1	10.3	6.7	1.6	6.8	6.1	8.7	41.1	26.4	40.6	2.4	0.98	1.02	0.45	0.33	0.82	32.0
UNS 00450	F	89.4	27.9	29.6	13.8	7.4	11.9	6.4	1.8	6.5	6.2	7.6	42.7	28.3	43.1	5.4	1.06	0.94	0.48	0.31	0.86	34.5
AMS R 176399	M	75.4	25.1	24.5	11.9	7.7	7.8	5.7	1.6	5.9	5.2	6.8	35.4	22.9	33.5	3.5	0.98	1.02	0.47	0.33	0.74	15.5
UNS 00451	M	72.3	25.4	24.2	12.4	7.1	8.7	5.4	1.6	5.6	6.0	7.4	33.5	22.7	34.0	3.4	0.95	1.05	0.46	0.35	0.76	15.0
ZFMK 92544	M	85.5	27.4	28.5	14.1	7.7	8.8	5.6	1.8	6.7	6.7	7.8	39.2	26.2	38.9	4.3	1.04	0.96	0.46	0.32	0.73	23.0
<i>Rhacophorus kio</i>																						
AMS R 173453	F	84.6	28.5	28.3	13.9	7.4	8.2	5.4	2.4	6.8	6.6	8.0	39.3	29.5	40.9	3.8	0.99	1.01	0.46	0.34	0.74	—
FMNH 252380	F	84.2	28.7	27.9	13.7	7.6	8.7	5.8	1.4	6.8	5.6	7.7	42.5	28.1	39.9	4.1	0.97	1.03	0.50	0.34	0.76	—
FMNH 252382	F	83.2	28.1	28.0	13.8	6.9	8.3	4.4	2.5	6.9	5.9	7.6	42.7	28.7	42.5	4.3	1.00	1.00	0.51	0.34	0.64	—
FMNH 252383	F	88.9	27.0	28.3	13.1	7.3	8.3	5.1	2.1	6.6	5.4	7.4	42.5	30.0	42.0	3.2	1.05	0.96	0.48	0.30	0.70	—
FMNH 252386	F	83.7	27.0	26.6	12.7	7.0	10.4	4.7	2.0	6.7	5.3	7.9	43.6	26.9	41.7	3.1	0.99	1.02	0.52	0.32	0.67	—
FMNH 255304	F	86.4	27.4	28.6	13.3	7.4	10.9	4.6	2.0	7.1	5.7	7.0	44.9	28.3	40.7	4.2	1.04	0.96	0.52	0.32	0.62	—
FMNH 252377	F	86.5	28.5	28.9	13.9	7.9	9.2	4.8	2.2	6.2	5.9	6.9	44.0	30.0	43.3	3.5	1.02	0.98	0.51	0.33	0.61	—
FMNH 252378	F	82.6	28.5	28.3	13.5	8.1	9.0	4.3	2.3	6.2	6.1	7.4	43.8	—	45.6	4.3	1.00	1.00	0.53	0.34	0.54	—
FMNH 252379	F	86.5	28.1	28.8	13.4	7.7	8.2	5.2	2.4	7.2	5.1	7.4	45.3	30.6	44.4	4.1	1.03	0.97	0.52	0.32	0.67	—
NCSM 77851	F	~78	28.0	28.2	13.1	7.7	—	4.9	1.5	6.5	5.5	6.9	40.9	—	—	3.1	1.01	0.99	0.52	0.36	0.64	—
AMS R 173451	M	66.6	23.0	20.9	10.5	6.5	5.7	4.9	1.1	5.5	4.8	6.1	30.6	20.9	31.4	2.6	0.91	1.10	0.46	0.35	0.76	—
AMS R 173452	M	69.1	24.2	22.4	11.5	7.4	6.5	4.1	1.8	6.3	4.8	6.3	33.1	21.8	31.2	3.7	0.93	1.08	0.48	0.35	0.55	—
FMNH 252381	M	69.5	24.3	22.7	10.6	7.0	6.6	4.2	2.0	5.4	5.1	5.6	34.8	22.2	33.2	2.9	0.93	1.07	0.50	0.35	0.59	—
FMNH 252384	M	66.9	21.2	21.7	10.0	7.1	7.5	4.1	1.5	5.1	4.4	5.8	32.8	22.4	31.9	2.9	1.02	0.98	0.49	0.32	0.57	—
FMNH 255305	M	69.2	22.9	21.5	11.3	6.3	8.5	3.3	1.7	5.7	4.8	6.3	33.4	21.8	32.0	2.8	0.94	1.07	0.48	0.33	0.52	—

^aSVL = snout-vent length; HDL = head length from tip of snout to rear of jaws; HDW = head width at the commissure of the jaws; SNT = snout length from tip of snout to the anterior corner of eye; EYE = diameter of the exposed portion of the eyeball; IOD = interorbital distance; TMP = horizontal diameter of tympanum; TEY = distance from anterior edge of tympanum to posterior corner of the eye; IN = intermarial space; NS = distance from nostril to tip of snout; EN = distance from front of eye to nostril; TIB = tibia length with the hindlimb flexed; ML = manus length from tip of third digit to base of tubercle on prepollex; PL = pes length from tip of fourth toe to base of the inner metatarsal tubercle; IML = length of inner metatarsal tubercle; weight measured in life.

individuals, with black on webbing of feet absent between toes I–II in AMS R 176399, UNS 00450, and ZFMK 92544. Distal margins of webbing (~2 mm) are pale yellow in males and pale green in females. Males have nuptial pads on the outside margins of the prepollex and finger I, slit-like vocal sacs openings at the edge of mouth opening, medial subgular vocal sac, and slightly loose skin in the gular region.

Distribution and Natural History.—*Rhacophorus helenae* is known only from Nui Ong Nature Reserve (Binh Thuan Province) and Tan Phu Forest (Dong Nai Province), two fragments of low-elevation, disturbed, mixed-forest in a highly modified agricultural landscape in southern Vietnam. The two known localities are approximately 30 km apart and are isolated from each other by agricultural land. The actual distribution of the new species is unknown but likely extends into other fragments of lowland forests in southern Vietnam and possibly into extreme eastern Cambodia (although no large, green rhacophorids have been recorded from Cambodia; Neang and Holden, 2008).

In contrast, re-examination of *R. kio* specimens and molecular data from throughout its range confirms that this species has a range encompassing over 1,000 km in longitude and ~900 km in latitude (Fig. 1). Throughout central and northern Vietnam, Laos, and southern China, *R. kio* inhabits forests at 200–1,800 m elevation (Orlov et al. 2002). *Rhacophorus kio* and *R. helenae* appear to have allopatric distributions. To our knowledge, neither species has been recorded from the ~380-km gap between the collection sites of the new species in southern Vietnam and the southernmost record of *R. kio* in Gia Lai Province in central Vietnam (Fig. 1).

Rhacophorus helenae was observed in small numbers at Tan Phu Forest in September (2008). Breeding pairs were observed in amplexus in trees above temporary pools at this site, but no calling was heard. A typical foam nest (sensu Altig and McDiarmid, 2007) was observed in the same tree, adjacent to the specimens collected, and was likely deposited by *R. helenae*. In addition, UNS 00450 deposited a foam nest overnight whilst in a plastic bag; eggs within the nests were small and unpigmented. During surveys at Tan Phu Forest in May (2008), no *R. helenae* were observed. In ten survey nights at Nui Ong Nature Reserve also in May (2008), only one *R. helenae* (the holotype) was observed.

Etymology.—The specific epithet is in honor of Mrs. Helen M. Rowley for her valuable support.

DISCUSSION

Re-examination of *Rhacophorus kio* specimens and molecular data from Vietnam, Laos, and southern China suggests that *R. helenae* is restricted to a highly fragmented habitat in southern Vietnam that is under increasing human pressures. Both collection localities for *R. helenae* are <120 km from the center of Ho Chi Minh City (<90 km for the Tan Phu Forest locality), a city with a population of over 7 million (GSO, 2009). Although the protected areas are separated by as little as 12 km, the habitat in-between is densely populated and presents a barrier to any potential dispersal for *R. helenae*.

Lowland forests are some of the most threatened habitats in the world, suffering high levels of anthropogenic disturbance due to their accessibility (Curran et al., 2004, Hansen et al., 2009). In southern Vietnam, lowland forests have been particularly degraded over the last century (Durand, 1994). It is likely that *R. helenae* was once widespread over southern Vietnam but now persists in small fragments of remaining

lowland forest. The continued survival of *R. helenae* is threatened by further habitat loss and degradation due to encroachment (e.g., livestock grazing and collection of forest products) and habitat isolation. Given the likely, relatively small range of the new species, the high human population density within that range, and the highly and increasingly fragmented state of lowland forests in the area, we recommend that the new species be considered Threatened following IUCN's Red List categories (IUCN, 2001).

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APPENDIX 1

Additional Specimens Examined

- Rhacophorus annamensis*.—Vietnam, Quang Nam Province, Song Thanh Nature Reserve, AMS R 171793–171800; Cambodia, Mondulkiri Province, Seima Biodiversity Conservation Area, AMS R 174027–174030.
- Rhacophorus exochopygus*.—Vietnam, Quang Nam Province, Song Thanh Nature Reserve, AMS R 171788–171792.
- Rhacophorus feae*.—Vietnam, Kon Tum Province, Ngoc Linh Nature Reserve, AMS R 173809; Vietnam, Nghe An Province, Pu Hoat Proposed Nature Reserve, AMS R 173194–173197.
- Rhacophorus kio*.—Vietnam, Nghe An Province, Pu Hoat Proposed Nature Reserve, AMS R 173451–173453; Vietnam, Gia Lai Province, FMNH 252377–252384, FMNH 252386; Laos, Khammouane Province, FMNH 255304; Laos, Houaphan Province, FMNH 255305; Laos, Sekong Province, NCSM 77851.
- Rhacophorus orlovi*.—Vietnam, Nghe An Province, Pu Mat National Park, AMS R171731–171735; Vietnam, Nghe An Province, Pu Hoat Proposed Nature Reserve, AMS R 173199.
- Rhacophorus reinwardtii*.—Indonesia, Java, Bogor, AMS R 6856.
- Rhacophorus rhodopus*.—Vietnam, Binh Thuan Province, Nui Ong Nature Reserve UNS00417/AMS R 173325, UNS00418/AMS R 173326, AMS R 173327–173328, UNS00419/AMS R 173329.
- Rhacophorus vampyrus*.—Vietnam, Lam Dong Province, Bidoup-Nui Ba National Park AMS R 173127 (holotype), AMS R 173126, UNS 00103/AMS R 173128, UNS 00104/AMS R 173129, NCSM 77318, UNS 00105, ZFMK 91076, AMS R 173507 (paratypes).