A new species of the genus Raorchestes (Anura: Rhacophoridae) from Yunnan Province, China

DEAR EDITOR,

A new bush frog species, Raorchestes cangyuanensis sp. nov., from Cangyuan, Yunnan Province, China, is described based on morphological and molecular analyses. It differs from all known congeners by a combination of the following characters: body size small, adult snout-vent length (SVL) 16.1–20.0 mm in males (n=3); tympanum indistinct; tips of all fingers and toes expanded into discs with circummarginal grooves; rudimentary webbing between toes; fingers and toes with lateral dermal fringes; inner and outer metacarpal tubercles present; heels meeting when limbs held at right angles to body; crotch with a distinct black patch; discs of fingers and toes orange; male with external single subgular vocal sac and reddish nuptial pad at the base of first finger.

The genus Raorchestes Biju, Shouche, Dubois, Dutta, and Bossuyt, 2010 ranks in the top two most speciose genera of Rhacophoridae. Raorchestes is characterized by adult snout-vent lengths between 15.0 mm and 45.0 mm, no vomerine teeth, transparent/translucent vocal sac while calling, direct development without free-swimming tadpoles and nocturnal lifestyles (Biju et al., 2010; Vijayakumar et al., 2016). Raorchestes currently contains 62 species, ranging from the southern tip of the Indian Peninsula to northeastern India, Indo-China, and southwestern China (Frost, 2019). Thirty-two new species have been described in the last decade, entirely in India (Biju et al., 2010; Biju & Bossuyt, 2009; Padhye et al., 2013; Seshadri et al., 2012; Vijayakumar et al., 2014; Zachariah et al., 2011). Southeast Asia and southern China (SEA-SC) contain only four known species which are R. gryllus, R. parvulus, R. menglaensis, and R. longchuanensis, with no new species records for Raorchestes in SEA-SC in recent years. The high density of recently described species in other regions suggests that cryptic lineages may exist in more depauperate areas of Raorchestes distribution that have received less investigative attention.

Southwestern China is a global biodiversity hotspot that harbor a high diversity of amphibian species (AmphibiaChina, 2019; Myers et al., 2000), owed largely to its complicated topography (altitude ranges from <2 000 m in some valleys to 7 558 m a.s.l. at the summit of Gongga Mountain) and variety of habitats and climates. The amphibian fauna in southwestern China is rich in terms of species count and endemism (AmphibiaChina, 2019; Frost, 2019). In recent years, several cryptic and new species of amphibians have been described (Chen et al., 2017, 2018; Li et al., 2018; Lyu et al., 2019; Wang et al., 2019; Yang et al., 2016; Yang & Chan, 2018; Yu et al., 2019; Yuan et al., 2018). These results suggest that the rich amphibian diversity in the region still remains underestimated. During fieldwork in Cangyuan, Yunnan Province, southwestern China, we collected specimens that superficially resembled R. menglaensis, R. longchuanensis, and R. parvulus, which potentially occur in this region. We evaluated these individuals using molecular and morphological phylogenetic analyses. Based on an integrative taxonomic approach, we identified a distinct evolutionary lineage and describe it as a new species of the

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The new species of the genus Raorchestes, the Cangyuan population and other described species of the genus Raorchestes were obtained from previous publications (Fei et al., 2009; Padhye et al., 2018; Kuramoto & Joshy, 2009). Measurements followed Tamura et al. (2007) in MEGA v6.0.6 (Tamura et al., 2007). The topologies recovered by both ML and BI analyses were essentially identical, with relatively robust support for most terminal clades (Figure 1B). The monophyly of Raorchestes was strongly supported and in agreement with the results of Biju et al. (2010) and Vijayakumar et al. (2016). Among other members of the genus Raorchestes, the Cangyuan population represented a distinct phylogenetic lineage with strong support (Bayesian posterior probabilities=1; bootstrap support=100; Figure 1B). The genetic distance between the Cangyuan population and other described species of the genus ranged from 5.4% (with R. gryllus) to 14.1% (with R. archeos) (Supplementary Table S2). A 3% P-distance value for 16S rRNA is considered a useful indicator for new candidate species in frogs (Vieites et al., 2009). Thus, genetic divergence between the Cangyuan population and its congeners exceeds the proposed threshold for species-level differentiation in frogs. Morphologically, the newly identified matriline differed from all named species. Thus, we describe the new species of the genus Raorchestes below.

**Taxonomic account**

*Raorchestes cangyuanensis* sp. nov. Wu, Suwannapoom, Xu, Murphy et Che (Figure 1; Table 1)

**Holotype:** Adult male (KIZ015856) from Cangyuan County, Yunnan Province, China (N23.22542°, E99.22509°, 1 272 m a.s.l.), collected by Da-Hu Zou and Kai Xu on 25 May 2016.

**Paratypes:** Two males KIZ015855 and KIZ015857, collected by Da-Hu Zou and Kai Xu. Location and date are the same as those of the holotype.

**Diagnosis:** *Raorchestes cangyuanensis* sp. nov. is diagnosed as a member of the genus *Raorchestes* by the following morphological characters: small body size; vomerine teeth absent; nocturnally active; tips of all fingers and toes expanded into discs with circummarginal grooves. The new species is distinguished from geographically and molecularly relevant congeners by the following combination of characters: (1) body size small, adult SVL 16.1–20.0 mm in males (n=3); (2) tympanum indistinct; (3) tongue pyriform, with a deep notch at the posterior tip; (4) tips of all fingers and toes expanded into discs with circummarginal grooves; (5) no webbing between fingers; (6) rudimentary webbing between toes; (7) fingers and toes with lateral dermal fringes; (8) inner and outer metacarpal tubercles present; (9) inner metatarsal tubercle oval, outer metatarsal tubercle absent; (10) heels meeting when limbs held at right angles to body; (11) tibiotarsal articulation reaching anterior of eye when hindlimb is stretched along the side of the body; (12) dark brown interorbital triangle between eyes; (13) crotch with a distinct black patch; (14) discs of fingers and toes orange; (15) dorsal surface brown with a dark *°*'-shaped marking; (16) supratympanic fold distinct, from posterior corner of eye to above insertion of arm; (17) iris golden brown; (18) male with external single subdigital vocal sac; and (19) reddish nuptial pad at the base of first finger.
Table 1  Measurements (mm) of Raorchestes cangyuanensis sp. nov.

| Catalog No. | KIZ015855 | KIZ015856* | KIZ015857 |
|-------------|-----------|------------|-----------|
| Sex         | Male      | Male       | Male      |
| SVL         | 19.3      | 20.0       | 16.1      |
| HDL         | 6.4       | 6.6        | 5.5       |
| HDW         | 7.2       | 7.2        | 6.2       |
| SNT         | 2.5       | 2.4        | 2.3       |
| DNE         | 1.4       | 1.5        | 1.1       |
| IND         | 1.9       | 2.0        | 1.9       |
| IOD         | 2.5       | 2.4        | 2.2       |
| UEW         | 1.8       | 1.6        | 1.7       |
| ED          | 2.8       | 2.6        | 2.3       |
| SN          | 1.1       | 1.3        | 0.9       |
| IFE         | 3.8       | 3.5        | 2.6       |
| IBE         | 6.7       | 6.8        | 4.7       |
| FAL         | 4.2       | 4.5        | 3.9       |
| HL          | 5.3       | 5.4        | 4.9       |
| THL         | 9.0       | 8.9        | 8.2       |
| TL          | 9.0       | 9.1        | 7.7       |
| FL          | 7.3       | 7.5        | 6.2       |
| FL1         | 1.6       | 1.8        | 1.0       |
| FLII        | 2.1       | 2.1        | 1.9       |
| FLIII       | 3.3       | 3.7        | 3.5       |
| FLIV        | 2.4       | 2.7        | 2.1       |
| TL          | 1.2       | 1.2        | 1.2       |
| TLII        | 1.9       | 1.7        | 1.7       |
| TLIII       | 3.1       | 2.9        | 2.0       |
| TLIV        | 3.4       | 4.2        | 3.3       |
| TLV         | 2.1       | 2.5        | 2.0       |

For abbreviations, see text and Supplementary Methods. Asterisk (*) indicates holotype.

For color of holotype in life see Figure 1. Dorsal surface brown, with golden brown band between eyes; dorsal surface with a dark* (-shaped marking; dark brown interorbital triangle between eyes; upper and lower lips with white and black dots; supratympanic fold dark brown; iris golden brown; dorsal parts of limbs with dark brown crossbars; crotch with a distinct black patch bordering large creamy white plaque below the black patch near the groin; dorsal thigh orange with two black crossbars; ventral surface body and limbs brown, with small black and white spots; discs of fingers and toes orange (Figure 1).

Color of holotype in preservative: Dorsum faded to dark brown; golden brown band between eyes still clear; a dark* (-shaped marking faintly present on dorsum; black patch present at crotch still distinct; large creamy white plaque below the black patch still clear; cross bands present on dorsal side of forelimbs and hind limb still clear; discs of fingers and toes fades to brown; throat, chest, abdomen and ventral surface of limbs dark brown, mottled with white dots (Supplementary Figure S1).

Male secondary sexual characteristics: Adult males possess nuptial pads covering the dorsal surface of the base of Fl; external single subungual vocal sac; slit-like opening at posterior of jaw.

Variation: Morphometric measurements of holotype and two paratypes are given in Table 1. Paratypes generally agree with the holotype morphologically, with the following exceptions: smaller body length for one of the three adult male specimens (KIZ015857); body length of KIZ015857, KIZ015855, and KIZ015856 16.1 mm, 19.3 mm, and 20.0 mm, respectively. KIZ015855 and KIZ015856 have light brown band between eyes, which is absent in paratype KIZ015857.

Distribution and habitat: Raorchestes cangyuanensis sp. nov. is known only from a single locality, Cangyuan County, Yunnan, China (N23.22542°, E99.22509°). The new species was found at an elevation of 1 272 m a.s.l. in shrubbery near streams.

Comparisons: Based on morphology, we compared Raorchestes cangyuanensis sp. nov. with morphologically,
Raorchestes cangyuanensis sp. nov. differs from R. longchuensis in the following characters: tympanum indistinct in males (vs. distinct); lateral dermal fringes on all fingers and toes (vs. only on first and second fingers with lateral dermal fringes, lateral dermal fringes of toes absent); rudimentary webbing between toes (vs. 1/4 webbing); iris golden brown (vs. reddish brown).

Raorchestes cangyuanensis sp. nov. differs from R. menglaensis by the following combination of characters: male with external single subgular vocal sac (vs. internal single subgular vocal sac); all fingers and toes with lateral dermal fringes (vs. absent); outer metatarsal tubercle absent (vs. present); discs of fingers and toes orange (vs. not orange).

Raorchestes cangyuanensis sp. nov. differs from R. gryllus by the following combination of characters: SVL of adult male 16.1–20.0 mm (vs. 25.0–27.0 mm); rudimentary webbing between toes (vs. little more than half webbed); outer metatarsal tubercle absent (vs. present), Raorchestes cangyuanensis sp. nov. differs from R. parvulus in the following characters: tympanum indistinct (vs. distinct); toes with lateral dermal fringes (vs. lateral dermal fringes of fifth toe indistinct); SVL of adult male 16.1–20.0 mm (vs. 20.1–23.2 mm); supernumerary tubercles absent (vs. present on third finger); relative toe lengths: I<II<IV<III (vs. relative toe lengths: I<II<III<IV).

Raorchestes cangyuanensis sp. nov. differs from R. ghatii in the following characters: reddish nuptial pad at the base of first finger (vs. absent); relative finger lengths: I<II<IV<III (vs. relative finger lengths: I<IV<II<III).
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Supplementary data to this article can be found online.

AUTHORS' CONTRIBUTIONS

J.C., C.S., and Y.H.W. designed the study. J.M.C. and K.X. collected specimens in the field. J.Q.J and H.M.C. performed molecular experiments. Y.H.W. performed data analyses, and wrote the manuscript. J.C., R.W.M., and C.S. revised the manuscript. All authors read and approved the final version of the manuscript.

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