Matrilineal History of the *Rana longicrus* Species Group (*Rana*, Ranidae, Anura) and the Description of a New Species from Hunan, Southern China

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**Abstract** Fieldwork in Jiemuxi National Nature Reserve, Hunan, China, discovered morphologically indistinguishable groups of brown frogs that bred at different, exclusive times of the year. A principal components analysis of morphometoric data, molecular analyses, and the exclusive breeding season suggested the occurrence of two species. The population that breeds during the winter was found to be an undescribed species and was subsequently described as *Rana jiemuxiensis* sp. nov. The new cryptic species can be identified from its congeners at the same locality by having a different breeding season and a divergent DNA barcode. Six major lineages of brown frogs were resolved with high nodal support. *Rana japonica*, *R. chaochiaoensis*, *R. omeimontis*, and *R. jiemuxiensis* have independent maternal lineages. *Rana hanluica* and *R. maoershanensis* have essentially identical maternal lineages and they appear to represent the same species. The validity of the species *R. longicrus*, *R. zhenhaiensis*, and *R. culaiensis* and their genealogical relationships are not resolved and deserve further study. The genealogy indicates that sympatric cryptic lineages probably widely exist in the *R. longicrus* group. This highlights the importance of future more fine-scaled samplings and the inclusion of nuclear genes.

**Keywords** *Rana jiemuxiensis*, *Rana japonica* group, phylogeny, genealogy, DNA barcode, cryptic species

**1. Introduction**

Brown frogs of the genus *Rana* Linnaeus, 1758 (type species: *Rana temporaria* Linnaeus, 1758), also known as wood frogs, are mainly distributed from temperate Eurasia into Indochina, and western North America (Frost, 2010). This genus contains 48 species. Morphological similarities make many species notoriously difficult to identify (Che *et al.*, 2007; Liu and Hu, 1961), especially when closely related species have overlapping distributions (personal observation).

Presently, 22 species of *Rana* are recognized from China (Frost, 2010; Yang *et al.*, 2010), six of which are assigned to the *R. longicrus* group by Fei *et al.*, (2009) (also called the *R. japonica* group) as follows: *R. chaochiaoensis* Liu; *R. chevronata* Hu et Ye; *R. hanluica* Shen, Jiang et Yang; *R. longicrus* Stejneger; *R. omeimontis* Ye et Fei, and *R. zhenhaiensis* Ye, Fei et Matsui. In addition, two recently described species, *R. culaiensis* Li, Lu et Li and *R. maoershanensis* Lu, Li et Jiang, are considered to be junior synonyms of *R.
zhenhaiensis and R. chaochiaensis, respectively (Fei et al., 2009).

The R. longicrus group is widely distributed in southern China including Taiwan. Pope and Boring (1940) consider most of the species as R. japonica Boulenger 1879. The recent surge in new species descriptions (Li et al., 2008; Lu and Li, 2002; Lu et al., 2007; Shen et al., 2007) suggests that diversity within the group may be underestimated, especially in poorly investigated forested regions of southern China. The morphology appears to be highly conserved, assuming that the group contains many cryptic species. Alternatively, many of the newly described species may not be valid. The reconstruction of their evolutionary history should shed light on the problem. Herein, DNA barcoding with the cytochrome c-oxidase subunit I (COI) (Hebert et al., 2003) and cytochrome b (Cyt b) genes is used to identify genetically distinct lineages worthy of more intense taxonomic study (Smith et al., 2008) and to hypothesize the groups’ matrilineal history.

During recent herpetofaunal surveys in Hunan province, we collected brown frogs in Jiemuxi National Nature Reserve during two mutually exclusive breeding seasons at one locality. This suggested either the presence of two breeding seasons for the species, or two cryptic species with different breeding seasons. The frogs that bred at different seasons could not be readily distinguished morphologically. To differentiate between these possibilities, we formed a testable hypothesis. Our null hypothesis of conspecificity could not be rejected by DNA barcodes alone because mitochondrial DNA cannot document the absence or presence of gene flow. The null hypothesis could be rejected by using DNA barcodes as an indicator of distinctiveness, matrilineal history to infer the co-occurrence of lineages in breeding frogs, and morphological and ecological data as indicators of niche partitioning. For example, the presence of exclusive lineages during a particular breeding season would indicate lineage segregation and thus serve to reject the null hypothesis. Either the absence of discrete lineages or the sharing of lineages in the two breeding seasons would fail to reject the null hypothesis. Similarly, morphological distinctiveness can also be used to reject the null hypothesis. The investigation involved all the other species in the R. longicrus group widely distributed in southern China, except for R. chevronata, and Japan.

2. Materials and Methods

2.1 Sampling From 2008 to 2010, field surveys were conducted in southern China covering the entire range of the R. longicrus group including Shandong, Henan, Jiangsu, Zhejiang, Anhui, Jiangxi, Hubei, Hunan, Fujian, Guangdong, Guangxi, Yunnan, Sichuan, and Guizhou provinces plus Chongqing Municipality. Adults, juveniles, and tadpoles were collected. Following euthanization,
tissues dissected from adult specimens were preserved in 95% ethanol. Voucher specimens, including juveniles and tadpoles, were fixed in 10% buffered formalin, and all metamorphosed frogs were later transferred to 70% ethanol. All specimens were deposited in the Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ). Comparative tissues from Taiwanese samples were provided by the National Museum of Natural Science (NMNS) Taiwan, China. Sampling data, which involved type localities, were summarized in Table 1 and Figure 1. *Rana chensinensis*, *R. huanrenensis*, *R. kukunoris*, *R. dybowskii*, and *R. amurensis* were used as outgroup taxa in the genealogical analyses according to their phylogenetic positions as hypothesized by Che et al. (2007).

### 2.2 Morphometrics

A total of 109 specimens including five species were examined (Table 2); *R. chevronata* has not been seen since its description. Measurements were
Table 2  Linear measurements (in mm) of five Chinese species of brown frogs, genus Rana used in principal components analysis. The feature abbreviations are provided in text

<table>
<thead>
<tr>
<th>Feature</th>
<th>R. chaochiaoensis</th>
<th>R. hanluica</th>
<th>R. jiemuensis</th>
<th>R. omeimontis</th>
<th>R. zhenhaiensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVL</td>
<td>40.22–59.91</td>
<td>49.89±6.94</td>
<td>46.41–68.58</td>
<td>58.97±7.66</td>
<td>34.11–53.57</td>
</tr>
<tr>
<td>HL</td>
<td>12.12–18.51</td>
<td>15.84±2.09</td>
<td>14.61–25.16</td>
<td>18.54±2.65</td>
<td>10.37–17.38</td>
</tr>
<tr>
<td>SL</td>
<td>6.64–9.36</td>
<td>7.97±0.88</td>
<td>7.47–11.27</td>
<td>9.50±1.21</td>
<td>4.05–12.50</td>
</tr>
<tr>
<td>EL</td>
<td>4.37–6.37</td>
<td>5.11±0.59</td>
<td>4.26–6.57</td>
<td>5.71±0.60</td>
<td>2.82–6.16</td>
</tr>
<tr>
<td>IND</td>
<td>2.72–4.70</td>
<td>3.67±0.54</td>
<td>3.40–5.21</td>
<td>4.25±0.42</td>
<td>2.54–3.81</td>
</tr>
<tr>
<td>IOD</td>
<td>5.16–8.24</td>
<td>6.67±0.95</td>
<td>6.44–9.67</td>
<td>7.85±0.84</td>
<td>4.31–7.50</td>
</tr>
<tr>
<td>TYE</td>
<td>2.96–5.24</td>
<td>3.73±0.56</td>
<td>3.15–5.61</td>
<td>4.50±0.69</td>
<td>2.54–4.08</td>
</tr>
<tr>
<td>TLE</td>
<td>3.00–5.53</td>
<td>4.45±0.85</td>
<td>3.18–5.94</td>
<td>4.83±0.64</td>
<td>2.48–4.11</td>
</tr>
<tr>
<td>FL1</td>
<td>5.08–7.68</td>
<td>6.18±0.84</td>
<td>5.27–9.19</td>
<td>7.09±1.15</td>
<td>3.78–7.26</td>
</tr>
<tr>
<td>FL2</td>
<td>4.12–6.90</td>
<td>5.29±0.75</td>
<td>4.48–7.53</td>
<td>5.93±0.90</td>
<td>2.77–6.76</td>
</tr>
<tr>
<td>FL3</td>
<td>6.45–9.57</td>
<td>7.53±0.82</td>
<td>6.93–12.04</td>
<td>8.94±1.16</td>
<td>5.40–9.37</td>
</tr>
<tr>
<td>FL4</td>
<td>4.34–6.65</td>
<td>5.38±0.73</td>
<td>4.33–7.25</td>
<td>5.83±0.79</td>
<td>3.16–6.32</td>
</tr>
<tr>
<td>FEL</td>
<td>22.17–35.22</td>
<td>29.91±4.72</td>
<td>27.78–43.75</td>
<td>36.51±6.52</td>
<td>18.27–31.51</td>
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<tr>
<td>TL</td>
<td>20.29–37.29</td>
<td>31.39±5.11</td>
<td>10.70–47.26</td>
<td>38.43±7.29</td>
<td>19.98–34.77</td>
</tr>
<tr>
<td>FOL</td>
<td>22.99–34.07</td>
<td>28.47±3.46</td>
<td>25.78–36.87</td>
<td>33.06±7.21</td>
<td>15.54–34.58</td>
</tr>
<tr>
<td>TL1</td>
<td>2.38–3.46</td>
<td>2.67±0.28</td>
<td>2.18–4.39</td>
<td>3.52±0.56</td>
<td>1.75–3.51</td>
</tr>
<tr>
<td>TL2</td>
<td>3.11–6.56</td>
<td>4.58±0.87</td>
<td>4.20–7.20</td>
<td>5.71±0.79</td>
<td>2.96–7.12</td>
</tr>
<tr>
<td>TL3</td>
<td>6.88–10.77</td>
<td>8.94±1.18</td>
<td>8.23–12.76</td>
<td>10.8±1.28</td>
<td>3.12–10.13</td>
</tr>
<tr>
<td>TL4</td>
<td>12.79–19.95</td>
<td>16.24±2.21</td>
<td>15.43–24.00</td>
<td>19.47±2.17</td>
<td>10.30–19.58</td>
</tr>
<tr>
<td>TL5</td>
<td>7.58–12.73</td>
<td>9.58±1.47</td>
<td>9.01–14.36</td>
<td>12.08±1.56</td>
<td>4.12–11.66</td>
</tr>
</tbody>
</table>

made by one person using dial calipers with a precision of 0.01 mm. Twenty-three linear measurements were taken as follows: SVL (snout-vent length), HL (head length), HW (head width), SL (snout length), EL (eye length), IND (interdorsal distance), IOD (interorbital distance), UWE (upper eyelid width), TYE (tympanic outer diameter), LAL (lower-arm length), FL1 (first finger length), FL2 (second finger length), FL3 (third finger length), FL4 (fourth finger length), FEL (femur length), TL (tibia length), TFL (length of foot and tarsus), FOL (foot length), TL1 (first toe length), TL2 (second toe length), TL3 (third toe length), TL4 (fourth toe length), and TL5 (fifth toe length). Because only three of the 23 morphological characters differed significantly between females and males (TYE, FL2, and FL3; t-test, P < 0.05), data from females and males were combined for a principal components analysis (PCA) implemented in SPSS 13.0.

2.3 Extraction, amplification and sequencing

Total genomic DNA was extracted from liver or muscle tissue using the standard phenol–chloroform protocols (Sambrook et al., 1989). A fragment of mitochondrial cytochrome oxidase subunit I (COI), the standard barcoding marker, was used to amplify all samples, together with Cyt b. Amplification was performed in a 25 μL volume reaction with the following procedures: initial denaturation step for 5 min at 95 °C, 35 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 50 °C for Cyt b and 46°C for COI, extension for 1 min at 72 °C. Final extension at 72 °C was conducted for 10 min. The primers for COI are available from the authors upon request. Sequences of Cyt b were amplified with the primers 14850-1 (Tanaka-Ueno et al., 1998), CytbB and CytbC (Bosuuyt and Milinkovitch, 2000). PCR products were purified with Gel Extraction Mini Kit, and sequenced in both directions with BigDye Terminator Cycle Sequencing Kit and ABI PRISM 3730. The sequence data were submitted to a BLAST search in GenBank to confirm identity. Nucleotide sequences were aligned using Clustal X 1.81 (Thompson et al., 1997) with default parameters, and then optimized by eye in MEGA 4.0 (Tamura et al.,...
2.4 Phylogenetic analyses Genealogical reconstructions using Bayesian inference (BI) and maximum parsimony (MP) considered COI and Cyt b separately and concatenated into a single partition. BI analysis was executed in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) using models estimated with MrModeltest v2.3 (Nylander, 2004). Consensus frequencies, i.e., Bayesian posterior probabilities (BPP), were used to estimate nodal support. Four separate runs were performed with four Markov chains. Each run was conducted for 3 000 000 generations and sampled every 100 generations. Log-likelihood scores were tracked for stabilization and the first 25% of the trees were discarded as burn-in. A 50% majority rule consensus tree was calculated from the remaining trees. MP analyses were implemented using PAUP 4.0b10a (Swofford, 2003). The heuristic MP searches were executed for 1000 replicates with all characters treated as unordered and equally weighted. One thousand pseudoreplicates were implemented for bootstrap analyses.

3. Results and Discussion

3.1 Morphometrics The measurements of all specimens were summarized in Table 2. The first three factors explained 87.3% of the total variance. The first factor alone explained 81.9% of the variance, with large loading for the following 13 characters (the load factor > 0.5): SVL, HL, HW, SL, IND, IOD, UEW, FL1, FL2, FL3 FL4, FEL, and FTL. The second factor explained another 3.2% of the total variance, with the following 15 large loading characters: SVL, HL, HW, FEL, IND, IOD, TYE, LAL, EL, TL, FTL, FOL, TL3, TL4, and TL5. For the third factor, 2.3% variance was explained with large loading for the following 11 characters: SVL, LAL, FL3, FEL, FTL, FOL, TL1, TL2, TL3, TL4, and TL5. The plots from PC1 and PC2 did not completely define the five species. However, the new population from Jiemuxi National Nature Reserve (open squares) was clearly resolved and well differentiated from R. hanluica (X-pattern; Figure 2). This analysis indicated that the two breeding groups did not constitute a single species.

3.2 Genealogy and taxonomy Eighty unique sequences were newly determined and deposited in GenBank (Table 1). The sequences contained about 735 bp of coding sequences from Cyt b and 561 bp from COI. No indels were observed. Alignment of the concatenated sequences contained 1296 bp of which 444 sites were variable and 385 were potentially phylogenetically informative.

The MP and BI analyses produced very similar trees; only the Bayesian tree was shown (Figure 3). The

![Figure 2](image-url) Principal components analysis (PCA) of 23 morphometric variables for 109 individuals of five species (R. jiemuxiensis sp. nov., R. oneimontis, R. hanluica, R. chaochiaens, and R. zhenhaiensis). Positive PC 1 scores indicate a proportionately longer trunk, larger head, longer snout, larger internarial distance, larger interorbital distance, wider upper eyelid, and longer finger. Positive PC 2 scores correspond to a proportionately longer trunk, larger head, longer eye, larger internarial distance, larger interorbital distance, larger tympanum, longer lower-arm, longer foot and toe.
Figure 3 A Bayesian inference tree based on the combined mitochondrial CO1 and Cyt b nucleotide sequence data. Bayesian posterior probabilities > 90% are shown near the respective branches. Localities of corresponding samples are indicated in brackets. * = type locality.
longicrus complex, including *R. japonica* from Japan, was resolved as an exclusive lineage with high nodal support. Six distinct lineages (I–VI) were recovered within this group but with unresolved relationships among them. *Rana japonica*, *R. chaochiaoensis*, and *R. omeimonitis* constituted exclusive lineages I, II, and IV, respectively.

The two breeding groups from Jiemuixi National Nature Reserve were exclusively assigned to lineages III and V. There was no mixing of haplotypes within the breeding groups at the nature reserve. Lineage V exhibited substantial divergence from lineage III and the other groups (Figure 2). The null hypothesis of conspecificity required non-exclusive maternal lineages within the reserve because lineage segregation is not expected within a species. The presence of two exclusive lineages that breed at different seasons, combined with the morphometric analysis, required rejection of the null hypothesis and the description of a new species, as provided below.

The genealogical relationships were not only informative with respect to the identity of species at Jiemuixi National Nature Reserve, but they also had additional implications for the validity of some other species. Within lineage III, some individuals from Jiemuixi National Nature Reserve clustered with *R. hanluica* and *R. maoershanensis*. Genetic samplings of the named taxa are from their respective type localities. The identical and nearly identical haplotypes in these three species suggested extremely recent isolation events, introgressive hybridization, or on-going gene flow. In the first case, species recognition was not justified because isolation, if present, was likely to be ephemeral; isolation likely owed to the current interglacial period. In the hybridization scenario, the absence of genetic and morphological differentiation did not support this possibility. Consequently, our analyses suggested that all members of lineage III were best considered to be one species. Based on rules of priority in the Code, we recognized lineage III as *R. hanluica*.

*Rana maoershanensis* was initially reported as a new distribution record of *R. chaochiaoensis* from Guangxi (Lu et al., 2006), but later described as a new species (Lu et al., 2007). Fei et al. (2009) did not recognize *R. maoershanensis* and our study suggested it is a junior synonym of *R. hanluica*. This documentation contrasts sharply with that of Yang et al. (2011) who used the mitochondrial gene 16S to evaluate the status of *R. maoershanensis*. Unfortunately, their analysis recovered only one species in the region and the data base contained only 58 potentially informative sites for 14 species of frogs. As a consequence of the limited data, most nodes at the base of their BI, MP and maximum likelihood trees were not supported. This precluded a meaningful comparison of our tree with their tree. Nevertheless, their report of *R. maoershanensis* outside of the *R. japonica* complex suggests that the region might contain another species of *Rana*.

Cryptic species may be common within the complex. *Rana chaochiaoensis* (lineage II) is very distinct (Figure 2) and this suggests that the species is valid. Although an assessment of nuclear genes is required to confirm the species’ validity, the association of *R. maoershanensis* with *R. chaochiaoensis* (Lu et al., 2006) suggests that clear morphological diagnoses may not be common in the group. The absence of morphological differentiation does not preclude recognition of species; cryptic species are a reality and their recognition serves both evolutionary and conservation biology.

Our genetic analyses discover that the taxonomy of the brown frogs is far more complicated than originally imagined. Two genetic lineages that constitute two species of brown frogs from Maelershan (III, VI) are clearly recovered herein, and these correspond to our field observations of two different breeding seasons. Frogs in lineage III at Maelershan initiate breeding around October although the exact time varies depending on local climate. Within this group, breeding can continue to December. We have observed tadpoles under the ice during winter, which is consistent with the observations of Lu et al. (2007). The breeding season of specimens in lineage VI starts in February and the same pattern occurs at Yangmingshan. Here, it also appears that two cryptic species occur sympatrically and this may be a general pattern.

Lineage VI covers a wide range in China. Specimens of *R. longicrus*, *R. zhenhaiensis*, and *R. culaiensis* from their respective type localities all nest in this group. Their conservative morphology makes the correct determination of species challenging, especially in southern China. Most often, these species are identified by locality data only. *Rana zhenhaiensis* occurs in most regions of southeastern China (Fei et al., 2009). Our analyses suggest that specimens of *R. zhenhaiensis* from Fujian and Guangdong provinces might best be assigned to *R. longicrus* because the mtDNA is virtually identical, and *R. longicrus* branches off within *R. zhenhaiensis*. Further, two individuals of *R. zhenhaiensis* from Hubei and Henan cluster with *R. culaiensis*. Wood frogs from Xinhua, Yangmingshan (Hunan), and Maelershan (Guangxi) cluster together within lineage VI. *Rana zhenhaiensis* from type locality (Ningbo) forms its own sublineage within
lineage VI. Although this pattern is interesting, our sparse geographic sampling within lineage VI, and the exclusive use of mtDNA sequences, hinders an evaluation of the taxonomic status of these species, especially considering that sympatric cryptic lineages are common. Previous studies based on limited sampling (e.g., Jiang et al., 2001; Yang et al., 2001; Che et al., 2007) must be verified and collections should be made at different seasons of the year. Molecular data should be combined with morphology, ecology, and acoustic studies. This approach will likely reveal additional cryptic species.

A more fine-scale sampling from southern China should be undertaken in order to understand the diversity and relationships among the species of wood frogs in the R. longicrus species group. Inclusion of additional genes, especially nuclear genes, would be essential. mtDNA data alone cannot assess introgression and gene flow.

4. Taxonomy

Rana jiemuxiensis sp. nov. (Figures 4, 5)

Holotype KIZ 05553 (Figure 5), an adult male from Jiemuxi National Nature Reserve (28°52′45″ N, 110°24′608″ E, elevation 723 m), western Hunan, China. Collected by Fang YAN and Zhongmei ZHANG from 10 to 19 March 2010.

Paratypes KIZ05245–KIZ05269, KIZ05270–KIZ05272, KIZ05551, KIZ05554–KIZ05556 with the same collection date and locality as the holotype. A total of 32 adult individuals, including 22 males and 10 females.

Diagnosis Rana jiemuxiensis sp. nov. can be distinguished from all other species of the genus Rana by the following combination of characters: (1) dorsolateral fold from posterior canthus to crotch, slight curving above the tympanic membrane; (2) tips of fingers obtuse without disks; (3) three metacarpal tubercles in males, inner one covered partly by the nuptial pad at the base of the first finger, the two outer ones combined at the base of the third and fourth fingers; (4) gray-whitish nuptial pad developed, and divided into three groups; (5) five to six wide brownish-black bands at thigh and tibia; (6) lineae musculinae absent in males; (7) tibio-tarsal extends beyond the snout; (8) breeding season from the end of late February to middle March.

Etymology The species is named for the type locality, Jiemuxi, Yuanling County, Hunan.

Description of holotype SVL 48.48 mm. Head slightly longer than broad, snout pointed and projecting; from the anterior orbit to snout tip much longer than eye diameter; distance between nostril and anterior orbit longer than distance between snout-tip and nostril; interorbital distance equal to upper eyelid width; tympanum diameter slightly shorter than eye diameter; tympanic rim narrow, but prominent; loreal region concave, sloping outwards; vomerine teeth in short oblique series, anterior edges in line with centers of choanae; tongue notched posteriorly, not deeply; internal vocal sacs and lineae musculinae absent.

Forearm robust, tips of fingers obtuse, not expanded, without circummarginal grooves; relative length of fingers: II < IV < I < III; one prominent subarticular tubercle on fingers I and II, two small subarticular tubercles on fingers III and IV; inner metatarsal tubercle strong and larger, ovoid, partly covered by nuptial pad at base of finger I; two outer tubercles combined at base of fingers III and IV, flat and weak; strong nuptial pad divided into three groups, basal one covering anterior inner metatarsal tubercle, largest one ventrally extending from base of palmar onto medial dorsal surface of finger I to anterior edge of subarticular tubercle, the smallest one extending from anterior of largest nearly to tip of finger; nuptial pad with off-white dense spinules.

Hindlimb long, tibio-tarsal joint beyond snout-tip; indistinct skin ridge on dorsum of tibia; heels overlapping more when legs are held perpendicular to body; tips of toes similar to fingers; relative length of toes: I < II < III < V < IV; toes webbed fully, the web of toe IV reaching the second joint from tip; subarticular tubercles distinct, especially joint of toe and metatarsus; inner metatarsal tubercle andovoid, small but well developed; outer metatarsal tubercle indistinct.

Skin rather smooth; some small granules on body side or near vent; temporal fold distinct, a somewhat triangular gray patch behind eye and anterior to temporal fold; thickened dorsolateral fold from posterior canthus to crotch, slightly curved at upper tympanum and crossing temporal fold; elongate rectal gland reaching temporal fold and expanding; ventral surface smooth; some somewhat whitish granules on the posterior ventral surface of femurs.

Coloration Dorsum of body and body side grayish brown (gray in ethanol), with scattered black spots; the side of snout and upper lip before tympanum brown (grayish black in ethanol); lower lip whitish with black spots; forelimb grayish brown (gray in ethanol), two indistinct black bars on dorsum of each arm; dorsum of hand with white areas; dorsum of thigh and tibia grayish brown (gray in ethanol), with four and three black bars, respectively;
Figure 4 (a) Lateral view of mating *R. jiexiixiensis* on land during the breeding season. (b) Lateral view of mating *R. jiexiixiensis* underwater during the breeding season. (c) Lateral view of a male *R. jiexiixiensis*. (d) Lateral view of a female *R. jiexiixiensis*.

Figure 5 Holotype specimen of *R. jiexiixiensis*. (a) Dorsal view. (b) Ventral view. (c) Details of right hand showing the nuptial pad. (d) Details of right foot showing the extent of webbing.
anterior thigh and leg with black spots; tarsus and foot side grayish brown (gray in ethanol), tarsus with three black bars; dorsum of foot whitish with black mottling; throat and belly whitish with black mottling.

**Habitat and breeding behaviour** The breeding season is from February to March with a peak in early March. The exact timing can be influenced by either precipitation or human agricultural activities. Breeding individuals grouped at a nearby rice paddy, and the water came from either rainfall or irrigation (Figure 6). Since 2008, breeding lasted only for about ten days. The number of attending males always exceeded that of females. Males, which possess an obvious nuptial pad, always occupied a location to attract females with calls. Egg masses contained 200–1500 eggs. No parental care was observed. In 2010, froglets appeared in May, suggesting that development to metamorphosis required about two months.

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