A new species of *Rana* from the Dabie Mountains in eastern China (Anura, Ranidae)

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Abstract

A new species *Rana dabieshanensis* sp. n. is described from the Dabie Mountains in Anhui Province, China, based on morphological character differences and molecular analyses. The new species can be distinguished from its congeners by a combination of diagnostic characters. The results of phylogenetic analyses (based on 12s rRNA, 16s rRNA, ND2, Cyt b, RAG1, BDNF and Tyr) and genetic distances (based on Cyt b) indicate that the new species belongs to the *Rana longicrus* group, and is placed as the sister taxon to *R. hanluica*.

Keywords

Amphibians, morphology, molecular phylogeny, taxonomy

Introduction

The true frogs of the genus *Rana* Linnaeus are broadly distributed across Eurasia and the Americas (Amphibia Web 2017, Frost 2016, Yuan et al. 2016). Because of their body coloration and habitat preferences, the species of this genus are colloquially known as brown frogs or wood frogs (Boulenger 1920, Yan et al. 2011). To date, more than 100 species

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are attributed to this genus, with their distribution ranges across Asia (32 species), Europe and the Near East (12 species), and the Americas (57 species) with several lineages still being not formally described (Yuan et al. 2016, Amphibia Web 2017). In China, the genus *Rana* contained 17 species (Fei et al. 2012) that were divided into three groups (*R. longicrus* group, *R. chensinensis* group, and *R. amurensis* group) based on morphology and distribution (Fei et al. 2009). Molecular phylogenies, however, indicate presence of *R. longicrus*, *R. amurensis*, *R. chensinensis*, *R. sauteri*, *R. johnsi*, *R. shuchinae*, and *R. weinigensis* groups within the Chinese *Rana* sensu lato (Che et al. 2007, Yuan et al. 2016). And 24 species are contains in Chinese *Rana* genus (AmphibiaChina 2017, Yuan et al. 2016, Zhao et al. 2017). The species of the *R. longicrus* group are widely distributed in southern and eastern China, and the recent surge in new species descriptions suggests that these still insufficiently explored regions may contain many undescribed cryptic species (Lu and Li 2001, Lu et al. 2007, Shen et al. 2007, Li et al. 2008, Yan et al. 2011).

From 2015 to 2016, we collected 17 specimens of *Rana* sp. in montane forests of the Dabie Mountains. The specimens exhibited comparatively large body size and a straight dorsolateral fold from posterior corner of eye to groin. Initially, they were identified as *R. omeimontis* (according to the identification key by Ye et al. 1993). However, from the further detailed studies indicated that this record might represent a yet undescribed cryptic species of *Rana*.

Generally, the brown frogs are difficult to identify in the field because of their close morphological similarities especially when closely related species have overlapping distributions (Stuart et al. 2006, Che et al. 2007). According to the results of subsequent molecular analyses and morphological identification, we confirmed that the specimens of *Rana* sp. from Dabie Mountains were distinct from any species presently recognized in the genus of *Rana* and herein we describe them as a new species.

**Materials and methods**

**Sampling**: In total, 17 specimens of *Rana* sp. were collected from Yaoluoping National Nature Reserve in Dabie Mountains, Yuexi County, Anhui Province, China (30°58′16.92″N, 116°04′11.88″E, elevation 1150 m a.s.l.) (Fig. 1), in August 2015 and August 2016. Those individuals were dipped in 10% formalin (10 seconds) for fixation and subsequently transferred into 75% ethanol for storage. Before fixing in formalin, liver tissues from all individuals were sampled and preserved in 100% ethanol for molecular analyses. All specimens and tissue samples were deposited in the Anhui University Museum, Research Center for Biology.

**DNA extraction, PCR amplification and sequencing**: Genomic DNA was extracted from liver tissues of seven *Rana* sp. specimens using the standard proteinase K/phenol-chloroform protocol (Sambrook et al. 1989). Four mitochondrial genes (12S rRNA, 16S rRNA, ND2, and Cyt b) and three nuclear DNA markers (Tyr, BDNF and RAG1) were sequenced for one individual, while for six remaining specimens only Cyt b mtDNA gene was sequenced. The primers used for PCR and sequencing are summarized in Table 1. All
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**Figure 1.** Distribution of *Rana dabieshanensis* sp. n. in Dabie Mountains (Anhui, Hubei, and Henan provinces, central China). Occurrence record is marked with green mark.

**Table 1.** Primers used for PCR and sequencing.

| Locus | Primer Name | Sequences (5' end 3' end) | Temperature (°C) | Source |
|-------|-------------|---------------------------|------------------|--------|
| 12S   | L2519       | AAAGTGGAGTAGATACCCCACTAT  | 50               | Kocher et al. (1989) |
|       | H3296       | GCTAGACCATATGCAAGGGA      | 50               | Kocher et al. (1989) |
| 16S   | 16SAR       | AAGCTAAGATGAGCCCTAAGTTTCT| 50               | Kocher et al. (1989) |
|       | R16         | ATAGTGAGTTATCCTCCAGTTT    | 50               | Sumida et al. (2000) |
| ND2   | HERP322     | TCGAAGACAGAGTTRRAG        | 42               | Yuan et al. (2016)   |
|       | HERP323     | CAYACGGRGCYATGAA          | 42               | Yuan et al. (2016)   |
| Cyt b | HERP328     | GAAAARCTCGGTCTTTCAACTA    | 50               | Yuan et al. (2016)   |
|       | HERP329     | CTACKGGTGTCCTCCACTATG     | 50               | Yuan et al. (2016)   |
| Tyr   | Tyr1G       | TGCTGGGCGCTGCTCCCTCCCA    | 50               | Bossuyt and Milinkovich (2000) |
|       | Tyr1B       | AGGTCCTCGTTRAGGAAAGGATG   | 50               | Bossuyt and Milinkovich (2000) |
| RAG1  | AmpF2       | ACNGGNGCGRATCTTYCARCC     | 52               | Hoegg et al. (2004)  |
|       | AmpR2       | GTGTTTYAACACATCTCCATYTCR  | 52               | Hoegg et al. (2004)  |
| BDNF  | BDNF_2F     | GAGTTGGCAAGAGGAGG         | 41               | Zhou et al. (2012)   |
|       | BDNF_2R     | ACTGCGTAGTCCGGCATT        | 41               | Zhou et al. (2012)   |

PCRs were performed with the same conditions in 50 μL: 20 to 80ng of genomic DNA, 25 μL 2×EasyTaq PCR SuperMix polymerase (TransGen Biotech, containing 1.25U Ex Taq, 0.4mM dNTP, 4mM Mg²⁺) and 0.4 μM of primers. Reactions were performed with
| Species | Locality | Voucher | GenBank No. | Reference |
|---------|----------|---------|-------------|-----------|
| **R. dabieshanensis**<sup>sp. n. (1)</sup> | China: Anhui Province: Dabie Mountains area | AHU2016R001 | MF172963 MF172964 MF172974 MF172971 MF172972 MF172973 | This Study |
| **R. dabieshanensis**<sup>sp. n. (2)</sup> | China: Anhui Province: Dabie Mountains area | AHU2016R002 | N/A MF172965 N/A N/A N/A N/A | This Study |
| **R. dabieshanensis**<sup>sp. n. (3)</sup> | China: Anhui Province: Dabie Mountains area | AHU2016R003 | N/A MF172966 N/A N/A N/A N/A | This Study |
| **R. dabieshanensis**<sup>sp. n. (4)</sup> | China: Anhui Province: Dabie Mountains area | AHU2016R004 | N/A MF172967 N/A N/A N/A N/A | This Study |
| **R. dabieshanensis**<sup>sp. n. (5)</sup> | China: Anhui Province: Dabie Mountains area | AHU2016R005 | N/A MF172968 N/A N/A N/A N/A | This Study |
| **R. dabieshanensis**<sup>sp. n. (6)</sup> | China: Anhui Province: Dabie Mountains area | AHU2016R006 | N/A MF172969 N/A N/A N/A N/A | This Study |
| **R. dabieshanensis**<sup>sp. n. (7)</sup> | China: Anhui Province: Dabie Mountains area | AHU2016R007 | N/A MF172970 N/A N/A N/A N/A | This Study |
| **R. amurensis** | Russia: Tomskaya: Tegul-detskii district | MSUZP-SLK-RUS49 | KX269203 KX269349 KX269418 KX269568 KX269278 KX269795 | Yuan et al. 2016 |
| **R. arvalis** | Russia: Mordovia: Chamzinskii district | MSUZP-SLK-MKR21 | KX269197 KX269344 KX269413 KX269562 KX269272 KX269789 | Yuan et al. 2016 |
| **R. asiatica** | China: Xinjiang: 47tuan | KIZ-JX0251 | KX021945 KX021945 KX021945 KX269565 KX269275 KX269792 | Yuan et al. 2016 |
| **R. choaichaenensis** | China: Sichuan: Zhaojue | SCUM0405170CJ | KX269192 KX269339 KX269408 KX269557 KX269267 KX269800 | Yuan et al. 2016 |
| **R. chensinensis** | China: Shaanxi: Huxian | KIZ-RD05SHX01 | KX269186 KX269333 KX269402 KX269551 KX269261 KX269779 | Yuan et al. 2016 |
| **R. culaiensis** | China: Shandong: Culaishan shan | KIZ-SD080501 | KX021986 KX021986 KX021986 KX269555 KX269265 KX269783 | Yuan et al. 2016 |
| **R. dybowskii** | Russia: Primorye region: Khasanskii District | MSUZP-IVM-1d | KX021949 KX021949 KX021949 KX269553 KX269263 KX269781 | Yuan et al. 2016 |
| **R. hanluica** | China: Guangxi: Maershan shan | KIZGX07112915 | KX269191 KX269338 KX269407 KX269556 KX269266 KX269784 | Yuan et al. 2016 |
| **R. huanrenensis** | South Korea | MMS 231 | KX021944 KX021944 KX021944 KX269548 N/A KX269776 | Yuan et al. 2016 |
| Species          | Locality                        | Voucher          | GenBank No.         | Reference            |
|------------------|---------------------------------|------------------|---------------------|----------------------|
| R. japonica      | Japan: Isumi-shi: Chiba Prefecture | KIZ-YPX11775     | KX269220 KX269364 KX269434 KX269585 KX269295 KX269811 | Yuan et al. 2016     |
| R. jiexiannian   | China: Hunan: Jiexi             | KIZ-HU0708013    | KX269221 KX269365 N/A KX269586 KX269296 KX269812 | Yuan et al. 2016     |
| R. kukunorii     | China: Qinghai: Qinghai Lake    | KIZCJ06102001    | KX021947 KX021947 KX021947 KX269550 KX269260 KX269778 | Yuan et al. 2016     |
| R. kunyuensis    | China: Shandong: Kunyu Shan     | KIZ-HUI040001    | KX269201 KX269347 KX269416 KX269566 KX269276 KX269793 | Yuan et al. 2016     |
| R. longicrus     | China: Taiwan: Xiangtianhu Miaosu | NMNS15022         | KX269189 KX269336 KX269405 KX269554 KX269264 KX269782 | Yuan et al. 2016     |
| R. omeimontis    | China: Sichuan: Zhangcun Hongya | SCUM0405196CJ    | KX021946 KX021946 KX021946 KX269558 KX269268 KX269785 | Yuan et al. 2016     |
| R. zhengi        | China: Sichuan: Hongya: Zhangcun | SCUM0405190CJ    | KX269206 KX269352 KX269421 KX269571 KX269798 KX269495 | Yuan et al. 2016     |
| R. johnsi        | Vietnam: Lam Dong: Loc Bao      | ABV 00203         | KX269182 KX269328 KX269398 KX269546 KX269774 KX269471 | Yuan et al. 2016     |
| R. shuchinae     | China: Sichuan: Zhaojue         | CIB-HU040009      | KX269210 KX269356 KX269425 KX269575 DQ360057 KX269499 | Yuan et al. 2016     |
| R. weiningensis  | China: Sichuan: Weineng         | SCUM0405171       | KX269217 KX269362 KX269432 KX269582 KX269808 KX269506 | Yuan et al. 2016     |
| Pelophylax nigromaculatus | China: Sichuan: Hongya       | SCUM-045199CJ    | KX269216 KX269361 KX269431 KX269581 KX269807 KX269505 | Yuan et al. 2016     |
the following profile: PCR cycles were 5 min at 95 °C followed by 35 cycles of 30 s at 95 °C, 30 s at appropriate annealing temperature (Table 1), and 1 min at 72 °C, with a final extension at 72 °C for 10 min. The PCR products were purified using an EasyPure PCR Purification Kit (TransGene), and sequenced directly using the primers used in PCRs and the BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems) following the manufacturer’s instructions on an ABI Prism 3730 automated sequencer.

**Phylogenetic analyses:** 147 sequences were used for genetic analysis, which include 135 sequences within the Chinese *Rana* were downloaded from NCBI and 12 sequences in this study. The data are summarized in Table 2. All nucleotide sequences were aligned using MUSCLE (Edgar 2004) with default parameters and checked manually with MEGA 5.0 (Tamura et al. 2011), the length of the fragments was trimmed; newly obtained sequences were deposited in GenBank (Table 2). Nucleotide sites with ambiguous alignments were deleted from the analyses. Bayesian inference (BI) and maximum likelihood (ML) analyses were conducted using the six concatenated gene fragments. BI analyses were performed in MRBAYES v3.1.2 (Ronquist and Huelsenbeck 2003) using the optimal partitioning strategy and best-fit nucleotide substitution model for each region (Table 3) selected by PARTITIONFINDER v1.1.1 (Lanfear et al. 2012). MRBAYES analyses simultaneously initiated two Markov Chain Monte Carlo (MCMC) model runs to provide additional confirmation of convergence of posterior probability distributions. Analyses were run for 10,000,000 generations. Chains were sampled every 1000 generations. The first 25% of the total trees were discarded as "burn-in" and the remaining trees were used to generate a majority-rule consensus tree and to calculate Bayesian posterior probabilities. Nodal support was further assessed with a maximum-likelihood (ML) analysis in RAXML V.7.0.3 with 1000 bootstraps. *Pelophylax nigromaculatus* sequences were downloaded from GenBank and used as outgroup.

**Table 3.** Sequence information (a) and results of model selection by PartitionFinder (b). “V” and “PI” indicated the variable sites and parsimony-informative sites of each locus, respectively.

| (a) | Sequence name | Sequence length (bp) | V  | PI  |
|-----|----------------|----------------------|----|-----|
| 12-16s rRNA | 1743 | 555 | 336 |
| Cyt b | 834 | 306 | 262 |
| ND2 | 726 | 392 | 323 |
| RAG1 | 1191 | 158 | 86 |
| Tyr | 456 | 73 | 31 |
| BDNF | 454 | 31 | 10 |

| (b) | Best fit model | Partitions |
|-----|----------------|------------|
| GTR+I+G | 12S-16S, ND2-2nd |
| HKY+I | RAG-3rd, Tyr-1st, Tyr-3rd, BDNF-1st, BDNF-2nd |
| SYM+I+G | Cyt b-1st |
| HKY+I+G | ND2-3rd, RAG1-1st, RAG1-2nd, Cyt b-2nd, Tyr-2nd |
| GTR+I | ND2-1st, Cyt b-3rd |
| K80+I | BDNF-3rd |
Apart from phylogenetic tree-based methods, we also calculated pairwise sequence divergence based on uncorrected \( p \)-distance using MEGA 5.0 (Tamura et al. 2011) to determine the genetic distance between species. The analysis compared the 7 individuals of \emph{Rana} sp. from the Dabie Mountains to other 22 species of the genus \emph{Rana} inhabiting China.

**Morphological analyses:** The morphometric data were examined for 10 individuals. Measurements were made by Yanan Zhang using a vernier caliper with a precision of 0.1 mm. 17 linear measurements (Fei et al. 1999) were taken as follows:

- **SVL** (snout-vent length, from tip of snout to vent);
- **HL** (head length, from posterior corner of mandible to tip of snout);
- **HW** (head width, the greatest cranial width);
- **SL** (snout length, from tip of snout to the anterior corner of the eye);
- **IN** (internarial distance);
- **ED** (horizontal eye diameter);
- **IO** (interorbital distance, the minimal distance between upper eyelids);
- **UE** (upper eyelid width, the maximal width of upper eyelid);
- **TD** (horizontal tympanic diameter);
- **LAHL** (length of lower arm and hand, from the tip of finger III to the elbow joint);
- **HAL** (hand length, from proximal end of outer palmar tubercle to tip of the third finger);
- **LAD** (diameter of lower arm);
- **HLL** (hind limb length, from the tip of the toe IV to groin);
- **TL** (tibia length);
- **TW** (tibia width, the greatest width of tibia);
- **FL** (foot length, from the proximal end of the inner metatarsal tubercle to the tip of the toe IV) and
- **TFL** (length of tarsus and foot, from the proximal end of tarsus to the tip of the fourth toe IV).

The description of toe webbing followed Savage (1975) (Table 4). The morphological characters of the individuals of \emph{Rana} sp. from Dabie Mountains were compared with the members in the \emph{R. longicrus} group, \emph{R. chaochiaiensis} Liu, 1946, \emph{R. culaiensis} Li, 2008, \emph{R. hanluica} Shen, Jiang & Yang 2007, \emph{R. jiemuxiensis} Yan, 2011, \emph{R. longicrus} Stejneger, 1898, \emph{R. maoershanensis} Lu, 2007, \emph{R. omeimontis} and \emph{R. zhenhaiensis} Ye, 1995. We also compared individuals collected in this area with other \emph{Rana} species distributed in other parts of China \emph{R. arvalis} Gislén, 1959 (previously listed as \emph{R. altaica}, identification following Yang et al. 2010), \emph{R. amurensis} Boulen-ger, 1886, \emph{R. luanchuanensis} Zhao, 2017, \emph{R. asiatica} Bedriaga, 1898, \emph{R. chensinensis} David, 1875, \emph{R. dybowskii} Guenther, 1876, \emph{R. huanrenensis} Liu, 1993, \emph{R. kukunoris} Nikolsky, 1918 and \emph{R. kunyuensis} Lu, 2002. Considering the restrictions of samples, the morphological characteristics of the species in \emph{Rana} were obtained from literature (Fei et al. 2009, Fei et al. 2010, Fei et al. 2012, Zhao et al. 2017, Li et al. 2008, Yan et al. 2011, Inger et al. 1989).
Table 4. Measurements [in mm; mean±SD (range)] of adult specimens of Rana dabieshanensis sp. n.

| Character | Holotype | Males (8) | Mean±SD | Females (2) | Mean |
|-----------|----------|-----------|---------|-------------|------|
| SVL       | 62.8     | 50.9–62.8 | 57.1±4.1 | 53.0–68.3   | 60.7 |
| HL        | 17.8     | 16.0–19.0 | 17.8±1.1 | 18.0–19.7   | 18.8 |
| HW        | 17.6     | 15.3–18.9 | 17.4±1.1 | 16.7–18.8   | 17.8 |
| SL        | 8.4      | 7.5–9.5   | 8.4±0.8  | 7.9–8.5     | 8.2  |
| IN        | 5.5      | 3.9–5.5   | 4.5±0.6  | 4.3–4.6     | 4.5  |
| IO        | 5.5      | 3.9–5.5   | 4.5±0.6  | 4.3–4.5     | 4.4  |
| UE        | 3.6      | 2.9–3.8   | 3.4±0.4  | 3.6–3.7     | 3.6  |
| ED        | 4.8      | 4.1–5.7   | 4.8±0.6  | 4.6–4.7     | 4.5  |
| TD        | 4.0      | 3.5–5.2   | 4.4±0.6  | 4.2–4.3     | 4.3  |
| LAHL      | 27.6     | 21.4–27.6 | 24.7±2.1 | 23.4–26.7   | 24.5 |
| LAD       | 8.1      | 5.6–8.1   | 6.6±1.2  | 4.9–8.1     | 5.2  |
| HAL       | 14.8     | 13.1–14.8 | 13.8±0.7 | 13.3–13.6   | 13.5 |
| HLL       | 129.1    | 100.4–129.1| 115.5±10.6| 102.4–121.4| 111.9|
| TL        | 40.6     | 31.4–40.6 | 35.1±3.3 | 30.4–37.6   | 34.0 |
| TW        | 8.4      | 5.9–8.4   | 7.5±0.9  | 6.4–6.7     | 6.5  |
| TFL       | 53.2     | 43.1–53.2 | 48.6±3.4 | 44.3–51.2   | 47.7 |
| FL        | 35.5     | 27.6–35.1 | 32.8±2.8 | 27.8–35.6   | 31.7 |

Results

Molecular phylogenetic analyses: The BI and ML phylogenetic tree were constructed based on concatenated DNA sequences of the mitochondrial genes and nuclear genes (12S rRNA, 16S rRNA, ND2, Cyt b, Tyr, BDNF, and RAG1) with a total length of the final alignment 5414 bp. Besides, the variable sites and potentially parsimony informative sites are listed in Table 3. The results by BI and ML displayed the same topologies and strong node supporting values (Fig. 2). The major clades were similar to previous studies (Yuan et al. 2016). The individuals of Rana sp. from Dabie Mountains, clustered in the R. longicrus group and are reconstructed as a sister species of R. hanluica with high node support values (1.0/100 for BI posterior probabilities / ML bootstrap, respectively) (Fig. 2).

Furthermore, the nucleotide sequence divergences based on uncorrected pairwise distances model among the 23 brown frog species examined are shown in Table 5. The sequence divergences among the new populations from Dabie Mountains were 0.2%. Sequence divergences between the new populations from Dabie Mountains congeners ranged from 8.6% (R. culaiensis) to 27.7% (R. weiningensis). Within the R. longicrus group, divergence between were from 8.6% (R. culaiensis) to 16.0% (R. chaochiaoeensis). Sequence divergence between the individuals of Rana sp. from Dabie Mountains and the sister species R. hanluica is 8.8% (Table 5).

Based on phylogenetic analysis of both nuDNA and mtDNA genetic markers and genetic distances in Cyt b mtDNA gene, it is demonstrated that the population of Rana sp. from the Dabie Mountains represents a phylogenetically independent evolutionary lineage, and a member of R. longicrus group. It represents a previously undescribed species which is described herein.
Table 5. The pairwise uncorrected $p$-distance (%) of the Cyt $b$ partial sequence (834 bp) used in this study. 1: *Rana dabieshanensis* sp. n; 2: *R. culaiensis*; 3: *Rana longicrus*; 4: *R. zhenbaitiensis*; 5: *R. chaochaoensis*; 6: *R. hanluica*; 7: *R. huanrenensis*; 8: *R. japonica*; 9: *R. jiemuxiensis*; 10: *R. omeimontis*; 11: *R. chensinensis*; 12: *R. dybowskii*; 13: *R. kukunoris*; 14: *R. amurensis*; 15: *R. arvalis*; 16: *R. asiatica*; 17: *R. coreana*; 18: *R. johnsi*; 19: *R. kunyuensis*; 20: *R. sauteri*; 21: *R. shuchinae*; 22: *R. weiningensis*; 23: *R. zhengi*. The number in bold present the distance between *Rana dabieshanensis* sp. n. and the species of *Rana* analyzed in this study.

|    | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18  | 19  | 20  | 21  | 22  | 23  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1  | 0.002 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 2  | 0.086 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 3  | 0.101 | 0.042 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 4  | 0.100 | 0.032 | 0.053 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 5  | 0.160 | 0.148 | 0.159 | 0.141 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 6  | 0.088 | 0.086 | 0.086 | 0.091 | 0.133 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 7  | 0.172 | 0.197 | 0.208 | 0.190 | 0.171 | 0.186 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 8  | 0.148 | 0.139 | 0.161 | 0.141 | 0.144 | 0.138 | 0.172 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 9  | 0.104 | 0.109 | 0.112 | 0.116 | 0.156 | 0.093 | 0.193 | 0.145 |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 10 | 0.093 | 0.090 | 0.102 | 0.106 | 0.167 | 0.085 | 0.193 | 0.150 | 0.111 |     |     |     |     |     |     |     |     |     |     |     |     |
| 11 | 0.178 | 0.178 | 0.190 | 0.176 | 0.156 | 0.180 | 0.063 | 0.160 | 0.186 | 0.191 |     |     |     |     |     |     |     |     |     |     |
| 12 | 0.164 | 0.173 | 0.178 | 0.169 | 0.183 | 0.181 | 0.121 | 0.162 | 0.174 | 0.188 | 0.132 |     |     |     |     |     |     |     |     |     |
| 13 | 0.172 | 0.173 | 0.181 | 0.171 | 0.156 | 0.175 | 0.059 | 0.151 | 0.178 | 0.182 | 0.066 | 0.112 |     |     |     |     |     |     |     |     |     |
| 14 | 0.184 | 0.207 | 0.219 | 0.213 | 0.194 | 0.186 | 0.198 | 0.196 | 0.193 | 0.172 | 0.182 | 0.191 | 0.187 |     |     |     |     |     |     |     |
| 15 | 0.206 | 0.206 | 0.226 | 0.213 | 0.201 | 0.204 | 0.174 | 0.166 | 0.214 | 0.211 | 0.186 | 0.177 | 0.168 | 0.188 |     |     |     |     |     |     |
| 16 | 0.160 | 0.157 | 0.172 | 0.166 | 0.184 | 0.162 | 0.163 | 0.148 | 0.172 | 0.166 | 0.152 | 0.167 | 0.146 | 0.155 | 0.136 |     |     |     |     |     |
| 17 | 0.199 | 0.197 | 0.206 | 0.195 | 0.194 | 0.190 | 0.209 | 0.204 | 0.191 | 0.196 | 0.191 | 0.189 | 0.189 | 0.144 | 0.199 | 0.180 |     |     |     |     |
| 18 | 0.185 | 0.183 | 0.187 | 0.177 | 0.196 | 0.194 | 0.208 | 0.181 | 0.184 | 0.192 | 0.189 | 0.186 | 0.187 | 0.210 | 0.192 | 0.191 | 0.219 |     |     |     |     |
| 19 | 0.201 | 0.199 | 0.208 | 0.200 | 0.199 | 0.197 | 0.212 | 0.199 | 0.202 | 0.193 | 0.197 | 0.196 | 0.190 | 0.152 | 0.200 | 0.185 | 0.022 | 0.220 |     |     |
| 20 | 0.193 | 0.192 | 0.209 | 0.193 | 0.192 | 0.181 | 0.197 | 0.169 | 0.204 | 0.201 | 0.183 | 0.182 | 0.177 | 0.176 | 0.192 | 0.155 | 0.199 | 0.222 | 0.192 |     |
| 21 | 0.191 | 0.207 | 0.235 | 0.210 | 0.220 | 0.210 | 0.193 | 0.205 | 0.211 | 0.197 | 0.198 | 0.189 | 0.182 | 0.202 | 0.194 | 0.182 | 0.206 | 0.197 | 0.194 | 0.204 |
| 22 | 0.277 | 0.279 | 0.262 | 0.266 | 0.255 | 0.267 | 0.271 | 0.252 | 0.283 | 0.259 | 0.267 | 0.254 | 0.261 | 0.277 | 0.237 | 0.273 | 0.292 | 0.270 | 0.273 | 0.259 |
| 23 | 0.203 | 0.195 | 0.202 | 0.185 | 0.192 | 0.201 | 0.198 | 0.194 | 0.193 | 0.211 | 0.191 | 0.203 | 0.185 | 0.217 | 0.192 | 0.194 | 0.226 | 0.046 | 0.222 | 0.229 | 0.204 | 0.282 |
Taxon description

*Rana dabieshanensis* sp. n.
http://zoobank.org/B2595A92-AD54-433B-9F0A-C05DF5E6836

**Holotype.** Specimen AHU2016R001, an adult male (Figures 3, 4) from the Yaoluoping National Nature Reserve, Yuexi County, Anhui Province, China (30°58′16.92″N, 116°04′11.88″E, elevation 1150 m a.s.l.) (Fig. 1), leg. Lifu Qian, 8 August, 2016.

**Paratypes.** Seven males: AHU2016R002, AHU2016R003, AHU2016R004, AHU2016R005, AHU2016R006, AHU2016R007 and AHU2016R008, collected from the same locality as the holotype by Chencheng Wang between 15 and 20 August 2015. Two adult females, AHU2016R009 and AHU2016R010 collected by Lifu Qian at the same locality and time as the holotype.

**Diagnosis.** The new species is assigned to the genus *Rana* based on the morphological characteristics typical for this genus, including the possession of a prominent dorsolateral folds, dark temporal mask, and a body that is counter-shaded in various shades of brown. The species can be distinguished from its congeners by the following combination of morphological characteristics: (1) comparatively large body size (SVL 50.9–62.8 mm in males, N = 8 and females 53.0–68.3 mm, N = 2); (2) snout obtusely pointed in lateral view; (3) temporal fold distinct; (4) canthus rostralis distinct; (5) dark mask covering tympanum; (6) tympanum diameter equal to eye diameter (7) head length almost equal with head width (8) distinct transverse grayish brown bars on dorsal surface of lower arms, tarsus, thighs, and tibia; (9) dorsal skin smooth, small granules on legs, large tubercles absent; (10) tips of fingers not expanded, relative finger lengths III > I > IV > II, fingers webbing absent, toes two third webbed, toes webbing...
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Figure 3. Dorsal (A) and lateral view (B) of the holotype (AHU2016R001, male) of *Rana dabieshanensis* sp. n. in life.
formula I 2–1– II 2– 1† III 3–2 IV 2–2† V; (11) gray-blackish nuptial pad prominent and forming two groups in males, with minute nuptial spines; (12) external vocal sac absent; (13) a straight dorsolateral fold from temporal area to groin. (14) dorsum coloration varies from golden to brown.

**Description of Holotype.** SVL 62.8 mm. Head length is approximately equal to the head width (HL/HW = 1.01); snout long and rounded in profile, projecting a little beyond the lower jaw; internarial space equal to the interorbital space (INS/IOS = 1); diameter of the eye larger than the width of upper eyelid (ED/UE=1.33); canthus rostralis distinct; tympanum rounded, with the obvious tympanic rim; tongue deeply notched behind; external vocal sacs not discernable; pupil horizontal.

**Forelimbs:** forearm robust, fingers slender, finger webbing absent; fingertips obtuse with no expansion and lacking circummarginal grooves; relative finger lengths III > I > IV > II; one prominent subarticular tubercle on fingers I and II, two small subarticular tubercles on fingers III and IV; the inner metatarsal tubercle oval-shaped and elongated; outer metatarsal tubercle small rounded; the nuptial pad appeared on the finger I, covered by small black spines and divided into two groups, one near tip larger than the other one.

**Hindlimbs:** hind limbs long (HLL 129.1 mm, 205.7% of SVL), about 4.7 times than length of forelimbs (LAHL 27.6 mm, 43.9% of SVL); heels overlapping when limbs are held at right angles to body; the tibio-tarsal joint reaches beyond the snout-tip when the hind limb is stretched forward; the relative toe lengths IV > III > V > II > I; toes two third webbed, toes webbing formula: I 2 – 1– II 2– 1† III 3 – 2 IV 2 – 2† V and the webbing of the toe IV reaches as far as the penultimate distal joint; toe tips rounded, lacking circummarginal grooves; three tubercles on the IV toes, two tubercles on II III and V toes, one tubercle on I toe; the inner metatarsal tubercle ovoid, small but distinct; without outer metatarsal tubercles.

**Skin:** skin on dorsum is smooth while some small tubercles present on the body flanks and mouth angle; a mass of small tubercles on the dorsal surfaces of thighs and shanks while little warts on forelimb basis; a triangular gray patch behind the eye and anterior to the temporal fold; temporal fold distinct, extending from posterior margin of eye above and behind tympanum to above arm insertion; dorsolateral fold obvious and straight from the temporal area to groin; the throat, chest, belly and ventral surfaces of thighs being smooth with irregular black spots.

**Coloration:** in life and in preservative: in life, the iris is golden with a black pupil, two dark spots near pupil edges in the anterior and posterior edges of eye and a dark vertical bar in the lower half of iris; the color of the dorsal side changes according to environment, from golden to light brown; lip is golden brown with darker brown markings lasting from the area under the eye towards nostrils and snout tip; the mandible whitish with unclear gray spots; large triangular brown patch behind the eye and anterior to temporal fold; forelimbs dorsally the same color as the dorsal surface of body, with four faint ash black stripe in the forearm; the dorsum of the thigh and tibia is a grayish brown, with nine ash black stripes; the sides of the tarsus and foot are grayish brown with three ash black bars; throat, chest, and belly white.
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Figure 4. **A** volar view of the left hand of the holotype in life (AHU2016R001, male) **B** volar view of the right hand of female paratype in preservative (AHU2016R010, female) **C** thenar view of the right foot of the holotype in life (AHU2016R001, male) **D** thenar view of the right foot of the female paratype in preservative (AHU2016R010, female).

with irregular black spots; nuptial pad grayish brown. In preservative, dorsal surface gray-brown; all ash black fade to black; throat, chest, and abdomens fade to creamy white, with gray spots.
**Variation and sexual dimorphism.** Morphometric data were summarized in Table 4. Body size of males (SVL 50.1–62.8 mm) is smaller than that of females (SVL: 53.0–68.3 mm). Their dorsal color varied from golden to dark brown. Number of grayish brown crossbars on dorsal surface of lower arms, tarsus, thighs, and tibia varied. Nuptial pads are absent only in females.

**Measurements (in mm) of the holotype.** SVL: 62.8; HW: 17.6; HL: 17.8; SL: 8.4; IN: 5.5; IO: 5.5; UE: 3.6; ED: 4.8; TD: 4.0; LAHL: 27.6; LAD: 8.1; HAL: 14.8; HLL: 129.1; TL: 40.6; TW: 8.4; TFL: 53.2; FL: 35.5.

**Etymology.** The epithet of the new species “dabieshanensis” is a Latinized toponymic adjective derived from the Dabie Mountains in central China where the new species was discovered.

**Common names.** We recommend the “Dabie Mountain Brown Frog” as a common name of the new species in English; “Da Bie Shan Lin Wa” in Chinese.

**Ecological notes.** *Rana dabieshanensis* sp. n. appears closely associated with high altitudes of the southeastern mountains environments. Specimens were found at night between 20:00 and 01:00 h around a water pool in Yaoluoping National Nature Reserve, Yuexi, Anhui province, China (Figure. 1). The surrounding habitat consists of small hardwoods, mixed with shrubs and vines. Most of the specimens were found in grass nearby the water, few frogs were in the water. Air temperature was about 13.6 to 17.1 °C and water temperature about 12.1 °C to 14.7 °C. The relative humidity in this area was from 62 to 81%. Other amphibian species include *R. chensinensis*, *Rhacophorus anhuiensis*, *Pelophylax nigromaculata*, *Fejervarya multistriata*, and *Yerana yei* was also recorded during field survey in Yaoluoping National Nature Reserve (Pan et al. 2014).

**Distribution.** Currently, *Rana dabieshanensis* sp. n. is only found in the Yaoluoping National Nature Reserve (Anhui Province). This species might be found in other regions of the Dabie Mountains.

**Comparisons.** *Rana dabieshanensis* sp. n. differ from the Chinese species of the genus *Rana* by following morphological characters: (1) without black glandular ridge in scapular region (vs. an inverted V-shaped black glandular ridge in scapular region in *R. chaochiaoensis*, *R. hanluica*, *R. longicrus*, *R. omeimontis*, *R. maoershanensis*, *R. huanrenensis*, *R. japonica*, and *R. jiemuxiensis*); (2) smooth dorsum without tubercles (vs. many tubercles on the dorsolateral surface in *R. arvalis*, *R. amurensis*, *R. asiatica*, *R. dybowski*, *R. japonica* and *R. kukunoris*); (3) tympanum diameter equal to eye diameter (TD 3.5–5.2 mm, ED 4.1–5.7 mm N=8) (vs. tympanum diameter being 2/3 times of eye diameter in *R. chaochiaoensis* (TD 3.0–5.5 mm, ED 4.3–6.3 mm N = 22), *R. hanluica* (TD 3.5–4.8 mm, ED 5.2–7.8 mm N = 16), *R. omeimontis* (TD 4.0–5.5 mm, ED 5.4–6.9 mm N = 20), *R. zhenhaiensis* (TD 2.5–4.0 mm, ED 5.0–6.4 mm N = 25) and *R. culaiensis* (TD 3.4–4.3 mm, ED 5.1–6.7 mm N = 5), *R. japonica* (described by Stejneger and Matsui in 1907); tympanum diameter being 1/2 times of eye diameter in *R. maoershanensis* (TD 3.4–3.8 mm, ED 6.1–6.7 mm N = 3), *R. huanrenensis* (TD 1.9–3.0 mm, ED 4.0–7.0 mm N = 15), *R. kunyuensis* and *R. chensinensis* (TD 2.5–3.0 mm, ED 5.3–6.0 mm N = 8); tympanum diameter being 3/4 times of the eye diameter in *R. jiemuxiensis* (TD 2.5–4.1 mm, ED 2.8–4.2 mm)); (4) internarial distances almost equal to interorbital distances (IOS 3.9–5.5 mm, INS 3.9–5.5 mm N = 8) (vs. interorbital distances larger than internarial distances of in *R. chaochiaoensis* (IOS 5.2–8.2 mm,
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Figure 5. Typical habitat of Rana dabieshanensis sp. n. in Dabie Mountains, Anhui Province, China.

INS 2.7–4.7 mm N=20), R. jiemuxiensis (IOS 4.3–7.5 mm, INS 2.5–3.8 mm); internarial distances larger than interorbital distances of in R. maoershanensis (IOS 3.1–3.3 mm, INS 4.5–5.5 mm, N = 3)); (5) interorbital distances larger than width of upper eyelid in R. dabieshanensis (IOS 3.1–5.5 mm, UE 2.9–3.8 mm N = 8) (vs. interorbital distances almost equal to upper eyelid in R. hanluensis (IOS 3.3–4.5 mm, UE 3.1–4.3 mm N = 16), R. huanrenensis (IOS 2.9–4.0 mm, UE 3.0–4.0 mm N = 15) and R. chensinensis (IOS 2.9–4.0 mm, UE 3.0–4.0 mm N = 8); upper eyelid interorbital larger to interorbital distances in R. zhenhaiensis (IOS 2.3–3.4 mm, UE 3.5–4.5 mm N = 25), R. kukunoris (IOS 2.4–3.1 mm, UE 3.8–4.9 mm N = 5), R. dybowskii (IOS 3.0–4.0 mm, UE 4.1–5.8 mm N = 25) and R. amurensis (IOS 2.6–3.5 mm, UE 3.5–5.2 mm N = 21)); (6) distinct canthus rostralis (vs. not distinct canthus rostralis in R. longicrus); (7) the relative finger lengths IV > I > III > II in Rana dabieshanensis sp. n (vs. finger lengths III > IV > I > II of R. hanluica, R. luanchuanensis, and R. longicrus); (8) thicker lower arm, LAD 4.9–8.1 mm in males (LAD/SVL radio 0.13) and 5.1–5.3 mm in females (LAD/SVL radio 0.10) (vs. LAD 3.6–4.5 mm (LAD/SVL 0.09) in males (N = 8) and 3.4–4.5 mm (LAD/SVL 0.08) in females (N = 7) of R. chensinensis). (9) long hind limb (HLL 100.4–129.1 mm N = 8) (vs. in R. chaochiaoensis (HLL 92.0–100.0 mm N = 20), R. longicrus (HLL 70.8–84.8 mm N = 20), R. zhenhaiensis (HLL 73.4–100.0 mm N = 25), R. chensinensis (HLL 80.0–97.0 mm N = 8), R. kukunoris (HLL 80.0–99.0 mm N = 5), R. arvalis (HLL 61.1–82.4 mm N = 16) and R. huanrenensis (HLL 61.4–84.5 mm N = 15); (10) toes being webbed on two thirds (vs. toes fully webbed in R. chaochiaoensis and R. huanrenensis); (11) larger body sizes, SVL: males 50.9–62.8 mm, N = 8 and females 53.0–68.3 mm, N = 2 (vs. SVL: males 35.6–49.9 mm and females 34.1–53.6 mm in R.
jiemuxiensis, SVL: males 39.0–46.9 mm, N = 15 and females 42.4–49.0 mm, N = 8 in R. huanrenensis, SVL: males 27.2–33.0 mm, N = 12 and females 23.7–41.2 mm, N = 25 in R. luanchuanensis; (12) a straight distinct dorsolateral fold lasting from the temporal region to groin in Rana dabieshanensis sp. n (vs. dorsolateral fold curved above the tympanum of R. longicrus, R. zhenhaiensis, R. maoershanensis, R. jiemuxiensis, R. chensinensis, R. huanrenensis, R. arvalis, R. amurensis, R. asiatica, R. dybowski, R. kukunoris, R. kunyuensis R. luanchuanensis, and R. culaiensis).

Discussion

The Chinese species of the genus Rana were divided into three species groups based on external morphology (Fei et al. 2009); however, previous studies have been hampered by sampling restricted geographic regions or limited species groups with limited gene markers, mostly based on mtDNA. For example, the European species R. arvalis and Central Asian species R. asiatica once were considered belong to R. chensinensis group (Fei et al. 2009), while phylogenetic analyses indicated that these two species belonged to the same clade with R. temporaria. The recent progress on multilocus phylogeny of the genus Rana (Yuan et al. 2017, Zhao et al. 2017) indicated seven groups within the Chinese Rana sensu lato which contained 24 species (AmphibiaChina 2017, Zhao et al. 2017). Species in the R. longicrus group mostly occur in the southern part of China and Taiwan with no significant changes, except that the number of species in this group has increased. The species of the R. longicrus group appear to have highly conserved morphological characteristics compared to other species groups of Chinese Rana, implying that this group contains many cryptic species (Yan et al. 2011). In recent years, many species were identified based on molecular identification methods, providing a new understanding of the taxa that were once misidentified. For example, R. jiemuxiensis was distinguished from R. hanluica based on results of molecular analyses and differing breeding habits (Yan et al. 2011).

In the last decades, different opinions have been proposed on the distribution of Rana species in the Dabie Mountains of central China. Initially, the brown frog species found in the Dabie Mountains was identified as R. japonica Boulenger, 1879 (Zhao and Wu 1974). Subsequently, R. japonica was often mentioned in reports and surveys on amphibian fauna of this area (Lu et al. 1999, Shi et al. 2011, Zhao and Wu 1974, Zhang et al. 2000). However, later it was shown that R. japonica is only distributed in Japan, while Chinese populations belong to a different species (Maeda and Matsui 1989). In 2011, the R. zhenhaiensis was recorded in the Jintangtai National Nature Reserve (Shi et al. 2011) and the Huangbaishan National Forest Park (Wang et al. 2011) during the monitoring of amphibians in the Dabie Mountains. Subsequently, R. zhenhaiensis was identified as R. culaiensis in Huangbaishan National Forest Park through phylogenetic analyses (Zhao et al. 2015). In addition, the species of R. chensinensis was also mentioned as inhabiting in Dabie Mountains during the monitoring of amphibians (Pan et al. 2014), and phylogeography analysis on R. chensinensis had never sampled in this mountains area (Che et al. 2007). So, with the help of phylogenetic analyses, three
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species (Rana dabieshanensis sp. n., R. culaiensis, and R. chensinensis) are now known to inhabit the Dabie Mountains (Fang 2011, Zhao et al. 2015, Zhou et al. 2012).

During recent research in the Dabie Mountains, many endemic species like Moschus anhuiensis (Su et al. 2000) and Protobothrops dabieshanensis (Huang et al. 2012) were discovered, indicating that natural resources and animal diversity of this area are still insufficiently studied. With the addition of Rana dabieshanensis sp. n the genus now contains 103 known species and 25 species in China. To date, the new species is only known from a small montane area in Anhui Province of central China. However, its range might include other montane areas of central and southern China, so further surveys are urgently needed for investigation of the current distribution and population status of this species.

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