LETTER TO THE EDITOR

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# New Distribution Records of *Rana jiemuxiensis* in Anhui Province

Min TANG[1], Xing YU[1], Jiahui LI[1], Taiyue LI[1], Wenrui ZHU[1], Jing CHE[2], Jianjun LIU[1], Huaxing ZHOU[3] and Liuwang NIE[1*]

[1] *The Anhui Provincial Key Laboratory of Biodiversity Conservation and Ecological Security in the Yangtze River Basin, College of Life Sciences, Anhui Normal University, Wuhu 241002, Anhui, China*

[2] *State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650201, Yunnan, China*

[3] *Anhui Key Laboratory of Aquaculture & Stock Enhancement, Fisheries Research Institution, Anhui Academy of Agricultural Sciences, Hefei 230001, Anhui, China*

## Dear Editor,

The true frogs classified under the genus *Rana*, with widespread distribution in Eurasia and North America, constitute a varied and intricate group (Yuan *et al*., 2016; Chen *et al*., 2022). In total, 52 species within this genus have been documented (IUCN, 2024), with 28 of those species found in China (AmphibiaChina, 2024). By virtue of morphological statistics and genetic analysis, a novel species identified as the Jiemuxi brown frog (*Rana jiemuxiensis*) has been recognized within the *R. longicrus* Species Group, being endemic to China (Yan *et al*., 2011). Previously, this species has been recorded in the Jiemuxi National Nature Reserve (28°52′45″ N, 110°24′608″ E) in Hunan, China, where it lives at an altitude of about 723 m in the mountains near the Yuanjiang River, a tributary of the Yangtze River basin (Yan *et al*., 2011).

In April 2022, we found 7 individuals (5 females and 2 males) and some developing tadpoles belonging to the genus *Rana* at Dalongwo Lake near the Yangtze River (31°15′23″ N, 118°12′44″ E) in Wuhu, Anhui Province, China, at an altitude of 0 m (Figure 1). The vernier caliper was used to record 23 morphological characteristics of 7 individuals collected from Anhui with an accuracy of 0.01 mm, and found that they had highly similar morphological characteristics to the type specimens of *R. jiemuxiensis*: (1) HL (head length) is slightly greater than HW (head width) (Mean±SE, 16.56±3.22 mm > 15.96±2.38 mm), (2) TL (tibia length) is longer than FTL (length of foot and tarsus) (Mean±SE, 28.4±5.57 mm > 12.59±3.18 mm), (3) LAL (lower-arm length) (Mean±SE, 25.63±5.34 mm) is nearly half of the SVL (snout-vent length) (Mean±SE, 47.47±7.68 mm), (4) several individuals had a dark spot shaped like "^" on the upper shoulder (Figure 1, Supplementary Table S1). Based on morphological comparisons, the morphological measurements were consistent with those reported for *R. jiemuxiensis* (Yan *et al*., 2011), and thus the species was preliminarily identified as *R. jiemuxiensis*.

To facilitate species identification, we utilized one native sample from Anhui to amplify *COXI* and *CYTB* of mitochondrial genes. The primer design was derived from the related species *R. longicrus* (GenBank No. OL681879) and *R. zhenhaiensis* (GenBank No. OL681880), which are available in the GenBank database (Supplementary Table S2). Combined with the gene sequence data from three samples in Hunan provided by Yan *et al*. (2011), using ModelTest-NG v0.1.7, the most appropriate nucleotide evolution model TIM2+I+G4 for *COXI* and *CYTB* of 34 *Rana* species (Supplementary Table S3) was estimated, and RAxML-NG v1.2.0 was used to generate maximum likelihood phylogenetic tree (Kozlov *et al*., 2019; Darriba *et al*., 2020). The results showed that *R. jiemuxiensis* individuals distributed in the Jiemuxi National Nature Reserve and Wuhu in Anhui, China clustered into a single branch with a bootstrapping support value of 100 (Figure 2).

To analyze the genetic differences between two geographic populations of *R. jiemuxiensis*, 10 native samples from Anhui and 6 samples from Hunan were used to amplify and sequence

* Corresponding author: Prof. Liuwang NIE, from Anhui Normal University, Wuhu, Anhui, China, with his research focusing on the evolutionary biology of amphibians and reptiles.
E-mail: lwnie@mail.ahnu.edu.cn
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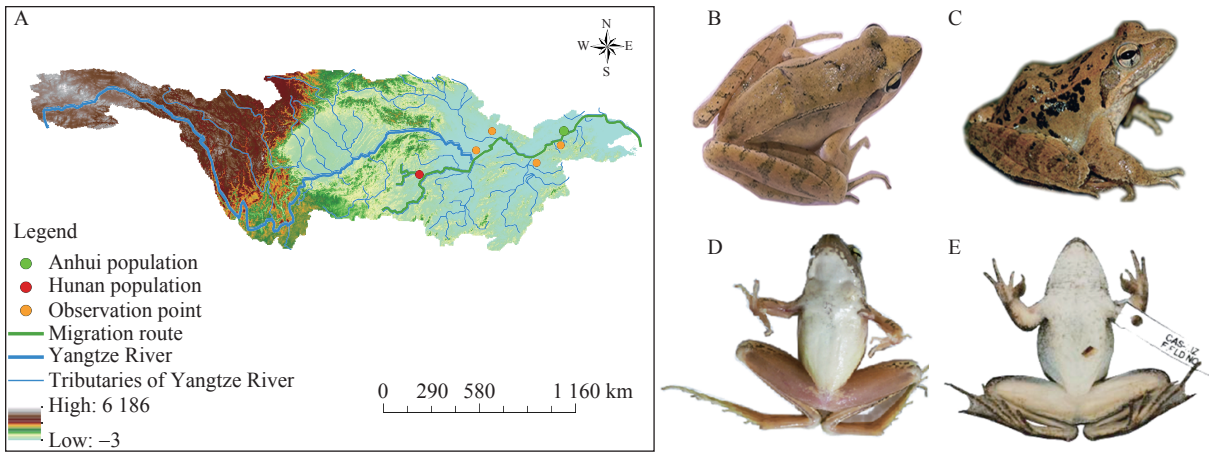



**Figure 1** Geographical distribution and specimens of *Rana jimuxixiensis* in China. The geographical distribution of *R. jimuxixiensis* in China (A), the red point is the distribution of Hunan population, the green point is the distribution of Anhui population, and yellow points are observation points, and there is no distribution of the *R. jimuxixiensis*. The side view (B) and ventral view (D) of *R. jimuxixiensis*, from Wuhu, Anhui Province, China. The side view (C) and ventral view (E) of *R. jimuxixiensis*, from the Jiemuxi National Nature Reserve (Yan *et al.*, 2011).

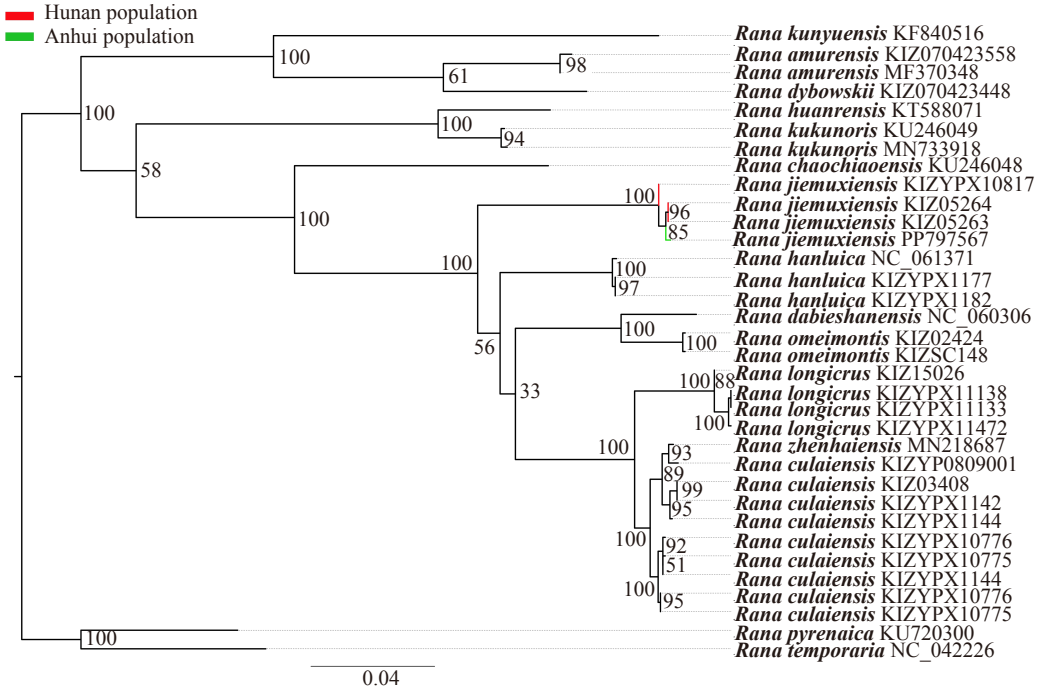



**Figure 2** Phylogenetic relationship based on *COXI* and *CYTB*. The phylogenetic analysis of 34 *Rana* was carried out by maximum likelihood method. The outgroups of the tree are *R. pyrenaica* KU720300 and *R. temporaria* NC_042226. The number on the node is the bootstrap value of maximum likelihood.

the mitochondrial D-loop region (Supplementary Tables S2, S4). These samples from Hunan were obtained through the Sample Application process at the Animal Genetic Resources Bank of the Southwest China Germplasm Bank of Wildlife. In total, 16 samples were after Multiple Alignment using Fast Fourier Transform (MAFFT) comparisons (Madeira *et al.*, 2022). Through MEGA X, the genetic distance between the two populations of *R. jiemuxiensis* was calculated using the Kimura-2 parameter distance function (Kumar *et al.*, 2018). The estimated results indicate that: (1) the genetic distance

between all samples was small, and there was no obvious genetic difference between the populations, (2) the maximum genetic distance was between *R. jiemuxiensis* WH22040710 and *R. jiemuxiensis* YPX10827 (value=0.03515), and the minimum genetic distance was between *R. jiemuxiensis* WH2204072 and *R. jiemuxiensis* WH2204077 (value=0.00320), and (3) the genetic distance between Wuhu individuals and Jinzhuxi individuals in Hunan was slightly smaller than that between Wuhu individuals and Shanmudong individuals in Hunan (Supplementary Table S5).

Based on morphological and molecular biology analyses, a second distribution of *R. jiemuxiensis* was identified, which is also a new distribution of *Rana* in Anhui Province. The linear distance between the two distribution intervals was estimated at 780.34 km by using ArcMap 10.8, and no significant population genetic differences were identified based on the mitochondrial D-loop region. Furthermore, observation points were established and surveyed along the Yangtze River, and no additional distribution sites were found. Rivers often serve as essential migration corridors for amphibians (Howell *et al*., 2018; Luo *et al*., 2021). The type specimens of *R. jiemuxiensis* are located near the Yuan River, a tributary of the Yangtze River. Consequently, we hypothesize that an egg mass of *R. jiemuxiensis* may be using the Yangtze River as a migration channel, enabling them to inhabit the Dalongwo Lake in Wuhu, Anhui (Dalongwo Lake is shaped like a spoon and the "handle" of the spoon is connected to the Yangtze River). We anticipate that our findings will contribute significantly to the conservation efforts for *R. jiemuxiensis* and enhance systematic evolutionary research on amphibians.

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**Supplementary information** Supplementary information is available at https://doi.org/10.3724/ahr.2095-0357.2024.0018

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