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 congeners by features such as the G1, which has a fold covering the surface of the entire subterminal article with a transparent 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, 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 mya, 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 the 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. Thus, the geographical distribution pattern of the genus *Bottapotamon* is also established gradually.

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1) All scientific names must be in italics or underlined for example:

Bottapotamon nanan or Bottapotamon nanan

2) The author(s) and year are not in italics.

3) If there are 2 authors, decide if you want to use ‘and’ or ‘&’.

4) If there are more than 3 authors, use ‘et al.’ after the first author. There is n need to type out all the names of all the authors in the text.

5) Please be consistent about et al or *et al* in the text.

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* ~~YZ, JX Lin & XQ Luo,~~ *et al.,* *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*, *B.*

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engelhardti, *B. yonganense*, *B. lingchuanense* (*Türkay & Dai, 1997*), *B. youxiense* (*Cheng et al., 2010*) and *B. nanan* (*Zhou et al., 2008*).

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 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, 1997*) (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 2120 m in height (*Gong ~~HL, Zhuang WY, Liao WB~~ et al., 2016*). The terrain the genus *Bottapotamon* now inhabits is geologically relatively stable and experienced little orogenic activity during the Cenozoic (*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.

By 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 authors (or who exactly? the first author) discovered two new species in Chenzhou City, Hunan Province, and Luxi County, Jiangxi Province. This paper compares the morphological features of eight species including two new species of the genus *Bottapotamon*, as well as 16S rRNA (*Crandall et al. 1996*), mtDNA COI (*Folmer et al., 1994*) and nuclear histone H3 (*Colgan et al., 1998*) gene fragments were 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

Specimen 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 author compared specimens with holotypes of the Institute of Zoology, Chinese Academy of Sciences. 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^{TM} 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.

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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*).

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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 divided into two major subfamilies, Potamiscinae and Potaminae, estimated to have a divergence time of 20.9-24.7 mya, which was set as calibration point 1 in our study (*Shih et al., 2010*). The Parathelphusidae subfamily, *Somanniathelphusa taiwanensis*, which is distributed in Taiwan Island and separated from *Somanniathelphusa amoyensis*, which is distributed in Fujian Province, approximately 0.27-1.53 mya (*Jia et al., 2018*). The results are consistent with the quaternary glacial period and interglacial period and agree 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 mya) (*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: PeerJ, PubMed Central and CLOCKSS.

Results

Systematics

Potamidae Ortmann, 1896
Bottapotamon Tüerkay et Dai, 1997
~~***Bottapotamon chenzhouense*** **sp.n.,** ***Bottapotamon luxiense*** **sp.n.**~~

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Bottapotamon chenzhouense **sp. n. (*Fig. 2-6*)**

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Materials examined

Holotype: 1 ♂ (25.72 × 15.69 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. Dingmei Luo, July 26th 2006. Paratypes: 1 ♀ (18.7 × 13.7 mm) (NCU MCP 643), the same data as the holotype.

Comparative materials

B. fukiense (*Türkay & Dai*, *1997*): 4 ♂ (25.21 × 15.02 mm, 25.03 × 14.97 mm) (NCU MCP 4089), Xiapu Village, Ningde County, Fujian; (26.08 × 15.45 mm) (NCUMCP4156), Shangshan Village, Zhenghe County, Fujian; (26.08 × 15.45 mm) (NCUMCP4090), Siqian Village, Shouning County, Fujian; 1 ♀ (26.01 × 15.57 mm) (NCU MCP 4156), Shangshan Village, Zhenghe County, Fujian. *B. engelhardti* (*Türkay & Dai*, *1997*): 5♂ (23.01 × 14.03 mm, 24.68 × 15.69 mm, 24.81 × 15.87 mm, 25.02 × 15.47 mm) (NCU MCP 4157), Tangsan Village, Youxi County, Fujian; (25.21 × 15.16 mm) (NCU MCP 4091), Chimu Village, Youxi County, Fujian; 1 ♀ (26.01 × 16.35 mm) (NCU MCP 4091),Chimu Village, Youxi County, Fujian. *B. yonganense* (*Türkay & Dai, 1997*): 1♂ (25.87 × 15.95 mm) (NCU MCP 4096), Sanming City, Fujian; *B. lingchuanense* (*Türkay & Dai, 1997*), *3*♂(24.78 × 14.89 mm, 25.04 × 15.06 mm) (NCU MCP4076), Yuanpu Village, Gongcheng County, Guangxi Zhuang Autonomous Region; (25.25 × 15.11 mm) (NCU MCP 3281), Bindong Village, Lingchuan County, Guangxi Zhuang Autonomous Region; 3♀ (25.48 × 14.92 mm, 25.14 × 15.09 mm, 25.78 × 14.79 mm), (NCU MCP 3281), Bindong Village, Lingchuan County, Guangxi Zhuang Autonomous Region. *B. youxiense* (*Cheng et al.*, *2010*): (24.91 × 15.72 mm) (NCU MCP 4092), (25.11 × 15.16 mm) (NCU MCP 4158), (25.34 × 15.52 mm) (NCU MCP 4059), Xiwei Village, Youxi County, Fujian; 1♀ (26.04 × 14.92 mm) (NCU MCP 4059), Xiwei Village, Youxi County. *B. nanan*

(*Zhou et al.*, *2008*):6♂（26.26 × 15.72 mm, 26.03 × 15.55 mm）(NCU MCP4090), Siqian Village, Shouning County, Fujian; (26.15 × 15.43 mm, 26.16 × 15.32 mm) (NCU MCP4038), Yongjia County, Zhejiang; (26.25 × 15.36 mm, 26.06 × 15.52 mm) (NCU MCP4039), Yongjia County, Zhejiang; 1♀(26.11 × 15.12 mm) (NCU MCP4039), Yongjia County, Zhejiang.

Diagnosis

Carapace approximately about 1.6 times broader than long, dorsal surface gently convex longitudinally and transversely; cervical groove indistinct, H-shaped groove between gastric and cardiac regions distinct. Male pleon triangular, sixth somite width 2.5 times length; telson triangular, tip rounded, with proximal width 1.7 times length. G1 long, tip of terminal segment reaching suture between thoracic sternites 4, 5 in situ; subterminal segment 1.3 times as long as terminal segment; terminal segment slightly elongated inward, distal part of terminal segment elongated with ventrally directed semicircular lobe. Female vulvae partially exposed anteriorly to the thoracic sternites 5, 6 in situ, ovate, deep, posteromesial margin with a law raised rim, opened inward.

Description

Carapace approximately about 1.6 times broader than long, dorsal surface gently convex, 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*).

The male sternum is relatively flat and has granular small pits. The first section is triangular and the second to fourth sections are fused. The interruption between sternite sutures is intermediate in depth and wide. The median longitudinal sutures of sternites 7/8 are shorter; the tubercle of abdominal lock is on the medial side of the fifth male ventral nail (*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, a fold covering the surface of the entire subterminal article with a transparent distal region.tip of terminal segment slightly reaching beyond sternal press-button in situ, subterminal segment about 1.3 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 2.3 times as long as terminal segment (*Fig. 5A, 6A*).

Remarks

The new species fits well within the morphological definition of the hitherto monotypic *Bottapotamon* (*Türkay & Dai*, *1997*; *Cheng et al.*, *2010*; *Zhou et al.*, *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 comparative specimens, 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; G1 is sturdy and blunt (*Table. 3*).

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, Chenzhou city, Hunan Province, China**.**

Bottapotamon luxiense **sp.n. (*Fig. 5-10*)**

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Materials examined

Holotype: 1 ♂ (18.72×15.69 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. Jiexin Zou, May 6th 2019. Paratypes: 1♂ (19.22 × 16.38 mm) (NCU MCP 4200). Others: 12 ♀ (16.7 × 15.7 mm, 15.41x15.36 mm, 14.23×12.98 mm, 15.63×14.52 mm, 16.13×15.86 mm, 16.23×14.97 mm, 13.65×12.33 mm, 14.56×13.15 mm, 15.27×14.10 mm, 16.02×15.43 mm, 15.89×15.01 mm, 13.13×12.46 mm) (NCU MCP 4200), 12♂ (15.66×13.89 mm, 14.21×13.11mm, 13.69×12.01 mm, 14.23×13.69 mm, 15.17×14.31 mm, 14.19×13.69 mm, 14.69×13.54 mm, 14.73×13.52 mm, 12.87×11.36 mm, 13.00×12.13 mm, 13.58×12.29 mm, 15.26×14.36 mm) (NCU MCP 4200), same data as holotype.

Comparative materials

Same as *Bottapotamon chenzhouense* sp. n.

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Diagnosis

Carapace about 1.4 times broader than long, dorsal surface gently convex longitudinally and transversely; cervical groove distinct, H-shaped groove between gastric and cardiac regions distinct. Male pleon triangular, sixth somite width 2.3 times length; telson triangular, tip rounded, with proximal width 1.6 times length. G1 long, 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 ventrally directed semicircular lobe. Female vulvae partially exposed anteriorly to the thoracic sternites 5, 6 in situ, ovate, deep, posteromesial margin with a law raised rim, opened inward.

Description

Carapace nearly ellipse, about 1.4 times broader than long, dorsal surface gently convex, surface slightly pitted. 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 ridged, external orbital angle triangular, outer margin smooth. Anterolateral margin cristate, epibranchial tooth pointed (*Fig. 7*).

Third maxilliped merus about 1.4 times as broad as long, trapezoidal; 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 and has granular small pits. The first section is triangular and the second to fourth sections are fused. The interruption between sternite sutures is medium in depth and wide. The median longitudinal sutures of sternites 7/8 are shorter; the tubercle of abdominal lock is on the medial side of the fifth male ventral nail (*Fig. 6B*).

Chelipeds slightly unequal; margins crenulated; outer surface of manus with convex 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 is sturdy and blunt tip of terminal segment slightly reaching beyond sternal press-button 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 (*Fig. 5B. 6B*).

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Remarks

The new species fits well within the morphological definition of the hitherto monotypic *Bottapotamon* (*Türkay & Dai*, *1997*; *Cheng et al.*, *2010*; *Zhou et al.*, *2008*): cervical groove indistinct, H-shaped groove between gastric and cardiac regions distinct, G1 long, 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 comparative specimens, by the carapace surface gently convex, cervical groove shallow and

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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, with spinule at base; G1, a fold covering the surface of the entire subterminal article with a transparent distal region (*Table. 3*).

Etymology

The species is named after the type locality: Yixiantian Wugongshan Mountain, Luxi county, Pingxiang city, Jiangxi Province, China.

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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

The combined mtDNA COI, 16S rRNA and nuclear histone H3 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*). *B. engelhardti*, *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. engelhardti*, *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 distributed in the Nanling Mountain Range. *B. fukiense* and *B. youxiense* are also distributed in the Wuyi Mountain Range, but they do not assemble with *B. engelhardti*, *B. yonganense* and *B. nanan.*

The divergence time estimation results are consistent with the four calibration points. The genus *Bottapotamon* diverged approximately 3.49-1.08 mya, *B. fukiense* and *B. youxiense* diverged 1.96 mya (95% confidence interval =2.65-1.31 mya), *B. luxiense* diverged 1.90 mya

(95% confidence interval =2.05-1.09 mya), *B. lingchuanense* and *B. chenzhouense* sp. n. diverged 1.51 mya (95% confidence interval =1.6-0.7 mya); *B. engelhardti* and *B. nanan* diverged 1.08 mya (95% confidence interval =1.76-0.80 mya).(*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. engelhardti* 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, 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* and *B. youxiense* is 1.96 mya (95% confidence interval =2.65-1.31 mya) (*Fig. 12*), which agrees well with records of the Pacific plate and Indian plate extrusion in the Neogene-Quaternary (1.64-23.3 mya) (*Li, 1984*). Therefore, these geological events may explain the distribution pattern of the genus *Bottapotamon* in the Wuyi Mountain Range. The ancestor of the genus *Bottapotamon* originated in an area close to the Wuyi Mountains, as the Wuyi Mountain Formation and smaller-scale mountain deformations resulted in sufficient geographic barriers to isolate populations; thus, the two species-groups were separated by the Wuyi Mountains.

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 mya, and *B. lingchuanense* and *B. chenzhouense* sp. n. diverged at 1.51 mya) (*Fig. 12*). The authors speculated that the Luoxiao Mountains continuously rose due to neotectonic movement and gradually became 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* was gradually isolated. 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.

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Conclusions

B. 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 *Bottapotam*on was formed at 3.49-1.08 mya. 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* formed gradually.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Data Availability

Regarding data availability: all specimens in this study are housed in the permanent collections at the Department of Parasitology, Medical College of Nanchang University (NCU MCP), and the raw DNA data are included in the supplemental files.

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