Two new species and the molecular phylogeography of the freshwater crab genus *Bottapotamon* (Crustacea: Decapoda: Brachyura: Potamidae)

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**ABSTRACT**

*Bottapotamon chenzhouense* sp. n. and *B. luxiense* sp. n. are described from Hunan Province and Jiangxi Province, respectively. These species both have diagnostic features of the genus *Bottapotamon* and discernible characteristics as new species. *B. chenzhouense* sp. n. can be distinguished from co-geners by features such as the G1, which has a fold covering the surface of the entire subterminal article with a distal region. *B. luxiense* sp. n. has an elliptical carapace, and a sturdy and blunt terminal article of G1. The molecular phylogeny and biogeography of the genus *Bottapotamon* (Decapoda: Brachyura: Potamidae) were studied, using mitochondrial cytochrome oxidase I (mtDNA COI), 16S rRNA and nuclear histone H3 gene fragments. The results support the assignment of the two new species to the genus *Bottapotamon*. In addition, the divergence time of the genus *Bottapotamon* was estimated to be 3.49–1.08 Ma, which coincided with various vicariant and dispersal events that occurred in the geological area where the genus *Bottapotamon* is commonly distributed. Mountains appear to have played an important role in the distribution of this genus. The Wuyi Mountains gradually formed offshore and inland of southeastern China by the compression of the Pacific plate and the Indian plate in the Neogene-Quaternary, and the Luoxiao Mountains formed continuously in the continued forming in the north-south direction because of neotectonic movement, have resulted in the geographical distribution pattern of the genus *Bottapotamon*, which was also established gradually.

**INTRODUCTION**

The genus *Bottapotamon* is a unique genus of freshwater crabs from the China mainland. In 1997, three species of the genus *Malayopotamon* on (*Bott*, 1967; *Cheng, Lin & Luo*, 1993; *Dai et al.*, 1979) and one new species were identified as *Bottapotamon* on the basis of its morphological characteristics, such as the form of carapace and first gonopod (G1) (*Türkay & Dai*, 1997). Until the current study, the genus *Bottapotamon* contained *B. fukiense* (*Dai et al.*, 1979), *B. engelhardti* (*Bott*, 1967), *B. yonganense* (*Cheng, Lin & Luo*, 1993) and *B. chenzhouense* sp. n. and *B. luxiense* sp. n.
The relatively low fecundity and poor dispersal abilities of freshwater crabs (Daniels et al., 2003; Yeo et al., 2008) mean that these crabs are easily isolated by barriers such as mountains or seas. Geographically isolated populations then become genetically natural distinct and result in allopatric speciation (Shih et al., 2006; Yeo et al., 2007). In mainland China, the distribution of the genus *Bottapotamon* is restricted within the area of the Wuyi Mountain Range; *B. engelhardti*, *B. yonganense*, *B. youxiense* and *B. nanan* are distributed east of the Wuyi Mountain Range, *B. fukiense* occurs on both sides of the Wuyi Mountains (Fujian and Jiangxi Provinces), and only *B. lingchuanense* has been isolated in the Nanling Mountain Range (Dai, 1999) (Fig. 1). The geographic barrier separating the Wuyi Mountains from the Nanling Mountains is the Luoxiao Mountain Range, which is the highest range in the area, exceeding 2,120 m in height (Gong, Zhuang & Liao, 2016). The terrain the genus *Bottapotamon* now inhabits is geologically relatively stable and experienced little orogenic activity during the Cenozoic Era (Yi, 1996; Zhou & Li, 2000). Therefore, we hypothesize that the current distribution of the genus *Bottapotamon* in mainland China was caused by the emergence of these mountains.

While organizing the existing specimens deposited at the Department of Parasitology of the Medical College of Nanchang University (NCU MCP) and the newly collected specimens, the first and third author discovered two new species collected from Chenzhou City, Hunan Province, and Luxi County, Jiangxi Province, respectively. This paper compares the morphological features of eight species including two new species of the genus *Bottapotamon*, as well as 16S rRNA (Crandall, Fitzpatrick & Faith, 1996), mtDNA
COI (Folmer et al., 1994) and nuclear histone H3 (Colgan et al., 1998) gene fragments that are used to support the establishment of new species in the genus Bottapotamon. The phylogenetic relationship, distribution pattern and possible association with major geological and historical events are also discussed.

MATERIALS & METHODS

Specimens collection

Specimens from Jiangxi, Zhejiang, Fujian and Guangxi, were recently collected and preserved in 95% ethanol. The remaining specimens used in this study were from and deposited at the Department of Parasitology of the Medical College of Nanchang University (NCU MCP), Jiangxi Province, China. The authors compared specimens with holotypes of the National Zoological Museum of China, Chinese Academy of Sciences (CAS). All 26 specimens were used for mtDNA COI, 16S rRNA and histone H3 gene fragment amplification (Table 1).

Phylogenetic analyses and Divergence time estimation

Genomic DNA was extracted from leg muscle tissue with an OMEGA EZNA™ Mollusc DNA Kit. The 16S rRNA, mtDNA COI, and histone H3 regions were selected for amplification by polymerase chain reaction (PCR) (Table 2). The amplification products were sent to the Beijing Genomics Institute for bidirectional sequencing, and the sequencing results were spliced manually to obtain the sequence data. DNA sequences of B. yonganense specimens collected from the suburb of Sanming City, Fujian Province, China, could not be amplified due to poor preservation.

The sequences of four individuals with the same primer sequences were selected from National Center for Biotechnology Information (NCBI) database, as the outgroups (Candidiopotamon rathbunae (GenBank accession numbers: mtDNA COI—AB290649, 16S rRNA—AB208609, histone H3—AB290668), Geothelphusa dehaani (GenBank accession numbers: mtDNA COI—AB290648, 16S rRNA—AB290630, histone H3—AB290667), Himalayapotamon atkinsonianum (GenBank accession numbers: mtDNA COI—AB290651, 16S rRNA—AB290632, histone H3—AB290670), and Ryukyum yaeyamense (GenBank accession numbers: mtDNA COI—AB290650, 16S rRNA—AB290631, histone H3—AB290669). After comparing and selecting the conservative regions, each sequence was 1323 bp in length. According to the Akaike information criterion (AIC), MrMTGui: ModelTest and MrModelTest (phylogenetic analysis using parsimony (PAUP)) determined the best models was GTR+I+G; MEGA 6.06 (Tamura et al., 2013) was used to establish a phylogenetic tree based on the maximum likelihood (ML) (Trifinopoulos et al., 2016). The Bayesian inference (BI) tree was established using MrBayes (Ronquist & Huelsenbeck, 2003).

The divergence times of genus Bottapotamon were estimated from the combined 16S rRNA and mtDNA COI sequences, based on the Bayesian evolutionary analysis sampling trees (BEAST) program, and four calibration points were used. The Potamidae family has been divided into two major subfamilies, Potamiscinae and Potaminae, estimated to have a divergence time of 20.9–24.7 Ma, which was set as calibration point 1 in our
Table 1  Specimens and GenBank accession numbers of genus *Bottapotamon*.

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Study (Shih, Yeo & Ng, 2010). From the Parathelphusidae subfamily, *Somanniathelphusa taiwanensis*, which is distributed in Taiwan Island and separated from *Somanniathelphusa amoyensis*, which is distributed in Fujian Province, for approximately 0.27–1.53 Ma (Jia...
Table 2  Primer sequences used in this study.

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<th>Gene</th>
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<td>GGTCAACAAATCATAAAGATATTGG</td>
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<td>Folmer et al. (1994)</td>
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<td>COI-2198</td>
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<td>16S rRNA</td>
<td>16S-1471</td>
<td>CCTGTTTANCAAAAAACAT</td>
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<td>Crandall, Fitzpatrick &amp; Faith (1996)</td>
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<td>16S-1472</td>
<td>AGATAGAAACCAACCTGG</td>
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<td>H3</td>
<td>H3-F</td>
<td>ATGGGCTCGTACCAAGCAGACVGCG</td>
<td>374bp</td>
<td>Colgan et al., 1998</td>
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<td>H3-R</td>
<td>ATATCCTTRGCGATRATRGTCGAC</td>
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This is consistent with the quaternary glacial period and interglacial period and agrees with the separation of Taiwan Island and Fujian Province; this time point was set as calibration point 2. In the geological area where genus *Bottapotamon* is distributed, the Wuyi Mountains gradually formed by the compression of the Pacific plate and the Indian plate in the Neogene-Quaternary (1.64–23.3 Ma) (*Li, 1984*); this time point was set as calibration point 3. A Yule speciation model was constructed for speciation within the genus *Bottapotamon*. We used a GTR+G model with parameters obtained from MrMTGui: ModelTest and MrModelTest (PAUP) for each gene. Seventeen independent MCMC chains were run for 200,000,000 generations, and every 20,000 generations were sampled. The convergence of the 17 combined chains was determined by the evolutionary stable strategy (ESS) (>200 as recommended) for each parameter in Tracer after the appropriate burn-in and cutoff (default of 10% of sampled trees). Trees in the 17 chains were combined using LogCombiner (v.1.6.1, distributed as part of the BEAST package) and were assessed using TreeAnnotator (v.1.6.1, distributed as part of the BEAST package). A chronogram was constructed by FigTree.

**Nomenclatural note**

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: [urn:lsid:zoobank.org:pub:211926FF-6950-4DFE-95C4-F5247CA9E0BA]. The online version of this work is archived and available from the following digital repositories: Peer J, PubMed Central and CLOCKSS.
RESULTS

Systematics

Potamidae Ortmann, 1896
Bottapotamon Tüerkay & Dai, 1997

*Bottapotamon chenzhouense* sp. n. Gao, Cui & Zou (*Figs. 2–6*)

urn: lsid:zoobank.org:art:E43C4BBB-E429-4C17-8ACD-E4295F426BCB

Materials examined

Holotype: 1♂ (20.67 × 15.60 mm) (NCU MCP 643), Huangcao Village, Chenzhou City, Hunan Province, China, 25°39'24.60" N, 113°30'4.07" E, 141 m asl. Coll. Ding-mei Luo, July 26th, 2006. Paratypes: 1♀ (18.64 × 14.62 mm) (NCU MCP 643), the same data as the holotype.

Comparative materials

*B. fukiense* (*Dai et al., 1979*): 2♂ (15.66 × 12.64 mm, 13.15 × 10.26 mm) (NCU MCP 4089), Xiapu Village, Ningde County, Fujian Province; 1♂ (13.26 × 11.05 mm) (NCU MCP 4156), Shangshan Village, Zhenghe County, Fujian Province; 1♂ (22.93 × 17.67 mm) (NCU MCP 4090), Siqian Village, Shouning County, Fujian Province; 1♀ (19.26 × 15.70 mm) (NCU MCP 4090), Shangshan Village, Zhenghe County, Fujian Province. *B. engelhardti* (*Bott, 1967*): 3♂♂ (15.32 × 11.90 mm, 17.08 × 13.46 mm, 18.85 × 15.01 mm) (NCU
**Figure 3** *Bottapotamon chenzhouense* sp. n. Holotype male (20.67 × 15.60 mm) (NCU MCP 643). (A) Left third maxilliped; (B) right fourth ambulatory leg; (C) outer view of chelipeds. Photograph taken by Jie-Xin Zou, November 2018.

MCP 4157), Tangsan Village, Youxi County, Fujian; 3♂♂ (16.23 × 13.78 mm, 17. 50 × 14.41 mm, 14.86 × 11.18 mm) (NCU MCP 4091), Chimu Village, Youxi County, Fujian Province; 1♀ (28.03 × 21.97 mm) (NCU MCP 4091), Chimu Village, Youxi County, Fujian Province. *B. yonganense* (Cheng, Lin & Luo, 1993): 1♂ (22.97 × 18.19 mm) (NCU MCP 4096), Sanming City, Fujian; *B. lingchuanense* (Türkay & Dai, 1997), 6♂♂ (24.36 × 19.51 mm, 22. 34 × 18.70 mm, 23.03 × 18.51 mm, 25.33 × 19.46 mm, 24.92 × 19.10 mm, 18.04 × 14.41 mm) (NCU MCP 4076), Yuanpu Village, Gongcheng County, Guangxi Zhuang Autonomous Region; 4♂♂ (19.36 × 15.55, 19.56 × 15.69 mm, 19.68 × 16.15 mm, 20.11 × 15.98 mm) (NCU MCP 3281), Bindong Village, Lingchuan County, Guangxi Zhuang Autonomous Region. *B. youxiense* (Cheng, Lin & Li, 2010): 4♂♂ (14.27 × 12.21 mm, 13.57 × 11.05 mm, 13.78 × 11.16 mm, 14.09 × 11.42 mm) (NCU MCP 4092), 2♂ (13.35 × 10.60 mm, 13.41 × 11.02 mm) (NCU MCP 4158) . *B. nanan* (Zhou, Zhu & Naruse, 2008): 2♂ (28.48 × 22.65 mm, 22.23 × 16.92 mm) (NCU MCP 4090), Siqian Village, Shouning County, Fujian Province; 3♂♂ (23.59 × 18.92 mm, 21.73 × 17.36 mm, 22. 98 × 17.38 mm) (NCU MCP 4038), Yongjia County, Zhejiang Province; 2♂ (17.49 × 13.60 mm, 21. 28 × 16.11 mm), Yongjia County, Zhejiang Province; 1♀ (20.01 × 15.01 mm) (NCU MCP 4039), Yongjia County, Zhejiang Province.
**Diagnosis**

Carapace subquadrate, flat, dorsal surface smooth (Fig. 2); approximately about 1.3 times broader than long; third maxilliped ischium about 1.5 times as long as broad, exopod without flagellum (Fig. 3A); male pleon triangular, sixth somite width 2.5 times length; telson triangular, tip rounded, with proximal width 1.7 times length; median groove of male thoracic sternum deep, interruption between sutures of sternites 4/5, 5/6, 6/7 broad (Fig. 4). G1 long, tip of terminal segment reaching beyond suture between thoracic sternites 4/5 *in situ*; subterminal segment 1.3 times as long as terminal segment; terminal segment slightly elongated, curved inward, distal part of terminal segment elongated with anterioventrally directed semicircular lobe. Female vulvae partially exposed anteriorly to the thoracic sternites 5/6 *in situ*, ovate, deep, posteromesial margin with a low raised rim, opened inward.

**Description**

Carapace approximately about 1.3 times broader than long, dorsal surface gently convex from frontal view, regions not prominently inflated; with surface slightly pitted. Cervical
groove shallow, indistinct. H-shaped groove between the gastric region and cardiac region shallow but distinct. Postfrontal lobe blunt, separated medially by a Y-shaped groove extending to frontal region; postorbital crest indistinct, postorbital region slight concave. Frontal region deflexed downwards. Dorsal orbital margin ridged, external orbital angle triangular outer margin smooth; Anterolateral margin cristate, epibranchial tooth pointed, indistinct, clearly demarcated from external orbital tooth (Fig. 2).

Third maxilliped merus about 1.3 times as broad as long; Ischium about 1.5 times as long as broad, with distinct median sulcus; exopod reaching proximal third of merus length, without flagellum (Fig. 3A).

Male sternum pitted, sternites 1, 2 fused to form triangular structure; sternites 2, 3 separated by continuous suture; boundary between sternites,3, 4 present. Male sternopleonal cavity broad, shallow, with narrow median interruption in sutures 4/5, 5/6, 6/7; median line between sternites 7, 8 moderately short; male pleonal locking tubercle on posterior third of sternite 5 (Fig. 4).

Cheliped slightly unequal; margins crenulated; carpus with sharp spine on inner distal angle, with spinule at base; outer surface of manus with convex granules, manus about 1.6 times as long as high, slightly longer than movable finger, gape wide when fingers closed, cutting edge lined with low teeth (Fig. 3C).

Ambulatory legs slender; margins of propodus smooth; last leg with propodus about 1.8 times as long as broad, slightly shorter than dactylus (Fig. 3B).

G1 slender, ventral flap with transparent protrusion, with a fold covering the surface of the entire subterminal. Tip of terminal segment slightly reaching beyond sternal pleonal locking structure in situ, subterminal segment about 1.3 times as long as terminal segment. G1 slightly curved anterioventrally; distal part of G1 terminal segment distinctly broader than proximal part. G2 subterminal segment about 2.3 times as long as terminal segment (Figs. 5A and 6A).

Remarks
The new species fits well within the morphological definition of the genus Bottapotamon (Türkay & Dai, 1997; Cheng, Lin & Li, 2010; Zhou, Zhu & Naruse, 2008): G1 is slender, tip of terminal segment reaching suture between thoracic sternites 4/5 in situ; terminal segment slightly elongated inward (Table 3). Nonetheless, the new species can be distinguished from co-genus, by the carapace surface gently convex, cervical groove indistinct; H-shaped groove shallow but distinct; epibranchial tooth pointed and indistinct, third maxilliped without flagellum; chelipeds carpus with sharp spine on inner distal angle; and the ventromedially curved G1, which subterminal segment about 1.3 times as long as terminal segment (Table 3). The most obvious specific character of the new species is that the ventral flap of G1 with transparent protrusion, with a fold covering the surface of the entire subterminal region (Figs. 5A and 6A).

Etymology
The species is named after the type locality: Chenzhou city, Hunan Province, China.
Distribution
*B. chenzhouense* sp. n. was found under stones in a mountain stream in Huangcao village, Sunxian District, Chenzhou City, Hunan Province, China.

*Bottapotamon luxiense* sp. n. Gao, Cui & Zou (*Figs. 5–10*)

Materials examined
Holotype: 1♂ (17.36 × 13.26 mm) (NCU MCP 4200), Yixiantian Wugongshan Mountain, Luxi County, Pingxiang City, Jiangxi Province, China, 27°28′56.16″N, 114°10′27.51″E, 1331 m asl. Coll. Song-bo Wang, May 6th, 2019. Paratypes: 1♀ (19.21 × 14.67 mm) (NCU MCP 4200). Others: 10♀♀ (17.51 × 13.89 mm, 14.43 × 11.30 mm, 17.93 × 14.23 mm, 18.08 × 14.39 mm, 19.61 × 15.58 mm, 16.77 × 12.74 mm, 15.88 × 12.00 mm, 17.40 × 13.77 mm, 16.36 × 12.93 mm, 19.09 × 15.02 mm) (NCU MCP 4200), 14♂♂ (17.33 × 13.76 mm, 16.10 × 12.93 mm, 14.61 × 12.10 mm, 15.03 × 11.27 mm, 12.01 × 9.24 mm, 12.01 × 9.48 mm, 10.59 × 8.33 mm, 12.61 × 10.39 mm, 13.53 × 10.89 mm, 14.12 × 11.24
mm, 12. 84×10.07 mm, 12. 15× 9.76 mm, 14. 31× 11.64 mm, 11. 71× 9.20 mm) (NCU MCP 4200), the same data as holotype.

**Comparative materials**
Same as *Bottapotamon chenzhouense* sp. n.

**Diagnosis**
Carapace about 1.3 times broader than long, subquadrate, flat, dorsal surface gently convex longitudinally; cervical groove distinct, H-shaped groove between gastric, cardiac regions distinct (Fig. 7); third maxilliped ischium about 1.5 times as long as broad, with flagellum (Fig. 8A); male abdomen broadly triangular, telson triangular, with about 1.6 times as broad as long (Fig. 6B); median groove of male thoracic sternum deep, interruption
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<th>B. fukiense</th>
<th>B. yonganense</th>
<th>B. engelhardti</th>
<th>B. nanan</th>
<th>B. youxiense</th>
<th>B. lingchuanense</th>
<th>B. chenzhouense sp. n</th>
<th>B. luxiense sp. n</th>
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<td>Carapace</td>
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<td>External orbital angle</td>
<td>Blunt</td>
<td>Triangle</td>
<td>Blunt</td>
<td>Blunt</td>
<td>Triangle</td>
<td>Triangle</td>
<td>Triangle</td>
<td>Triangle</td>
</tr>
<tr>
<td>Third maxilliped merus</td>
<td>Length to width ratio 1.3</td>
<td>Length to width ratio 1.1</td>
<td>Length to width ratio 1.2</td>
<td>Length to width ratio 1.4</td>
<td>Length to width ratio 1.1</td>
<td>Length to width ratio 1.2</td>
<td>Length to width ratio 1.3</td>
<td>Length to width ratio 1.4</td>
</tr>
<tr>
<td>Male abdomen</td>
<td>Broad triangular</td>
<td>Narrow triangular</td>
<td>Broad triangular</td>
<td>Broad triangular</td>
<td>Broad triangular</td>
<td>Broad triangular</td>
<td>Narrow Triangular</td>
<td>Broad triangular</td>
</tr>
<tr>
<td>Male abdomen telson</td>
<td>Width to length ratio 1.5</td>
<td>Width to length ratio 1.3</td>
<td>Width to length ratio 1.3</td>
<td>Width to length ratio 1.4</td>
<td>Width to length ratio 1.5</td>
<td>Width to length ratio 1.2</td>
<td>Width to length ratio 1.3</td>
<td>Width to length ratio 1.3</td>
</tr>
<tr>
<td>Immovable finger</td>
<td>Length to width ratio 1.3</td>
<td>Length to width ratio 1.7</td>
<td>Length to width ratio 1.4</td>
<td>Length to width ratio 1.7</td>
<td>Length to width ratio 1.4</td>
<td>Length to width ratio 1.4</td>
<td>Length to width ratio 1.4</td>
<td>Length to width ratio 1.8</td>
</tr>
<tr>
<td>G1</td>
<td>Stout, straight</td>
<td>Slender, distal segment tubular arcuate</td>
<td>Slender, distal dorsal lobe convex</td>
<td>Slender, distinct longitudinal groove</td>
<td>Slender, distal segment spacious and strong</td>
<td>Slender, terminal, segment tortuous slightly</td>
<td>Slender, ventral flap with transparent protrusion</td>
<td>Blunt</td>
</tr>
</tbody>
</table>

Table 3  Primer sequences used in this study.
between sutures of sternites 4/5, 5/6, 6/7 broad. G1 long and blunt, tip of terminal segment reaching suture between thoracic sternites 4/5 in situ; subterminal segment 1.2 times as long as terminal segment; terminal segment slightly elongated inward, distal part of terminal segment elongated with anterioventrally directed semicircular lobe. Female vulvae partially exposed anteriorly to the thoracic sternites 5/6 in situ, ovate, deep, posteromesial margin with a low raised rim, opened inward.

**Description**

Carapace nearly ellipse in shape, about 1.3 times broader than long, flat, dorsal surface punctate, glabrous; regions distinctly defined; epibranchial region rugose, mesogastric regions slightly convex. Cervical groove distinct. H-shaped groove between the gastric region and cardiac region shallow but distinct. Postfrontal lobe blunt; postorbital crest indistinct, postorbital region slight concave. Frontal region deflexed downwards. Dorsal orbital margin ridge, external orbital angle triangular, outer margin smooth. Anterolateral margin ridge, epibranchial tooth pointed (Fig. 7).

Third maxilliped merus trapezoidal about 1.4 times as broad as long; ischium about 1.5 times as long as broad, with distinct median sulcus; exopod reaching proximal third of merus length, with flagellum (Fig. 8A).

Thoracic sternum pitted; sternites 1/2 completely fused to form triangular structure; sternites 2/3 separated by continuous suture; boundary between sternites 3/4 present, indistinct. Sterno-pleonal cavity broad, shallow, with narrow median interruption in sutures 4/5, 5/6, 6/7; median line between sternites 7/8 moderately long (Fig. 9).

The male sternum is relatively flat with numerous small pits; sternites 1/2 fused triangular; transverse sulcus between sternites 2/3 suture; sternites 3/4 fused without obvious demarcation. Male sterono-pleonal cavity is medium in depth wide; median
longitudinal groove between sternites 7/8 short; male pleonal locking tubercle on posterior third of sternite 5 (Fig. 6B).

Chelipeds slightly unequal; outer surface of manus with granules, manus about 1.5 times as long as high, slightly longer than movable finger, gape wide when fingers closed, cutting edge lined with low teeth (Fig. 8B).

Ambulatory legs slender; margins of propodus smooth; last leg with propodus about 1.7 times as long as broad, slightly shorter than dactylus (Fig. 8C).

G1 blunt, tip of terminal segment slightly reaching beyond sternal pleonal locking structure in situ, subterminal segment about 1.4 times as long as terminal segment. G1 slightly curved ventrolaterally; distal part of G1 terminal segment distinctly broader than proximal part. G2 subterminal segment about 2.2 times as long as terminal segment (Figs. 5B and 6B).

Remarks
The new species fits well within the morphological definition of the genus *Bottapotamon* (*Türkay & Dai, 1997; Cheng, Lin & Li, 2010; Zhou, Zhu & Naruse, 2008*), especially similar to *B. fukiense*, and *B. lingchuanense* in shape of carapace and slender G1. With regards to the other species of genus *Bottapotamon*, they can be separated (Table 3). Adult male specimens of *B. luxiense* sp. n. have the gastric regions relatively smooth with the rest of the
surfaces also some rugose and granulose; H-shaped groove shallow but distinct (Fig. 7).
The G1 of *B. luxiense* sp. n. is also quite different with the terminal segment straight, slender
and blunting towards the tip (Figs. 5B and 6B); third maxilliped with flagellum; median
longitudinal groove between sternites 7/8 short; chelifeds carpus with sharp spine on inner
distal angle, with spines at base (Fig. 8B).

**Etymology**
The species is named after the type locality: Yixiantian Wugongshan Mountain, Luxi
County, Pingxiang City, Jiangxi Province, China.

**Living coloration**
The dorsal surfaces of the carapace and pereopods are dark purple-red, and the joints of
the cheliped merus and carpus the ambulatory legs are bright red. The inner surface of the
immovable finger and distal part of the movable finger are almost milky.

**Distribution**
*B. luxiense* sp. n. was found under stones in a mountain stream in Yixiantian Wugongshan
Mountain, Luxi County, Pingxiang City, Jiangxi Province, China (Fig. 10).

**Ecology**
*B. chenzhouense* sp. n. and *B. luxiense* sp. n. were collected in the Luoxiao mountains.
This region has a humid subtropical monsoon climate and is in the Xiangjiang River and
Ganjiang River watershed, which has rich biodiversity (Wang, 1998). Similar to the natural habitat of other Bottapotamon species, B. chenzhouense sp. n. and B. luxiense sp. n. can be found under small rocks in sandy creek beds in narrow mountain streams or highway drains with clear, slow flowing and cool water surrounded by dwarf shrubs or grasses (Fig. 10).

Phylogenetic analyses and Divergence time estimation

Within genus Bottapotamon, a 1323 bp segment (excluding the primer regions) of the combined mtDNA COI, 16S rRNA and nuclear histone H3 from all 25 specimens was analysed. The phylogenetic trees were constructed by ML analysis, and the corresponding support values were calculated by ML and BI analyses, both of which had high support values. The results showed that the genus Bottapotamon is monophyletic, and confirmed that B. chenzhouense sp. n. and B. luxiense sp. n. are new species of genus Bottapotamon and supported the relationship of the genus Bottapotamon (Fig. 11). With regard to the relationships among the all specimens, the phylogenetic tree also show some distinct geographical distribution (Fig. 1). B. engelhardtii, B. yonganense and B. nanan, which are mostly distributed in the Wuyi Mountain Range, form a clade; B. luxiense sp. n. forms a sister clade to the clade of B. engelhardtii, B. yonganense and B. nanan. The next sister clade is composed of B. chenzhouense sp. n., which is distributed in the Luoxiao Mountain Range, and the furthest sister clade is composed of B. lingchuanense, which is situated some distance from the Wuyi Mountain Range and Luoxiao Mountain Range, but near the Nanling Mountain. However, B. fukiense and B. youxiense are also distributed in the Wuyi Mountain Range, they do not assemble with B. engelhardtii, B. yonganense and B. nanan.

Based on the relaxed molecular clock estimation, the earliest divergence time for genus Bottapotamon was estimated to be 3.49–1.08 Ma. The divergence time estimation results are consistent with the four calibration points. B. fukiense and B. youxiense diverged 1.96 Ma (95% confidence interval = 2.65–1.31 Ma), B. luxiense diverged 1.90 Ma (95% confidence interval = 2.05–1.09 Ma), B. lingchuanense and B. chenzhouense sp. n. diverged 1.51 Ma (95% confidence interval = 1.6–0.7 Ma); B. engelhardtii and B. nanan diverged 1.08 Ma (95% confidence interval = 1.76–0.80 Ma) (Fig. 12).

DISCUSSION

In mainland China, the genus Bottapotamon is primarily distributed in the Wuyi Mountain Range area; B. luxiense sp. n., B. youxiense, B. nanan, B. engelhardtii and B. yonganense are restricted within an area east of the Wuyi Mountain Range (Fig. 1). There is no record of any of these five species in Jiangxi, despite extensive surveys of this area by the authors and their colleagues over many years (Dai, 1999; Shi et al., 2012). The altitude of the Wuyi Mountain Range is clearly high enough to prevent these species from reaching Jiangxi. B. fukiense occurs on both sides of the Wuyi Mountain Range (Fujian and Jiangxi Provinces), and is able to disperse across these mountains. The divergence time of B. fukiense is 1.96 Ma (95% confidence interval = 2.65–1.31 Ma) (Fig. 12), and the divergence time agrees well with records of the Pacific plate and Indian plate extrusion in the Neogene-Quaternary (1.64–23.3 Ma) (Li, 1984). Therefore, these geological events may explain the distribution
pattern of the genus *Bottapotamon* in the Wuyi Mountain Range. The ancestor of *B. fukiense* originated in an area close to the Wuyi Mountains, which probably dispersed across the Wuyi Mountain Range when it was still a lowland, before the Wuyi Mountain Formation and smaller-scale mountain deformations occurred and separated.

In the Nanling mountain range, unique karst formation and the south Asian subtropical humid monsoon climate conditions provide a good living environment for all types of wildlife, including freshwater crabs. However, only one species of the genus *Bottapotamon*, *B. lingchuanense*, was isolated in this area, and there is an 830 km gap between *B. lingchuanense* and other species distributed within the Wuyi Mountain Range (Fig. 1), which has always been the focus of researches on the genus *Bottapotamon*. This study reports two new species of genus *Bottapotamon*, *B. chenzhouense* sp. n., which was first discovered in Chenzhou City, Hunan Province, in south of Luoxiao Mountains, and *B. luxiense* sp. n., which is distributed in north of the Luoxiao Mountains (Fig. 1). Divergence time estimation results suggested that *B. chenzhouense* sp. n., *B. luxiense* sp. n., and *B. lingchuanense* were isolated at almost the same time (*B. luxiense* sp. n. diverged 1.90 Ma, and *B. lingchuanense* and *B. chenzhouense* sp. n. diverged at 1.51 Ma) (Fig. 12). The authors speculated that the Luoxiao Mountains continuously rose due to neotectonic movement and gradually formed the Xiangjiang River and Ganjiang River watershed (*Wang, 1998*). The ancestors of the genus *Bottapotamon* occurred on both sides of the Luoxiao Mountains during the
mountains formation process, and under the influence of karst landforms and the Danxia landform, gradually isolated *B. luxiense* sp. n., *B. chenzhouense* sp. n. and *B. lingchuanense*. In addition, the climatic conditions in this area are ideal for *Bottapotamon*. The authors speculate that many new species of the genus *Bottapotamon* are likely to exist in the region from the Wuyi Mountain Ranges to the Nanling Mountain Range, but get to be discovered.

**CONCLUSIONS**

*Bottapotamon chenzhouense* sp. n. and *B. luxiense* sp. n., two new species from the Luoxiao Mountains were reported in this paper. These two new species compensated for the geographical gap in the genus *Bottapotamon*, and confirm the independence and intra- and interspecific relationships of genus *Bottapotamon*. Combined with estimates of divergence times, this paper suggests that the genus *Bottapotamon* was formed at 3.49–1.08 Ma. Molecular evidence further supports the scientific hypothesis of the authors that genus *Bottapotamon* originated on both sides of the Wuyi Mountains and Luoxiao Mountains. In the geological area where the genus *Bottapotamon* is distributed, the Wuyi Mountains gradually formed offshore and inland of southeastern China by the compression of the Pacific plate and the Indian plate in the Neogene-Quaternary, and the Luoxiao Mountains formed continuously in the north-south direction because of neotectonic movement. Thus, the geographical
distribution patterns of the genus *Bottapotamon* was formed gradually with the various events.

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**ADDITIONAL INFORMATION AND DECLARATIONS**

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**Competing Interests**
The authors declare there are no competing interests.

**Author Contributions**
- Ning Gao conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Ying-Yi Cui conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, approved the final draft.
- Song-Bo Wang conceived and designed the experiments, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Jie-Xin Zou conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.

**DNA Deposition**
The following information was supplied regarding the deposition of DNA sequences:
The group genus Bottapotamon sequences are available at GenBank accession numbers MK795646 to MK795668, MK920079 to MK920101, MK952547 to MK952596, MK981408 to MK981409, MK993542 to MK993545.

**Data Availability**
The following information was supplied regarding data availability:
The raw data is available at Genbank: MK795646–MK993545.
All specimens in this study are housed in the permanent collections at the Department of Parasitology, Medical College of Nanchang University (NCUMCP). Specimen numbers can be found in Table 1.

**New Species Registration**
The following information was supplied regarding the registration of a newly described species:
Publication LSID: urn:lsid:zoobank.org:pub:211926FF-6950-4DFE-95C4-F5247CA9E0BA
Bottapotamon chenzhouense sp. n. LSID: urn:lsid:zoobank.org:art:E43C4BBB-E429-4C17-8ACD-E4295F426BCB.
Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.7980#supplemental-information.

REFERENCES


