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

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Abstract

Bottapotamon chenzhouense sp. n. and *B.luxiense* sp. n. described from Hunan Province and Jiangxi Province, respectively. They all have diagnostic features of genus *Bottapotamon*. *B. chenzhouense* sp. n. can be distinguished by features such as the G1, which has a fold covering the surface of the entire terminal second segment, with a clear corner transparent distal region?. And *B.luxiense* sp. n. has an elliptical carapace, the width of G1 is same (similar to ?)from base to terminal. The authors used mitochondrial cytochrome oxidase I, 16S rRNA and nuclear histone H3 gene fragments to explore the relationship between new species and other freshwater crabs belonging to genus *Bottapotamon*. The results support two new species assignment to genus *Bottapotamon* and a clear differentiation from the other analyzed species. In addition, the divergence time of genus *Bottapotamon* was estimated to be 3.49-1.08mya which coincided with ~~. In~~ the geological area where genus *Bottapotamon* is commonly distributed, the Wuyi Mountains gradually formed offshore and inland of southeastern China by the compression of the Pacific plate and the Indian plate in Neogene-Quaternary, and the Luoxiao Mountains continued forming in the north-south direction because of neotectonic movement, the geographical distribution pattern of genus *Bottapotamon* gradually formed.

Introduction

Genus *Bottapotamon* is an unique genus of freshwater crabs from the China mainland ~~that belong to Crustacea: Malacostraca: Decapoda: Brachyura: Potamidae~~. In the 1960s, and 1970s, genus *Bottapotamon* was divided into two genera, *Parapotamon* and *Malayopotamon* according to morphology ~~ical classification~~ by ?. In 1997, the genus *Bottapotamon* was identified on the basis of its morphological characteristics, such as the form? shape? of carapace and first gonopod (G1) (Tiirkay & Yun 1997). Should be Türkay & Dai 1997.

Until the beginning ~~start~~ of the current study, the genus *Bottapotamon* include *B.fukiense*, *B. engelhardti*, *B.yonganense*, *B. lingchuanense* (Tiirkay & Yun 1997), *B. youxiense* (Cheng et al. 2010) and *B. nanan* (Zhou et al. 2008). According to the book “Geographical Division of Chinese Animals (Zheng & Zhang 1965), their natural habitats include the geographical area of central China region (IIB) and south China region (IIIB, IIIC), ~~the three freshwater crab natural geographical area~~ in mainland China (Figure 15). Five ~~5 kinds of~~ species of ~~genus~~ *Bottapotamon* are located in the central China region (II) eastern hilly plains subregion (IIB), and only *B. lingchuanense* was isolated in the South China region (III) Guangxi-Guangdong subregion (IIIB). By organizing collections ?? and newly collected specimens, the author discovered two new species in Chenzhou city in Hunan Province, and Luxi County in Jiangxi Province. This paper compares the morphological features of eight species including the two new species from genus *Bottapotamon*, and use 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 analyze phylogenetic relationships and estimate divergence time to support the establishment of new species of genus *Bottapotamon*. ~~and to discuss~~ The phylogenetic relationship, distribution pattern and possible association with major geological and historical events will also be discussed.

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Materials & Methods

Specimen collection

All specimens were preserved in 95% ethanol and deposited at the Department of Parasitology of the Medical College of Nanchang University (NCU MCP), Jiangxi Province, China. The authors compared specimens with holotype 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*). Does your specimens include freshly collected specimens and older specimens in the NCU MCP?

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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.

After searching the National Center for Biotechnology Information (NCBI) database, we finally selected the sequences of four individuals with the same primer sequences 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, the length of the sequences is 1323 bp. According to the Akaike information criterion (AIC), MrMTGui: ModelTest and MrModelTest (phylogenetic analysis using parsimony (PAUP)) obtained the best models as 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 is 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). ~~Regarding~~

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The Parathelphusidae subfamily, ~~the authors associated with the NCU MCP found that~~ *Somanniathelphusa taiwanensis*, which is distributed in Taiwan Island, separated from *Somanniathelphusa amoyensis*, which is distributed in Fujian Province, approximately 0.27-1.53 mya (??? personal communication). The results are consistent with the quaternary glacial period and interglacial period and are in accordance with the separation between Taiwan Island and Fujian Province; this time point was set as calibration point 2 (Jia et al. 2018). 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 Neogene-Quaternary (1.64-23.3 mya) ((Zhenzhong 1984)), this time point was set as calibration point 3. A Yule speciation model was constructed for speciation within 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 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 (v. 1.3.1, Rambaut, 2009).

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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.**

Bottapotamon chenzhouense sp.n.

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

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), same data as holotype.

Description

Carapace nearly subquadrate; dorsal surface slightly convex, with small granular depressions; cervical groove between gastric and hepatic region shallow; branchial region inconspicuous (*Fig. 1A*). H-shaped groove between the gastric region and cardiac region deep, obvious. Postfrontal lobe and postorbital crest blunt, separated by a narrow gap in the middle of the frontal border; orbital border smooth with no obvious depression. Exorbital angle obtuse, almost in line with epibranchial tooth; antero-lateral border distinctly cristate, smooth, with curved-end abdomen; posterior border flat and angular to postero-lateral border (*Fig. 1B*).

Surface of the third maxilliped has inconspicuous granules; width of the merus approximately 1.3 times the length; length of ischium approximately 1.2 times the width; merus trapezoidal with median depression. Ischium trapezoidal, median sulcus distinct, exopod reaches 1/3 of the proximal end of the merus, end without flagellum. Dactylus extends downward beyond the junction of the merus and ischium (*Fig. 2A*).

Male cheliped surfaces rugose; carpus short; inner-distal angles with sharp spines without spinules at the base; length of palm of the larger chela is approximately 1.6 times the height. Immovable finger long and thin and forms a small gap when fingers closed; inconspicuous triangular tooth on inner margins of fingers (*Fig. 2B*).

The ambulatory legs are slender and the surface is smooth. The length of the propodus of the last pair of ambulatory legs is approximately 2.5 times the width; the length of the dactylus is 1.3 times that of the propodus (*Fig. 2C*).

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. 3A, 3B*).

G1 is slender, and the terminal segment is beyond the tubercle of the abdominal lock and beyond the fourth and fifth breastplate lines (*Fig. 4*); the second segment bends toward the back side. The most obvious feature is that there is a fold with a clear corner covering the surface of the second segment; the interval with the last segment is not continuous, and the length is approximately 5.75 times that of the end segment. There is obvious contraction near the base, and the base is broad (*Fig. 5A, 5B, 5C*). The G2 base is approximately twice as long as the whip-like end (*Fig. 5D*).

Remarks

~~Same~~ As in the genus *Bottapotamon*, *B. chenzhouense* sp. n. has a subquadrate carapace, with insignificant small granular depressions and a clear H-shaped groove in the gastric region and cardiac region. The interruption between sutures of sternites is deep and wide, and the median longitudinal suture of sternites 7 and 8 is short. The male abdomen forms a long, narrow triangle. G1 is slender and exceeds the tubercle of the abdominal lock. However, the postfrontal lobe and postorbital crest of *B. chenzhouense* sp. n. are dull. The epibranchial tooth is not obvious. The antero-lateral border is uplifted, the extremity curves to the carapace, and the third maxilliped ~~um~~ does not have a whip. The inner-distal angle of the male cheliped has an inconspicuous sharp spine but no small spines at the base. G1 is slender, with obvious contraction near the base. The most obvious feature is the white fold with a clear corner covering the surface of the segment next to last and the interval with the last segment is not continuous (*Table. 3*).

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Etymology

The species is named after the type locality, Chenzhou city, Hunan Province, China.

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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.**

urn: lsid zoobank. org: art: 1C1CC520-193A-405E-9A2D-DC79E7D4AA87.

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, 13.13×12.46mm) (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.

Description

Carapace nearly ellipse; dorsal surface slightly convex, with small granular depressions; cervical groove obvious. H-shaped groove between the gastric region and cardiac region deep and obvious. Postfrontal lobe and postorbital crest blunt; orbital border smooth with no obvious depression. Exorbital angle sharp; antero-lateral border distinctly cristate, smooth, with curved-end abdomen; posterior border flat and angular to postero-lateral border (*Fig. 6*). Surface of the third maxilliped has inconspicuous granulars; width of the merus approximately 1.4 times the length; length of ischium approximately 1.1 times the width; merus trapezoidal with median depression. Ischium trapezoidal, median sulcus distinct, exopod reaches 1/3 of the proximal end of the merus, end without a flagellum. Dactylus extends downward beyond the junction of the merus and ischium (*Fig. 7A*).

Male cheliped surfaces rugose; carpus short; inner-distal angles with sharp spines without spinules at the base; length of palm of the larger chela is approximately 1.8 times the height. Immovable finger long and thin and forms a wide gap when fingers closed; inconspicuous triangular tooth on inner margins of fingers (*Fig. 7B*).

The ambulatory legs are slender and the surface is smooth. The length of the propodus of the last pair of ambulatory legs is approximately 2.6 times the width; the length of the dactylus is 1.3 times that of the propodus (*Fig. 7C*).

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 obvious. 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. 8*).

G1 is slender, and the terminal segment is beyond the tubercle of the abdominal lock and beyond the fourth and fifth breastplate lines (*Fig. 9*); The most obvious feature is that the width of G1 is same from basis to terminal (*Fig. 10A, 10B, 10C*). The G2 base is approximately twice as long as the whip-like end (*Fig. 10D*).

Remarks

~~Same~~ As in the genus *Bottapotamon, B. luxiense* sp. n. has insignificant small granular depressions and a clear H-shaped groove in the gastric region and cardiac region. The interruption between sutures of sternites is deep and wide, and the median longitudinal suture of sternites 7 and 8 is short. The male abdomen forms a long, narrow triangle. G1 is slender and exceeds the tubercle of the abdominal lock. However, *B. luxiense* sp. n. has an elliptical carapace, exorbital angle has a sharp angle, and the third maxilliped ~~um~~ does not have a whip. G1 is slender, the width of G1 is same from base to terminal (*Table. 3*).

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Etymology

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

Live ~~iving~~ coloration

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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 (*Fig. 6*).

Variation

Adult specimens are usually much more brightly colored than juveniles. The terminal segment of the G1 may vary in proportionate length, while the angle at which it point varies ?? from around 30-45 degrees.

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.11*).

Ecology

B. chenzhouense sp. n. and *B. luxiense* sp. n.were collected in the Luoxiao Mountains, located in the central China region (II) western mountain plateau subregion (IIA) of Chinese freshwater crabs natural geographic area. This region has a humid subtropical monsoon climate and is in the Xiangjiang River and Ganjiang River watershed, which is rich in biodiversity and is one of the most important biological compounds ?? in eastern Asia (Chunlin 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. 12*).

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 confirmed that *B. chenzhouense* sp. n. and *B. luxiense* are new species of genus *Bottapotamon* and supported the relationship of genus *Bottapotamon*. ??? (*Fig. 13*) What kind of relationship?

The divergence time estimation results are consistent with the four calibration points (see above). Genus *Bottapotamon* has 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 1.90mya (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).(see *Fig. 14*)

Discussion

Two new species of Bottapotamon ~~*chenzhouense* sp. n.~~, collected from the Chinese Luoxiao Mountains in the central China (II) western mountain plateau subregion (IIA), are reported in this paper.

The cervical groove of *B. chenzhouense* sp. n. is shallow and insignificant. The postfrontal lobe and postorbital crest are broad, and the third maxilliped does not have a whip. G1 is slender, with obvious contraction near the base. The most obvious feature is a fold with a clear corner covering the surface of the second segment, and the interval with the last segment is not continuous.

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B. luxiense sp. n. has an elliptical carapace, exorbital angle has a sharp angle. G1 is slender, the width of G1 is same from basis to end. On the basis of the mtDNA COI, 16S rRNA and nuclear histone H3 gene fragments, the results also support the establishment of two new species and demonstrate its intramolecular affinity.

In the central China region (II) eastern hilly plain subregion (IIB), there are *B. fukiense*, *B. youxiense, B. nanan, B. engelhardti* and *B. yonganense* (lack of molecular data) (*Fig. 15*). On the east coast of Fujian Province, four species, excluding *B. fukiense*, are found only on the eastern side of the Wuyi Mountains, indicating that the mountains are a significant barrier to the species. *B. fukiense* is distributed on both sides of Wuyi Mountains; one of the possible reasons for the distribution of this species is that the low mountains and hilly regions may have created natural conditions for freshwater crabs to spread along the river system (Lin-bo et al. 2012). Another possible reason is the Pacific plate and Indian plate extrusion at the Neogene-Quaternary (1.64-23.3 mya) (Zhenzhong 1984). As the Wuyi Mountains was forming, the genus *Bottapotamon* species originally distributed in this area was also slowly separated; this event is also consistent with the *B. fukienense* divergence time (2.65-1.31 mya) (*Fig. 14*). The eastern side of the Wuyi Mountains is mountainous and hilly, which makes it extremely easy to isolate freshwater crabs with weak migration abilities; thus, other species of genus *Bottapotamon* (which species?) has evolved separately.

In the South China region (III) Guangxi & Guangdong subregion (IIIB). This region's unique karst formation and the south Asian subtropical humid monsoon climate conditions provide a good living environment for all kinds of wildlife, including freshwater crabs. However, only one species from the genus *Bottapotamon*, *B. lingchuanense*, was isolated in this area, and there is a 830 km gap between it and other species distributed within the Wuyi Mountain Range, which has always been the focus of genus *Bottapotamon* researches. In this current study, two new species of genus *Bottapotamon*, *B. chenzhouense* sp. n. was first discovered in Chenzhou city, Hunan Province, in south of Luoxiao Mountains, in central China region (II) western mountain plateau subregion (IIA), and *B.luxiense* sp. n. is distributed in north of Luoxiao Mountains (*Fig. 15*). 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* diverged 1.90mya, *B. lingchuanense* and *B. chenzhouense* sp. n. diverged 1.51 mya). The authors speculated that Luoxiao Mountains continued to rise due to neotectonic movement and gradually became the Xiangjiang River and Ganjiang River watershed (Chunlin 1998). The ancestors of genus *Bottapotamon* occurred on both sides of Luoxiao Mountains due to the formation of the mountains, 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*. ~~good living environment, and~~ The authors further speculate that in the region where genus *Bottapotamon* was found and in south China region (III) Fujian-Guangdong-Jiangxi subregion (IIIC), the many? new species of genus *Bottapotamon* are likely to exist.

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Conclusions

B. chenzhouense sp. n. and *B. luxiense* sp. n. two new species from Luoxiao Mountains were reported in this paper. These two new species compensated for the geographical gap of the genus *Bottapotamon*, and confirms the independence of genus *Bottapotamon* and its intra- and interspecific relationships. Combined with estimates of divergence times, it is suggested that genus *Bottapotam*on was formed at 3.49-1.08 mya. Molecular evidences further supported 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 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 Neogene-Quaternary and Luoxiao Mountains continued growing in the north-south direction because of neotectonic movement, the geographical distribution patterns of genus *Bottapotamon* was gradually formed.

ADDITIONAL INFORMATION AND DECLARATIONS

Acknowledgements

We thank Mao-rong Cai, Hua Guo for collecting the specimens of the new species. Special thank is expressed to Xin-nan Jia and Shu-xin Xv? for their help and advice on the manuscript?. We would also thank Professor Xian-min Zhou for his guidance in this study.

Data Availability

Regarding data availability: all specimens in this study are deposited 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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