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A New Species of *Buergeria* From the Southern Ryukyus and Northwestern Taiwan (Amphibia: Rhacophoridae)

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Abstract: *Buergeria japonica*, long thought to be a single species widely occurring on the Ryukyu Archipelago and Taiwan, proved to include three genetically differentiated clades, (1) the Northern and Central Ryukyu, (2) the Southern Ryukyu and Northern Taiwan, and (3) the Southern Taiwan clades. The Southern Taiwan clade has already been split from the others as a distinct species. A distinct heterospecific relationship of the Southern Ryukyu and Northern Taiwan clade from the Northern and Central Ryukyu clade was also clear from genetic evidence. Morphological comparison between specimens from the Yaeyama (the Southern Ryukyu and Northern Taiwan clade) and Amamioshima, the type locality of *B. japonica* (the Northern and Central Ryukyu clade), confirmed genetic differences and corroborate their independent species status. Thus, we describe the Northern Taiwan and Southern Ryukyu clade as *B. choui* sp. nov.

Key words: *Buergeria choui* sp. nov.; Mitochondrial DNA phylogeny; Morphometry; Northern Taiwan; Southern Ryukyu

INTRODUCTION

Buergeria japonica (Hallowell, 1861) was long considered a single, wide-ranging species occurring from the Tokara Islands of the Northern Ryukyus through the Central and the Southern Ryukyus, Japan, to Taiwan (Okada, 1931; Nakamura and Uéno, 1963; Maeda and Matsui, 1999). However, Tominaga et al. (2015) studied the phylogeography of this species across its entire distribu-

tional range, and recognized three major clades in it: (1) the Southern Taiwan, (2) the Northern Taiwan+Southern Ryukyu, and (3) the Central+Northern Ryukyu clades. Genetic distances found among these three clades (12.8–17.3% in cytochrome b [cyt b] and 5.2–6.9% in 16S rRNA) are regarded as quite large compared with reported values among various congeneric anuran species, and each of these clades was thought to represent a taxonomically independent species (Fig. 1).

Subsequently, the Southern Taiwan clade was split from the combined Central+Northern Ryukyu, and Northern Taiwan+Southern

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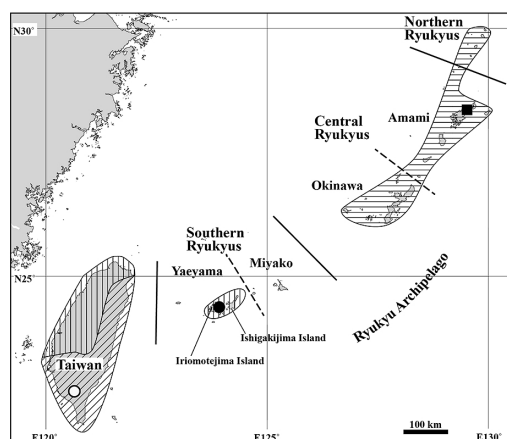


FIG. 1. Map of the Ryukyu Archipelago and Taiwan showing type localities and distributional ranges of *Buergeria choui* sp. nov. (closed circle and vertically hatched area, respectively); *B. japonica* (closed square and horizontally hatched area, respectively); *B. sp. ST* (open circle and obliquely hatched area, respectively).

Ryukyu clades as a distinct species *Buergeria otai* by Wang et al. (2017, 2018). However, this name is unfortunately not available taxonomically in the light of the International Code of Zoological Nomenclature (4th edition available at: <https://www.iczn.org/>), because no Zoobank ID was given in Wang et al. (2017), while neither type-fixation nor diagnosis was given in Wang et al. (2018). We admit the Southern Taiwan clade surely represents a good species, but until the appropriate formal taxonomic process is followed, we defer to use the name. In the present paper, we call it as *Buergeria* sp. Southern Taiwan (*B. sp. ST*), instead.

On the other hand, the taxonomic status of the Central+Northern Ryukyu and the Northern Taiwan+Southern Ryukyu clades has not been clarified yet. Because the type locality of *B. japonica* is assigned to Amamioshima Island (Is.) of the Central Ryukyus (Stejneger, 1907), populations composing the Northern Taiwan+Southern Ryukyu clade require a formal description. In this article, we compare morphological and acoustic characteristics of the Central

+Northern Ryukyu and the Northern Taiwan +Southern Ryukyu clades so as to formally describe the latter clade as a distinct species.

MATERIALS AND METHODS

We followed Tominaga et al. (2015) to classify samples of *B. japonica* based on their original localities. For specimens preserved in 70% ethanol and stored at the Ryukyu University Museum (Fujukan) (RUMF), the Faculty of Education, University of the Ryukyus (URE), and 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) snout–nostril length (S–NL); 4) nostril–eyelid length (N–EL); 5) snout length (SL); 6) eye length (EL, including eyelid); 7) tympanum diameter (TD); 8) head width (HW); 9) internarial distance (IND); 10) interorbital distance (IOD); 11) upper eyelid width (UEW); 12) lower arm and hand length (LAL); 13) third finger length (3FL), from the base point between second and third fingers to the tip; 14) hindlimb length (HLL); 15) tibia length (TL); 16) foot length (FL); and 17) inner metatarsal tubercle length (IMTL). All measurements were made to the nearest 0.1 mm with dial calipers, and using a binocular dissecting microscope, when necessary.

We compared SVL by the Tukey–Kramer test, while we performed Dunn’s multiple comparison test for ratio values and detection of the presence or absence of differences in the frequency distributions. Significance level of 5% was used in all statistical tests. All statistical analyses were performed by R 3.4.2 (R Core Team, 2017). In the following description, average values are shown with 1 standard deviation (\pm SD).

For examining overall morphological variation among each sex of three congeners, we also conducted multivariate analyses using \log_{10} -transformed metric values of all the 13 characters. We conducted canonical discriminant analysis (CANDISC) using the statistical

package of SAS (SAS, 2009).

For larvae preserved in 5% formalin, the following 14 measurements were taken to the nearest 0.1 mm using a binocular dissecting microscope equipped with a micrometer: (1) total length; (2) head-body length; (3) maximum head-body width; (4) maximum head-body depth; (5) eye-snout distance; (6) eyeball diameter; (7) interorbital distance; (8) snout-spiracle opening distance; (9) oral disk width; (10) tail length; (11) maximum tail depth; (12) maximum tail width; (13) maximum tail muscle depth; and (14) upper fin depth at middle of tail. For staging, we followed Gosner's (1960) table.

We recorded frog calls in the field using a digital recorder (Olympus LS-11) with an external Microphone (Olympus Compact Gun Microphone ME31) at 44.1 kHz/16 bits as uncompressed wave files, and analyzed recordings with Raven Lite 2.0 for Mac OS X on a Macintosh computer. We obtained temporal data from the oscillogram and frequency information from the audiospectrograms using Fast Fourier Transformation (1,024-point Hanning window). Definitions of acoustic parameters follow Matsui (1997) and Matsui and Dehling (2012).

Kuramoto (1986) tentatively classified calls of *B. japonica* (sensu lato) from Taiwan into three types; short trills, long trills, and whistles, that are distinct from each other. Of these, long trills emitted by frogs from Kuishanli (from the range of the Northern Taiwan + Southern Ryukyu clade of *B. japonica*) include two phases, the first of which is identical with the short trill, and quite different from long trills of Hualien (the Southern Taiwan clade, *B. sp. ST*), which are composed of short trills. Wang et al. (2017) reported a long trill unique to *B. sp. ST* (the Southern Taiwan clade), and use this as a diagnostic characteristic to split the Southern Taiwan clade, *B. sp. ST*, from the combined Northern and Central Ryukyu, and the Southern Ryukyu and Northern Taiwan clades of *B. japonica*. Thus, we studied the long trill.

RESULTS

Morphology

In all the Yaeyama (representing the Southern Ryukyu and Northern Taiwan clade), Amami (representing the Northern and Central Ryukyu clade), and *B. sp. ST* samples, females were significantly larger than males (32.6 ± 1.7 [30.2–35.8] mm [$n=10$] vs. 27.2 ± 1.1 [25.3–29.1] mm [$n=11$] in Yaeyama, 35.2 ± 2.2 [31.6–39.5] mm [$n=10$] vs. 26.7 ± 0.7 [25.5–27.7] mm [$n=10$] in Amami, and 33.9 ± 2.6 [29.2–36.9] mm [$n=10$] vs. 26.7 ± 2.2 [22.6–29.5] mm [$n=10$] in *B. sp. ST*) (Table 1).

Among samples from Yaeyama, Amami, and *B. sp. ST*, statistically significant differences were found in many combinations. In males, the three samples did not differ in SVL, but Yaeyama and Amami differed in RHL, RIND, REL, RTD, RLAL, RTL, and RHLL. Yaeyama and *B. sp. ST* differed in RHL, RHW, RSL, RTL, and RUEW. Males of Amami and *B. sp. ST* differed in RHL, RUEW, REL, RTD, RHLL, and RIMTL. In females, Yaeyama differed from Amami in SVL, RHW, RTD, RNEL, and R3FL. Yaeyama differed from *B. sp. ST* in RHW, RIOD, RUEW, RTD, RLAL, RFL, and RHLL. Amami and *B. sp. ST* were different in RLAL, RTL, RFL, RHLL, and R3FL.

Results of CANDISC of males revealed that Yaeyama and *B. sp. ST* were completely separated from Amami by the first and second axes (Fig. 2A). Yaeyama and *B. sp. ST* were separated from each other by the second axis, but they largely overlapped with each other by the third axis. The eigenvalues of the first (CAN1) and second (CAN2) axes accounted for 8.331 (proportion: 0.678) and 3.964 (proportion: 0.322), respectively. On the first axis, the highest absolute magnitude of the standardized canonical discriminant coefficients was -1.92 of SVL, followed by HL (1.88), TL (1.66), HLL (1.40), and LAL (-1.16). For the second axis, TL (2.74), HL (-1.48), HW (1.37), FL (-1.14), and UEW (-1.06) were high contributors.

TABLE 1. Measurements in three species of *Buergeria*. SVL (mean \pm SD, in mm) and medians of ratios (R) of other characters to SVL, followed by ranges in parentheses. See text for character abbreviations.

	Females				Males			
	Yaeyama (n = 10)	Amamioshima (n = 10)	B. sp. ST (n = 10)		Yaeyama (n = 11)	Amamioshima (n = 10)	B. sp. ST (n = 10)	
SVL	32.6 \pm 1.7	35.2 \pm 2.2	33.9 \pm 2.6	(29.2–36.9)	27.2 \pm 1.1	26.7 \pm 0.7	26.7 \pm 2.2	(22.6–29.5)
RHL	35.8	37.1	36.0	(34.5–38.4)	35.7	41.4	37.1	(34.9–40.0)
RHW	33.3	35.4	35.7	(34.4–36.7)	32.0	34.1	35.5	(32.8–38.3)
RIND	8.2	8.7	8.2	(6.8–10.7)	7.6	9.2	8.9	(6.2–10.2)
RIOD	7.3	7.8	8.3	(6.5–10.2)	7.6	8.5	8.0	(7.0–9.4)
RUEW	10.7	9.8	9.7	(9.0–10.6)	11.6	11.8	10.9	(9.4–11.1)
RSL	15.3	15.6	16.1	(14.9–17.8)	15.1	15.8	16.7	(14.4–17.9)
REL	15.5	15.3	16.1	(13.7–17.5)	16.3	19.0	16.8	(15.2–18.0)
RTD	7.2	8.1	6.2	(5.6–8.0)	7.4	8.6	7.2	(5.6–9.0)
RS-NL	7.0	7.6	7.7	(6.3–8.2)	7.4	8.1	8.1	(7.1–9.0)
RN-EL	7.1	7.7	7.8	(5.4–8.6)	7.9	8.0	8.3	(6.7–9.1)
RLAL	48.6	49.7	46.5	(44.6–47.9)	47.3	49.9	48.2	(47.2–51.1)
RTL	62.4	64.4	61.0	(59.1–63.3)	58.5	63.7	61.8	(56.2–66.5)
RFL	56.5	57.5	52.6	(50.9–55.8)	55.8	57.3	55.7	(49.1–58.2)
RHLL	192.5	197.5	189.0	(181.6–195.3)	187.1	198.9	189.9	(173.9–200.0)
RIMTL	4.7	5.2	5.2	(4.3–6.1)	5.1	4.7	5.3	(4.5–6.3)
R3FL	21.1	21.0	18.4	(16.4–21.1)	19.2	20.5	19.2	(16.1–21.6)

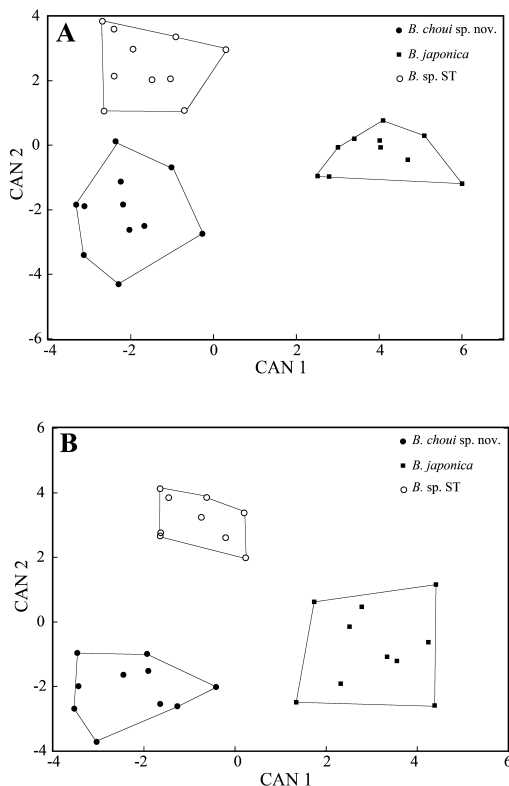


FIG. 2. Plot of first against second canonical variates from CANDISC for male (A) and female (B) samples of *Buergeria*. Closed circles: *Buergeria choui* sp. nov.; Closed squares: *B. japonica*; Open circles: *B. sp. ST*.

In females, results of CANDISC revealed that the three taxa were separated from each other by the first and second axes (Fig. 2B). The eigenvalues of the first (CAN1) and second (CAN2) axes accounted for 5.901 (proportion: 0.525) and 5.344 (proportion: 0.475), respectively. On the first axis, the highest absolute magnitude of the standardized canonical discriminant coefficients was -3.41 of SVL, followed by HW (2.99), TL (2.01), HLL (-1.39), and FL (1.37). HW (2.61), LAL (-1.97), SVL (-1.45), and IOD (1.15) were high contributors for the second axis.

In this way, samples of the Yaeyama (the Southern Ryukyu and Northern Taiwan clade), Amami (Northern and Central Ryukyu

clade), and *B. sp. ST* were morphologically clearly separated.

Acoustics

As already noted for *B. japonica* from Amamioshima (Kuramoto, 1975) and Taiwan (Kuramoto, 1986), and for *B. sp. ST* (Wang et al., 2017), our recordings of *B. japonica* (sensu lato) exhibited various types of calls that are not easy to compare. As stressed by Wang et al. (2017), frogs of the Ryukyus and the northwestern Taiwan (Japanese clade) have one kind of long trill (Type 1A) and lack Type 1B and Type 2 of frogs from eastern and southern Taiwan (Taiwanese clade). We thus mainly studied long trills of Yaeyama (representing the Southern Ryukyu and Northern Taiwan clade) and Amami samples (representing the Northern and Central Ryukyu clade), but parameters (call duration, number of pulses per call, pulse rate, and upper dominant frequency) of long trills greatly overlapped between the two samples and could not be used to differentiate them.

SYSTEMATICS

Buergeria choui sp. nov.

[Japanese name: Yaeyama-Kajika-Gaeru]

[English name: Yaeyama Kajika Frog]

(Figs. 3 and 4)

Polypedates japonicus: Van Denburgh, 1912, p. 205 (part); Okada, 1930, p. 192 (part); Okada, 1931, p. 207 (part); Okada, 1966, 178 (part).

Rhacophorus japonicus Inger, 1947, p. 346 (part); Nakamura and Uéno, 1963, p. 67 (part).

Buergeria japonica: Utsunomiya, 1979, p. 168 (part).

Buergeria japonica Northern Taiwan + Southern Ryukyu (NT/SR) clade: Tominaga et al., 2015, p. 240.

Buergeria japonica Japanese clade: Wang et al., 2017, p. 7.

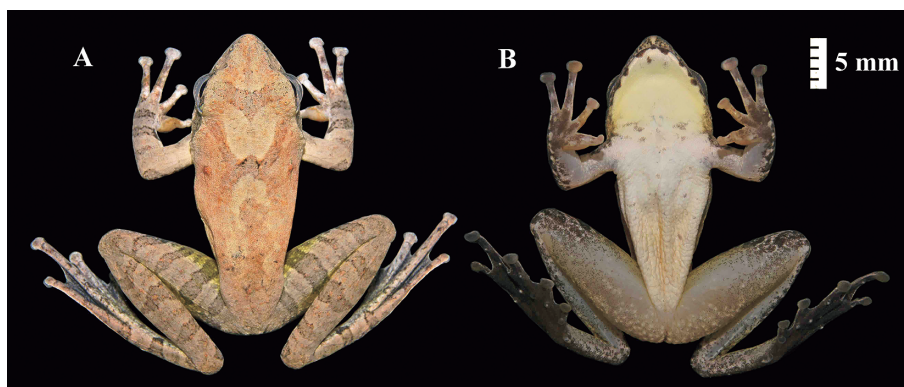


FIG. 3. Dorsal (A) and ventral (B) views of male holotype of *Buergeria choui* sp. nov. (RUMF-ZH-01032). Scale bar=5 mm.

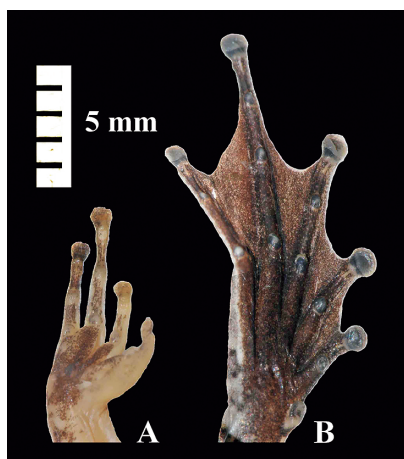


FIG. 4. Ventral view of right hand (A) and foot (B) of male holotype of *Buergeria choui* sp. nov. (RUMF-ZH-01032). Scale bar=5 mm.

Diagnosis

The new species is placed in *Buergeriinae* Channing, 1989, with distal end of the third metacarpal scarcely flattened, lacking a bony knob. It is assigned to a member of the genus *Buergeria* by having small body, becoming slender posteriorly; dorsum yellow to gray brown, never changing to green; pupil horizontally ellipsoid; tympanum evident; vomerine teeth degenerated; webs well developed among toes, but absent among fingers; digital disks with circummarginal grooves; tips of



FIG. 5. Dorsal (A), lateral (B), and ventral (C) views of a tadpole of *Buergeria choui* sp. nov. (RUMF-ZH-01032). Scale bar=5 mm.

distal digits T-shaped, not forked clearly; skin scattered with irregular tubercles without dorsolateral fold, but with supratympanic fold; ventral surface covered with rough circular tubercles; and a single vocal sac in males. *Buergeria choui* sp. nov. is very similar to *B. japonica* but is differentiated by morphometric characteristics and number and arrangements of dark spots on lower lips. It also is similar to *B. sp. ST* but differs in morphometric characteristics and markings on the thighs. Also, *B. choui* sp. nov., as well as *B. japonica*, lacks a long call unique to *B. sp. ST*. See *Comparisons* below for further details.

Etymology

The specific name is dedicated to Dr. Wen-Hao Chou, Professor of the National Museum of Natural Science, Taichung, Taiwan, who first paid attention to the variation and taxonomy of *B. japonica* (sensu lato) at the beginning of 1990s.

Holotype

Ryukyu University Museum (Fujukan) (RUMF-ZH)-01032 (former URE 5215), an adult male collected from Mihara, Taketomi-cho, Iriomotejima Is., Okinawa Pref., Japan (24°20'35.3" N, 123°55'19.4" E, 108 m asl) by A. Tominaga on 22 June 2019.

Paratypes

Iriomotejima Is., Okinawa Pref., Japan: RUMF-ZH-01033-01034 (former URE 361–362), a male and a female from Haimi, Taketomi-cho on 17 September 2011 by A. Tominaga; RUMF-ZH-01035 (former URE 395) a female from Airagawa, Taketomi-cho on 17 September 2011 by A. Tominaga; URE 369-371, three males from Shirahama, Taketomi-cho on 17 September 2011 by A. Tominaga. URE 1279, a male collected from Komi, Taketomi-cho (24°20'20.8" N, 123°54'58.7" E, 21 m asl) by J. Jiang and T. Kurita on 20 September 2012.

Referred specimens

Ishigakijima Is., Okinawa Pref., Japan: URE 364 a male from Banna-dake, Ishigaki-shi; URE 410, 411, 414, 416, 421, 422, four males and two females, Entrance to Mt. Omoto-dake on 19 September 2011 by A. Tominaga; URE 423, a female from Nosoko, Ishigaki-shi on 19 September 2011 by A. Tominaga; URE 1314, a female collected from Maesato, Ishigaki-shi (24°24'59.1" N, 124°11'29.6" E, 136 m a.s.l.) by A. Tominaga and J-P. Jiang on 22 September 2012.

Taiwan: KUHE 5155–5158, four specimens from Wushe, Ren-ai Township, Nantou County, on 28 July 1976 by T. Hikida; KUHE 5664–5677, 5898, 5899, 16 specimens from Lushan Hot Spring, Ren-ai Township,

Nantou County, on 26 July 1976 by T. Hikida.

Description of holotype (measurements in mm)

Snout-vent length (SVL) 28.0; habitus moderate (Fig. 3); head nearly as long (HL 9.9, 35.4%SVL) as broad (HW 10.0, 35.7%SVL); snout truncate without median projection, oblique in profile, projecting beyond lower jaw; eye length (EL 4.5, 16.1%SVL) nearly as long as snout length (SL 4.6, 16.4%SVL); canthus slightly rounded, constricted; lore vertical, slightly concave; nostril lateral, below canthus, nearer to eye (N-EL 1.9, 6.8%SVL) than to tip of snout (S-NL 2.3, 8.2%SVL); internarial distance (IND 2.5, 8.9%SVL) slightly wider than interorbital distance (IOD 2.2, 7.9%SVL), latter much narrower than upper eyelid (UEW 3.1, 11.1%SVL); pineal spot absent; tympanum conspicuous, oval, less than half length of eye (TD 2.1, 7.5%SVL); vomerine teeth in two very small groups; tongue oval, deeply notched posteriorly, without papillae; slit-like vocal openings on floor of mouth well anterior to jaw commissure on both sides.

Forelimb moderate (17.8, 63.6%SVL); fingers without web; finger length formula: I<II<IV<III (Fig. 4A); length of first, measured from distal edge of inner palmar tubercle (1FL 2.5, 8.9%SVL) much shorter than length of eye; tips of fingers expanded, rounded, forming small pads with circummarginal grooves; disc of third finger (3FDW 0.95, 3.4%SVL) less than half of tympanum; fingers not webbed at bases; inner palmar tubercle small (IPTL 0.50, 1.8%SVL), oval, slightly smaller than outer palmar tubercle (OPTL 0.59, 2.1%SVL); subarticular tubercles obscure; no supernumerary metacarpal tubercles.

Hindlimb slender, very long (HLL 50.8, 181.4%SVL) nearly three times length of forelimb; tibia long (TL 15.9, 56.8%SVL), heels meeting when limbs are held at right angles to body; tibiotarsal articulation of adpressed limb reaching to point far beyond

snout; foot (FL 14.5, 51.8%SVL) shorter than tibia; toe length formula $I < II < III < V < IV$; fifth toe slightly longer than third; tips of toes expanded (5TDW 0.97, 3.5%SVL); webbing fleshy, formula: $I \ 1-1\frac{1}{3} \ II \ 1-2 \ III \ 1-2\frac{1}{3} \ IV \ 2-1 \ V$ (Fig. 4B); subarticular tubercles present, but indistinct; an oval inner metatarsal tubercle, length (IMTL 1.33, 4.7%SVL), much shorter than first toe (1TOEL 3.5, 12.5%SVL); no outer metatarsal tubercle.

Dorsal skin rough scattered with blunt, short tubercles and ridges; cruciform ridge between shoulders especially clear (Fig. 3A); no dorsolateral fold, but supratympanic fold evident; dorsal surface of limbs scattered with small, low warts; tarsus without dermal ridge; throat and chest smooth, abdomen covered with rough circular tubercles (Fig. 3B); nuptial pads on the first and second fingers, the first one much larger than the latter, covering medial surface from its base to level of subarticular tubercle; a median internal subgular vocal sac.

Color

In life, dorsally clay brown with dark brown interorbital bar followed by dark cruciform pattern (Fig. 3A); another less clear brown marking from mid trunk to above cloaca; a blackish brown band from eye and surrounding tympanum; dark crossbars on dorsal surface of limbs; throat creamy yellow and chest creamy lightly tinged with brown; abdomen cream sparsely scattered with dark brown (Fig. 3B); ventral side of thigh scattered with light brown anteriorly, and white patches posteriorly; iris golden. In alcohol, color pattern slightly faded.

Variation

Variation in size and body proportions is given in Table 1. As shown above, females are significantly larger in SVL than males. Ground body color highly variable, from dorsum yellowish brown and grayish brown, to deep brown, but the marking is not much variable.

Larva

A total of five tadpoles of stages 35–36 (total length=26.4–30.6 [mean \pm SD=28.6 \pm 1.6] mm, head-body length=7.9–8.9 [8.4 \pm 0.5] mm) were closely examined. Head and body oval, slightly flattened above and below; width maximum at level of spiracle, 65–72 (median=68)% of head-body length; depth 75–82 (median=77)% of head-body width; snout rounded dorsally and in profile; eyes dorsolateral, not visible from below, eyeball diameter 11–14 (median=12)% of head-body length; interorbital distance moderate, 146–211 (median=178)% of eyeball diameter, subequal to eye-snout distance; nostril open, dorsolateral, rim not raised, midway between eye and tip of snout; internarial 75–94 (median=81)% of interorbital. Oral disc anteroventral, emarginate, width 35–50 (median=39)% of body width. Marginal papillae on upper labium with wide medial gap and short thick papillae at corners, submarginal papillae present: lower labium with short, thick papillae in one row with some distinctively enlarged submarginal papillae, or in two rows near corners and in one row medially; labial teeth row formula 1:3+3/2:1+1; finely serrated jaw sheaths forming wide arches with narrow black margins, upper jaw sheath with large medial convexity; lower jaw sheath V-shaped. Spiracle sinistral, on side, directed posterodorsally, tube fused to body wall; snout-spiracle distance 60–84 (median=70) % of head-body length. Vent tube dextral, attached to ventral fin. Tail lanceolate, upper margin weakly convex, tapering gradually to pointed tip, lower margin nearly straight; tail long, length 226–265 (median=236) % of head-body length, maximum height 16–19 (median=18) % of tail length; caudal muscle taller than either fin in proximal half of tail; origin of dorsal fin posterior to junction of body and tail, dorsal fin higher than ventral fin except near tip of tail. Neuromasts traceable. No glands present. In life, grayish brown dorsally; caudal muscle mottled with dark transverse bands dorsally, without pigment ventrally

(Fig. 5). Iridophores scattered throughout the body, tail, fins and eyes.

Range

Japan, Southern Ryukyus: Iriomotejima Is., Ishigakijima Is.; Taiwan, Northwestern regions: Yilan (Yilan Stream), Taipei (Xindian Stream), Taoyuan (Dahan Stream), Hsinchu (Taochien Stream), Miaoli (Zhonggang Stream), Taichung (Da-an Stream, Dajia Stream), Changhua (Dudu Stream), Nantou, Yunlin (Zhoushui Stream).

Natural history

Distributed widely from lowlands near seashore to mountain forests. Breeding season lasts long from March to November, when males are found calling near shallow pools, including slowly flowing streams and ditches. Eggs are laid scattered, and tadpoles hatched may spend their life in the water that may become very high in temperature as inferred from *B. japonica* (Komaki et al., 2016) and *B. sp. ST* (Chen et al., 2001).

Other frog species observed in Yaeyama immediately near the type locality were *Odorrana supranarina* (Matsui, 1994), *Odorrana utsunomiyaorum* (Matsui, 1994), *Nidirana okinavana* (Boettger, 1895), *Fejervarya sakishimensis* Matsui, Toda and Ota, 2007, *Kurixalus eiffingeri* (Boettger, 1895), *Rhacophorus owstoni* (Stejneger, 1907), *Polypedates leucomystax* (Gravenhorst, 1829), and *Microhyla okinavensis* Stejneger, 1901.

Calls

As stated above, parameters of the long trills greatly overlapped between *B. choui* sp. nov. from Yaeyama and *B. japonica* from Amami samples (Table 2). Calls of *B. choui* sp. nov. were recorded at Taketomi-cho, Iriomotejima Is. on 23 June 2019. Air temperature at the time of recording was 28.5°C. Calls were successively emitted and consisted of various combinations of notes with various intervals, from a very short one including one pulse, through short trills or whistles, to long trills. The long trill (Fig. 6) lasted 1.0–2.3

(mean \pm SD = 1.7 ± 0.4 , $n=17$) s and consisted of a series of 34–71 (53.5 ± 11.6 , $n=17$) pulses. Each pulse was emitted at an interval of 26–48 (33.3 ± 0.4 , $n=47$) ms, and the pulse repetition rate was 29.8–32.8 (31.1 ± 0.9 , $n=17$) per s. The frequency was spread over the 1.0–4.5 kHz range and the second dominant frequency was 3.4–3.6 (3.51 ± 0.11 , $n=17$) kHz. Harmonics were slightly clear and intensity modulation was weak.

Comparisons

Other than *B. japonica* and *B. sp. ST*, *B. choui* sp. nov. with male SVL of 25–29 mm and female SVL of 30–36 mm is characterized by much smaller body than the congeneric members: 37–44 mm in males and 49–69 mm in females in *B. buergeri* (Temminck and Schlegel, 1838) from Japan mainland; 34–38 mm in males and 60–68 mm in females in *B. oxycephala* (Boulenger, 1900 [1899]) from Hainan Is.; and 42–67 mm in males and 59–76 mm in females in *B. robusta* (Boulenger, 1909) from Taiwan (Matsui and Maeda, 2018; Fei et al., 2012).

Buergeria choui sp. nov. is very similar to *B. japonica* in morphology, but they are differentiated as follows: in males, *B. choui* sp. nov. is smaller than *B. japonica* in RHL, RIND, REL, RTD, RLAL, RTL, and RHLL, and in females, *B. choui* sp. nov. is smaller than *B. japonica* in SVL, RHW, RTD, and RNEL, but larger in R3FL. Additionally, they differ in the arrangements of dark markings on edges of lower lips. *Buergeria choui* sp. nov. has 9–18 (mean \pm SD = 13.67 ± 3.14 , $n=6$) dark spots, while *B. japonica* has 7–10 (8.33 ± 1.00 , $n=9$) spots that are arranged with wider space in between. Larval labial teeth row formula, 1:3+3/2:1+1 of *B. choui* sp. nov. also differs from that of *B. japonica* (1:4+4/2:1+1).

Buergeria choui sp. nov. is also similar to *B. sp. ST* from eastern and southern Taiwan (type locality: Donggang Stream, Pingdong County), but in males, it has smaller RHL, RHW, RSL, RTL, but larger in RUEW than *B. sp. ST*, and in females, it is smaller than

TABLE 2. Variation in long trills of *B. choui* sp. nov. from Iriomotejima, Ishigakijima, and Taiwan, and *B. japonica* from Amamioshima. Values are given in mean ± SD.

Locality	Air temperature	n	Call duration (s)	N of pulse/call	Pulse rate (/s)	Upper dominant frequency (kHz)
Iriomotejima	28.5C	17	1.75±0.40 (1.04–2.32)	53.5±11.6 (34–71)	31.1±0.9 (29.8–32.8)	3.51±0.11 (3.4–3.6)
Ishigakijima	26.7C	5	1.53±0.39 (0.95–1.98)	42.6±11.9 (27–56)	27.8±1.4 (26.3–29.6)	2.76±0.15 (2.5–2.9)
Kuishanli, Taiwan	25.0C*	8	2.14±0.21 (1.40–2.82)	70.6±6.9 (47–94)	32.1±0.3 (31.0–33.6)	3.13±0.09 (2.8–3.5)
Amamioshima	26.4C	5	1.92±0.21 (1.67–2.10)	55.0±6.7 (46–61)	28.1±1.2 (26.3–29.4)	3.02±0.01 (2.9–3.1)
Amamioshima	24.0C	6	1.55±0.36 (1.28–2.30)	36.1±7.9 (30–53)	23.4±0.8 (22.4–24.5)	3.09±0.03 (3.0–3.1)
Amamioshima	17.2C**	10	1.56±0.09 (1.22–2.07)	45.8±2.5 (35–60)	29.4±0.4 (28.0–32.0)	2.67±0.03 (2.6–2.8)

* Kuramoto (1986)
** Kuramoto (1975)

B. sp. ST in RHW, RIOD, but larger in RUEW, RTD, RLAL, RFL, and RHLL. In addition, *B. sp.* ST shows condensed, tiny, and white spots in a regular size and shape on the base of the thighs, in contrast to *B. choui* sp. nov., whose thigh is ventrally covered by irregular white patches with some variation in coverage ratio (Wang et al., 2017). *Buergeria* sp. ST is smaller than *B. japonica* in RHL, RUEW, REL, RTD, and RHLL, but larger in RIMTL in males, and is smaller than *B. japonica* in RLAL, RTL, RFL, RHLL, and R3FL in females.

Also, *B. choui* sp. nov. differs from *B. sp.* ST acoustically. The dominant frequency of *B. choui* sp. nov. was higher than that of *B. sp.* ST, and *B. choui* sp. nov., as well as *B. japonica*, lacks a long call unique to *B. sp.* ST, which represents faster call duration and shorter call rise time (Wang et al., 2017).

DISCUSSION

As suggested previously (Tominaga et al., 2015), *B. japonica* (sensu lato) is divided into three major clades, (1) the Northern and Central Ryukyu, (2) the Southern Ryukyu and

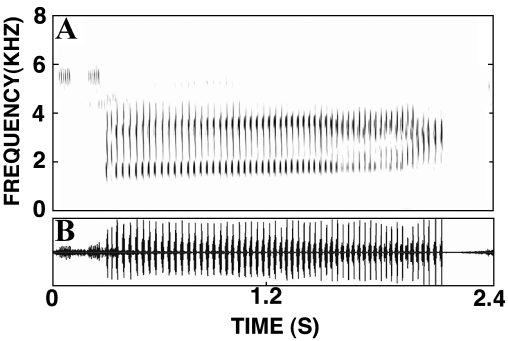


FIG. 6. Advertisement call of *Buergeria choui* sp. nov. from Taketomi-cho, Iriomotejima Is., Japan, recorded at an air temperature of 28.5°C, showing sonogram (A) and wave form (B).

Northern Taiwan, and (3) the Southern Taiwan clades. Wang et al. (2017) studied their acoustic characteristics and split the Southern Taiwan clade as a distinct species, *B. sp.* ST, but did not split the remaining clades and treated them as the Japanese clade.

The present study clarified that the Southern Ryukyu and Northern Taiwan clade could be distinguished from the Northern and Central Ryukyu clade (*B. japonica* sensu

stricto) in concordant with phylogenetic results (Tominaga et al., 2015) and named the former clade as *B. choui* sp. nov. This result is noteworthy from a biogeographic view point.

Among quite a few unique anuran species of the Ryukyu Archipelago, *Microhyla okinavensis* Stejneger, 1901 and *B. japonica* were long known to be widespread in both Yaeyama and most of the remaining islands of the archipelago (Maeda and Matsui, 1999; Matsui and Maeda, 2018). However, *M. okinavensis* has recently been taxonomically revised, and the Yaeyama population will soon be described as an endemic new species (Matsui and Tominaga, in press). Thus, the present description of *B. choui* sp. nov. as a species also confined to Yaeyama within the Ryukyus resulted in the complete absence of frogs widespread almost throughout the Ryukyu Archipelago, with an exception of dubious record from Iriomotejima of *Hyla hallowellii* Thompson, 1912, which is otherwise found in the Central Ryukyus (Matsui and Matsui, 1982).

Other than *B. choui* sp. nov., *Nidirana okinavana* (Boettger, 1895) and *Kurixalus eiffingeri* (Boettger, 1895), both confined to the Yaeyama Islands within Japan, are distributed in Taiwan (Matsui and Maeda, 2018). Although *Fejervarya kawamurai* Djong, Matsui, Kuramoto, Nishioka, and Sumida, 2011 may occur in western Taiwan (Djong et al., 2011), details still require further study.

In Taiwan, *N. okinavana* has a very restricted distribution range only in Nantou, Central region (Jang-Liaw and Chou, 2015), strongly contrasting to its relative *N. adenopleura* (Boulenger, 1909), which occurs widely on the island and also on the continent (Lyu et al., 2020). Japanese and Taiwanese populations of *N. okinavana* are only slightly differentiated genetically (Matsui, unpublished data), like the case of *B. choui* sp. nov., but unlike the latter, it is a relict species.

Kurixalus eiffingeri, widely occurring montane regions of Taiwan, has very close relatives, *K. berylliniris* Wu, Huang, Tsai, Li, Jhang, and Wu, 2016 from the southeastern

region and *K. wangi* Wu, Huang, Tsai, Li, Jhang, and Wu, 2016 from southern tip of Taiwan (Wu et al., 2016). *Kurixalus eiffingeri* from Northern Taiwan is genetically very close to Yaeyama, Japan, and together differ from the Central Taiwan population, which is as distant from them as the other two species. Thus the phylogeographic pattern of this group is comparable with that of *Buergeria japonica* (sensu lato) studied here.

Using the data from 16S rRNA, Tominaga et al. (2015) estimated the ancestral stock of *B. japonica* (sensu lato) to have first diverged at the periphery of the continent. Of the three major clades, now split at species level, *B. sp.* ST is distributed in Southern Taiwan with the deepest divergence from the other two, indicating the first divergence occurred in the terrestrial area of Taiwan, which was then a part of the eastern coast of the Continent, at the middle Miocene, 10.6 (95% CI=7.1–16.8) million years before present (MYBP). Later, *B. choui* sp. nov. of the Southern Ryukyu and *B. japonica* of the Central Ryukyu diverged at late Miocene to early Pliocene, 6.4 (4.4–8.8) MYBP, and finally, divergence between the Southern Ryukyu and North Taiwan populations of *B. choui* sp. nov. occurred at late Pliocene to middle Pleistocene, 3.3 (1.9–5.0) MYBP, both much older than the putative formation of the Kerama and Yonaguni Gaps in the Holocene (1.4–1.7 MYBP) from geological evidence (Osozawa et al., 2012). In order to clarify reasons for such discrepancies, further detailed studies are needed, especially for *Buergeria*, the pattern of whose distribution is very interesting as already noted long ago (Matsui and Orlov, 2004).

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