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

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Bottapotamon chenzhouense sp.n. and *B.luxiense* sp.n. described from Guangxi Zhuang Autonomous Region 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 white fold covering the surface of the entire terminal second segment, with a clear corner. And *B.luxiense* sp.n. has a round carapace, the width of G1 is same from basse 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. 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. The Luoxiao Mountains continued forming in the north-south direction because of neotectonic movement, and the geographical distribution pattern of genus *Bottapotamon* gradually formed.

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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. And *B.luxiense* sp.n. has an elliptical carapace, the width of G1 is same 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. 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 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 a 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 *Parapotamon* and *Malayopotamon* according to morphological classification. In 1997, the genus *Bottapotamon* was identified on the basis of its morphological characteristics, such as the carapace and first gonopod (G1)(Tiirkay & Yun 1997).

Until the start of the study, 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 geographical division of Chinese animal(Zheng & Zhang 1965), their habitat spans the natural geographical area, including central China region (IIB) and south China region (IIIB, IIIC), the three freshwater crab natural geographical area in mainland China. 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, Hunan Province, and Luxi County, Jiangxi Province. This paper compares the morphological features of 8 species including two new species of 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.

Materials & Methods

Specimen collection

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 author 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*).

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.

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 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. 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).

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 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 maxillium 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*).

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.**

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 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 maxillium does not have a whip. G1 is slender, the width of G1 is same from base to terminal (*Table. 3*).

Etymology

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

Living color

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, which belong to the central China region (II) western mountain plateau subregion (IIA) of Chinese freshwater crabs natural geographic area, which has a humid subtropical monsoon climate and is in the Xiangjiang River and Ganjiang River watershed, which has rich 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 grasse(*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*)

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

Discussion

Two new species of *B.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. *B.luxiense* sp.n. 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 on only 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 formed, the genus *Bottapotamon* species originally distributed in this area 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* evolved.

In the South China region (III) Guangxi & Guangdong subregion (IIIB). This region 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 of 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* research. In this 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, this area is a good living environment, and the authors speculate that in the region where genus *Bottapotamon* was found and in south China region (III) Fujian-Guangdong-Jiangxi subregion (IIIC), the new species of genus *Bottapotamon* are likely to exist.

Conclusions

B.chenzhouense sp.n. and *B.luxiense* sp.n., two new species from Luoxiao Mountains were reported in this paper. These new species compensate for the geographical gap of 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 formed at 3.49-1.08 mya. Molecular evidence supports the scientific hypothesis of the authors that genus *Bottapotamon* originated on both side 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* gradually formed.

ADDITIONAL INFORMATION AND DECLARATIONS

Acknowledgements

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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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Table 1(on next page)

Speciemens and GenBank accession numbers of genus *Bottapotamon*.

1

	Localities	Museum catalogue No.	Haplotypes	COI Accession No.	16S Accession No.	H3 Accession No.
Bottapotamon fukiense	Shangshan Village, Zhenghe County, Fujian	NCU MCP4156	Bfj1	MK920086	MK795653	MK952581
	Siqian Village, Shouning County, Fujian	NCU MCP4090	Bfj2	MK920097	MK795654	MK952582
	Xiapu Village, Ningde County, Fujian	NCU MCP4089	Bfj3	MK920088	MK795655	MK952583
		NCU MCP4089	Bfj4	MK920089	MK795656	MK952584
Bottapotamon youxiense	Xiwei Village, Youxi County, Fujian	NCU MCP4092	Byx1	MK920099	MK795666	MK952594
	Xiwei Village, Youxi County, Fujian	NCU MCP4158	Byx2	MK920100	MK795667	MK952595
	Xiwei Village, Youxi County, Fujian	NCU MCP4159	Byx3	MK920101	MK795668	MK952596
Bottapotamon engelhardti	Chimu Village, Youxi County, Fujian	NCU MCP4091	Bes1	MK920081	MK795648	MK952576
	Tangsan Village, Youxi County, Fujian	NCU MCP4157	Bes2	MK920082	MK795649	MK952577
		NCU MCP4157	Bes3	MK920083	MK795650	MK952578
		NCU MCP4157	Bes4	MK920084	MK795651	MK952579
		NCU MCP4157	Bes5	MK920085	MK795652	MK952580
Bottapotamon nanan	Siqian Village, Shouning County, Fujian	NCU MCP4090	Bna1	MK920093	MK795660	MK952588
		NCU MCP4090	Bna2	MK920094	MK795661	MK952589
	Yongjia County, Zhejiang	NCU MCP4038	Bna3	MK920095	MK795662	MK952590
		NCU MCP4038	Bna4	MK920096	MK795663	MK952591
	Yongjia County, Zhejiang	NCU MCP4039	Bna5	MK920097	MK795664	MK952592
		NCU MCP4039	Bna6	MK920098	MK795666	MK952593
	Bindong Village, Lingchuan	NCU MCP3281	Blc1	MK920090	MK795657	MK952585

Bottapotamon lingchuanense	County, Guangxi Zhuang Autonomous Region					
	Yuanpu Village, Gongcheng County, Guangxi Zhuang Autonomous Region	NCU MCP4076	Blc2	MK920091	MK795658	MK952586
		NCU MCP4076	Blc3	MK920092	MK795659	MK952587
***Bottapotamon chenzouense* sp.n.**	Zixing County, Chenzhou City, Hunan	NCU MCP643	Bcz1	MK920079	MK795646	MK952574
		NCU MCP643	Bcz2	MK920080	MK795647	MK952575
***Bottapotamon luxiense* sp.n.**	Yixiantian Wugongshan Mountain, Luxi County, Pingxiang City, Jiangxi	NCU MCP4200	Blx1	MK993542	MK981408	MK993544
		NCU MCP4200	Blx2	MK993543	MK981409	MK993545
Bottapotamon yonganense	Sanming City, Fujian	NCUMCP4096		Lack of sequence		

2

Table 2(on next page)

Primer sequences used in this study.

Gene	Primer name	Sequence (5'–3')	sequence length	Reference
COI	COI-1490	GGTCAACAAATCATAAAGATATTGG	750bp	Folmer et al., 1994
	COI-2198	TAAACTTCAGGGTGACCA AAAAATCA		
16S rRNA	16S-1471	CCTGTTTANCAAAAACAT	550bp	Crandall and Fitzpatrick, 1996.
	16S-1472	AGATAGAAACCAACCTGG		
H3	H3-F	ATGGCTCGTACCAAGCAGACVGC	374bp	Colgan et al.,1998
	H3-R	ATATCCTTRGGCATRATRGTGAC		

1
2

Table 3(on next page)

Differences between *Bottapotamon* species.

	B.fukiense	***B.yonganense***	***B.engelhardti***	***B.nanan***	***B.youxiense***	***B. lingchuanense***	***B.chenzhouense* sp.n**	***B.luxiense* sp.n.**
Frontal and gastric region	Protuberant	Protuberant	Protuberant	Protuberant	Protuberant	Protuberant slightly	Protuberant	Protuberant
Cardiac、intestinal and branchial region	Shallow	Protuberant	Protuberant	Shallow	Shallow	Protuberant slightly	Protuberant	Protuberant
Cervical groove	Low	Distinct	Low	Distinct.	Low	Low	Low	Distinct.
H shaped groove	Distinct	Distinct	Distinct	Distinct.	Distinct	Distinct	Distinct	Distinct.
postfrontal	Prominent	Prominent	Blunt	Prominent	Prominent	Prominent	Blunt	Blunt
Postorbital crest	Prominent	Blunt	Blunt	Blunt	Prominent	Blunt	Blunt	Prominent
Exorbital angle	Blunt	Triangle	Blunt	Blunt	Triangle	Triangle	Triangle	Triangle
Epibranchial tooth	Granular	Not prominent	Not prominent	Low but distinct	Not prominent	Not prominent	Blunt	Not prominent
Anter olateral regions	Ridgy	Ridgy	Ridgy	Ridgy	Ridgy	Long granular	Blunt	Blunt
Third maxilliped ischium	Length to width ratio 1.5	Length to width ratio 1.4	Length to width ratio 1.5	Length to width ratio 1.3	Length to width ratio 1.2	Length to width ratio 1.5	Length to width ratio 1.0	Length to width ratio 1.1
Third maxilliped merus	Length to width ratio 1.3	Length to width ratio 1.1	Length to width ratio 1.2	Length to width ratio 1.4	Length to width ratio 1.1	Length to width ratio 1.2	Length to width ratio 1.3	Length to width ratio 1.4
Male abdomen	Triangular	Narrow and long triangular	Triangular	Triangular	Triangular	Triangular	Narrow and long Triangular	Triangular
Male pleonal somite 6	Width to length ratio 2.1	Width to length ratio 1.9	Width to length ratio 2.2	Width to length ratio 2.0	Width to length ratio 1.8	Width to length ratio 2.1	Width to length ratio 2.5	Width to length ratio 2.5
Male telson	Width to length ratio 1.5	Wdth to length ratio 1.3	Tongue shape，width to length ratio 1.3	Triangular, width to length ratio 1.4	Tongue shape, width to length ratio 1.5	Width to length ratio 1.2	Width to length ratio 1.3	Width to length ratio 1.3
Immovable finger	Length to width ratio 1.3	Length to width ratio 1.7	Length to width ratio 1.4	Length to width ratio 1.7	Length to width ratio 1.7	Length to width ratio 1.4	Length to width ratio 1.4	Length to width ratio 1.8
Immovable finger and movable finger	Length ratio 1.4	Length ratio 1.3	Length ratio 1.2	Length ratio 1.7	Length ratio 1.6	Length ratio 1.2	Length ratio 1.7	Length ratio 1.6
G1	Subdistal segment contract，distal segment long and narrow	Distal Segment tabular arcuate	Distal segment long and narrow	With distinct longitudina groove	Subdistal segment narrow，Distal segment spacious and strong	Distal segment Contract，terminal Segment tortuous slightly	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.	G1 is slender, and the terminal segment is beyond the tubercle of the abdominal lock and beyond the fourth and fifth breastplate lines; the width of G1 is same from basis to end.

1

Figure 1

B.Chenzhouense sp.n. Holotype male (20.7 × 15.7 mm) (NCU MCP 643-1) .

(A) Overall habitus; (B) frontal view of cephalothorax. Photograph courtesy of Jie-Xin Zou, November 2018.

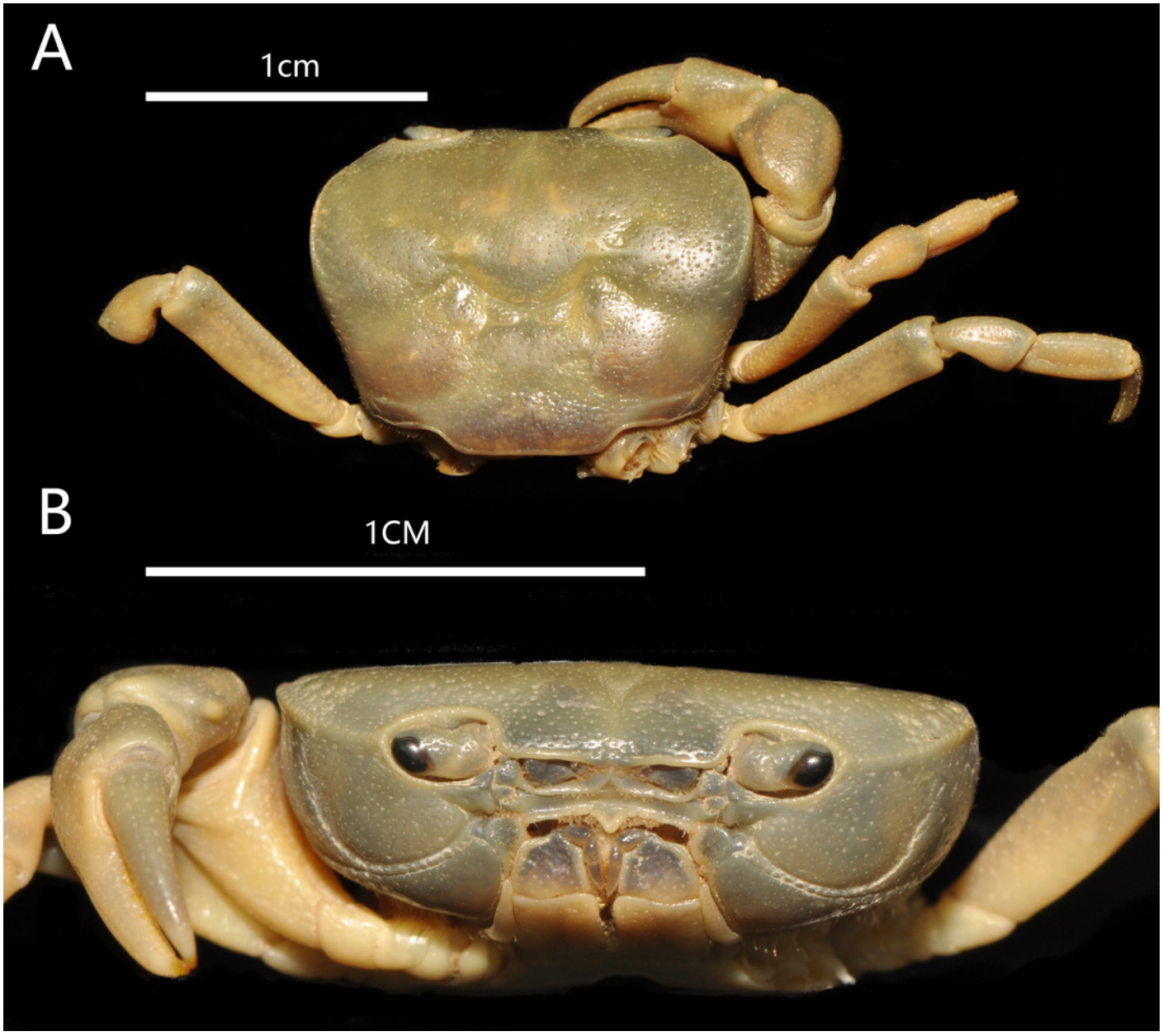

Figure 2

B.Chenzhouense sp.n. Holotype male (20.7 × 15.7 mm) (NCU MCP 643-1).

(A) left third maxilliped; (B) outer view of chelipeds; (C) right fourth ambulatory leg.

Photograph courtesy of Jie-Xin Zou, November 2018.

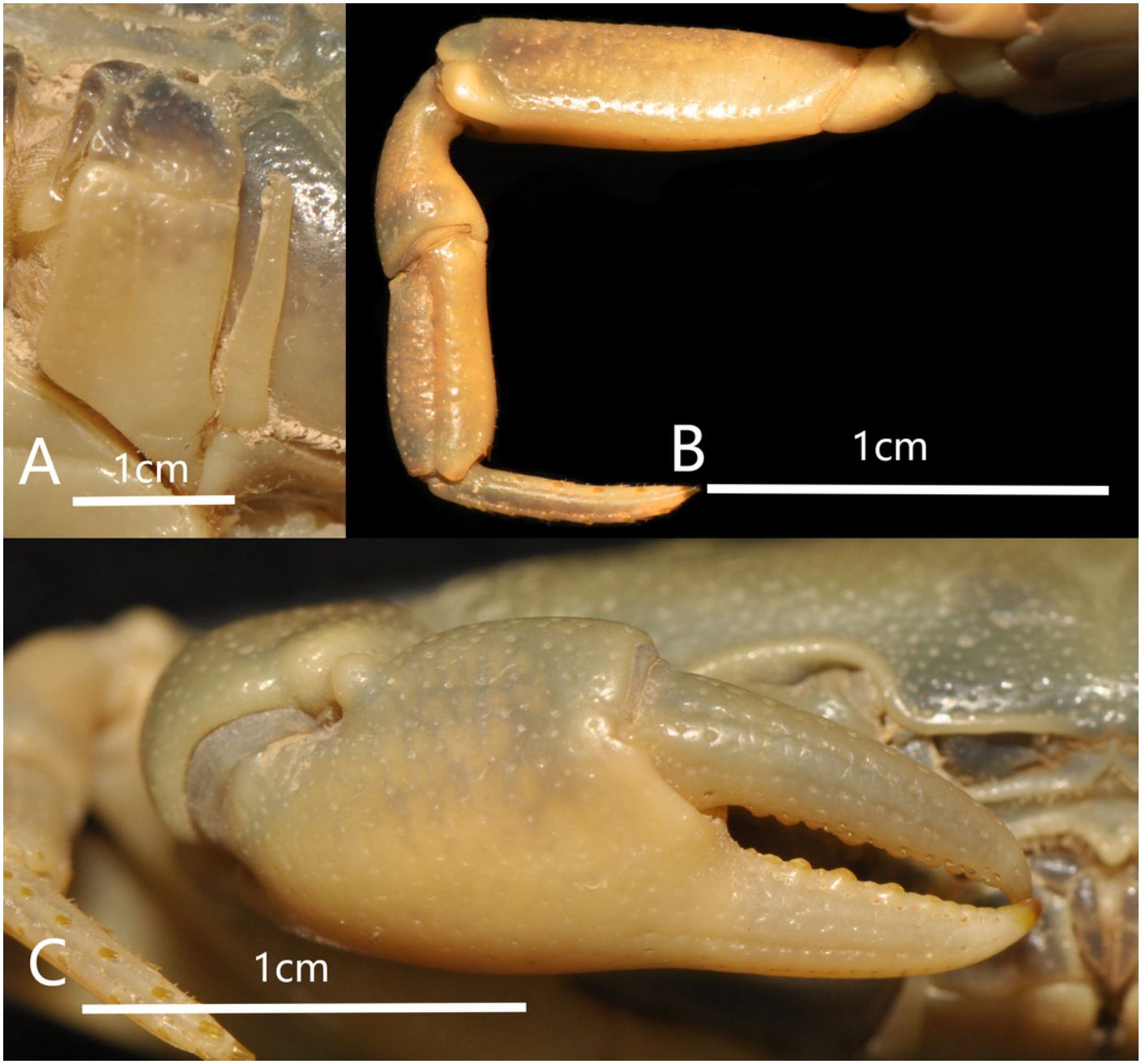

Figure 3

B.Chenzhouense sp.n. Holotype male (20.7 × 15.7 mm) (NCU MCP 643-1).

(A) male sternum. Interruption between sutures of sternites 4/5, 5/6, 6/7;tubercle of abdominal lock. (B) median logitudinal suture of sternites7, 8. Photograph courtesy of Jie-Xin Zou, November 2018.

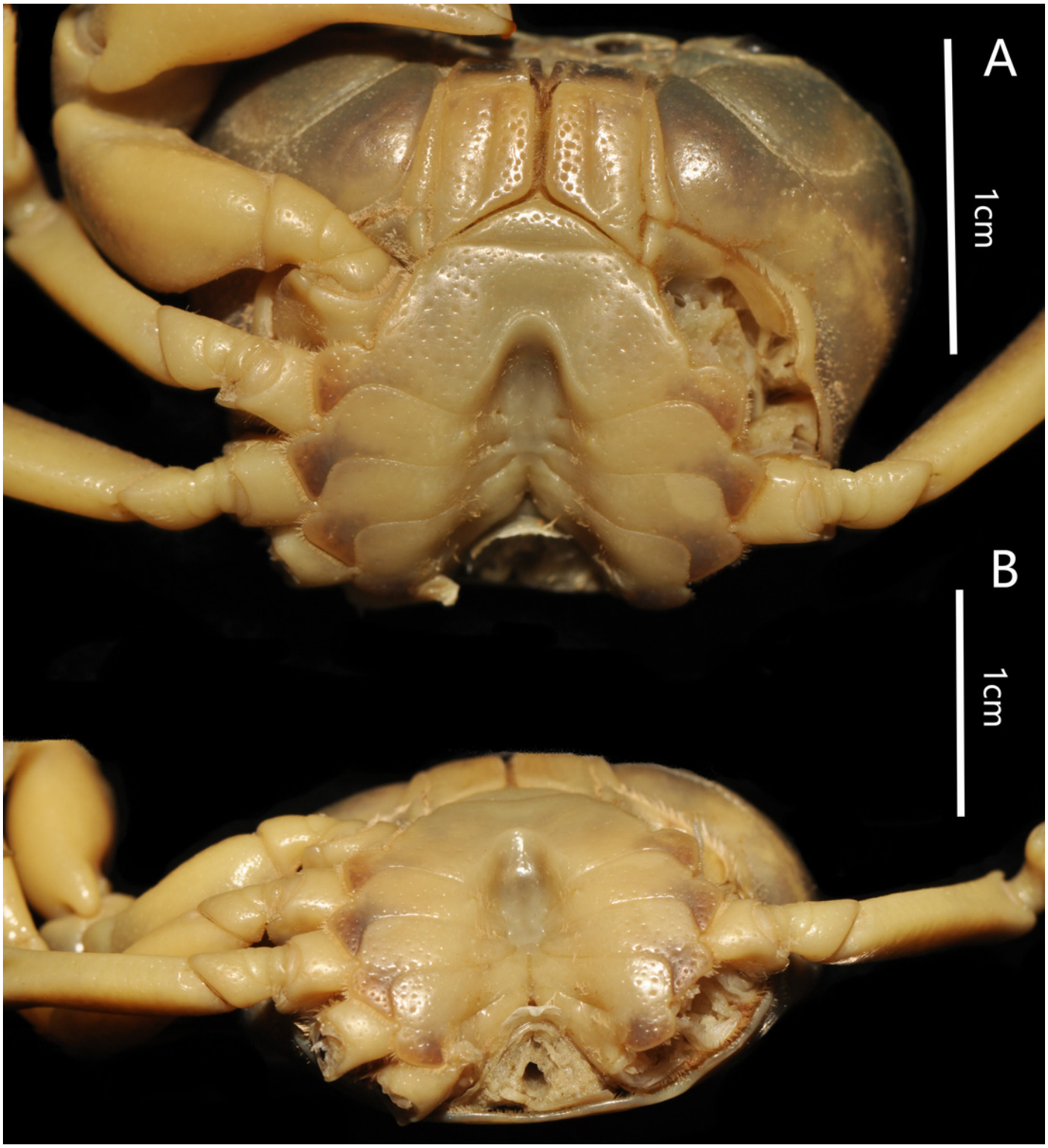
A
1cm
B
1cm

Figure 4

B.Chenzhouense sp.n. Holotype male (20.7 × 15.7 mm) (NCU MCP 643-1).

Natural position of male G1 and median longitudinal suture of sternites 7,8. Photograph courtesy of Jie-Xin Zou, November 2018.

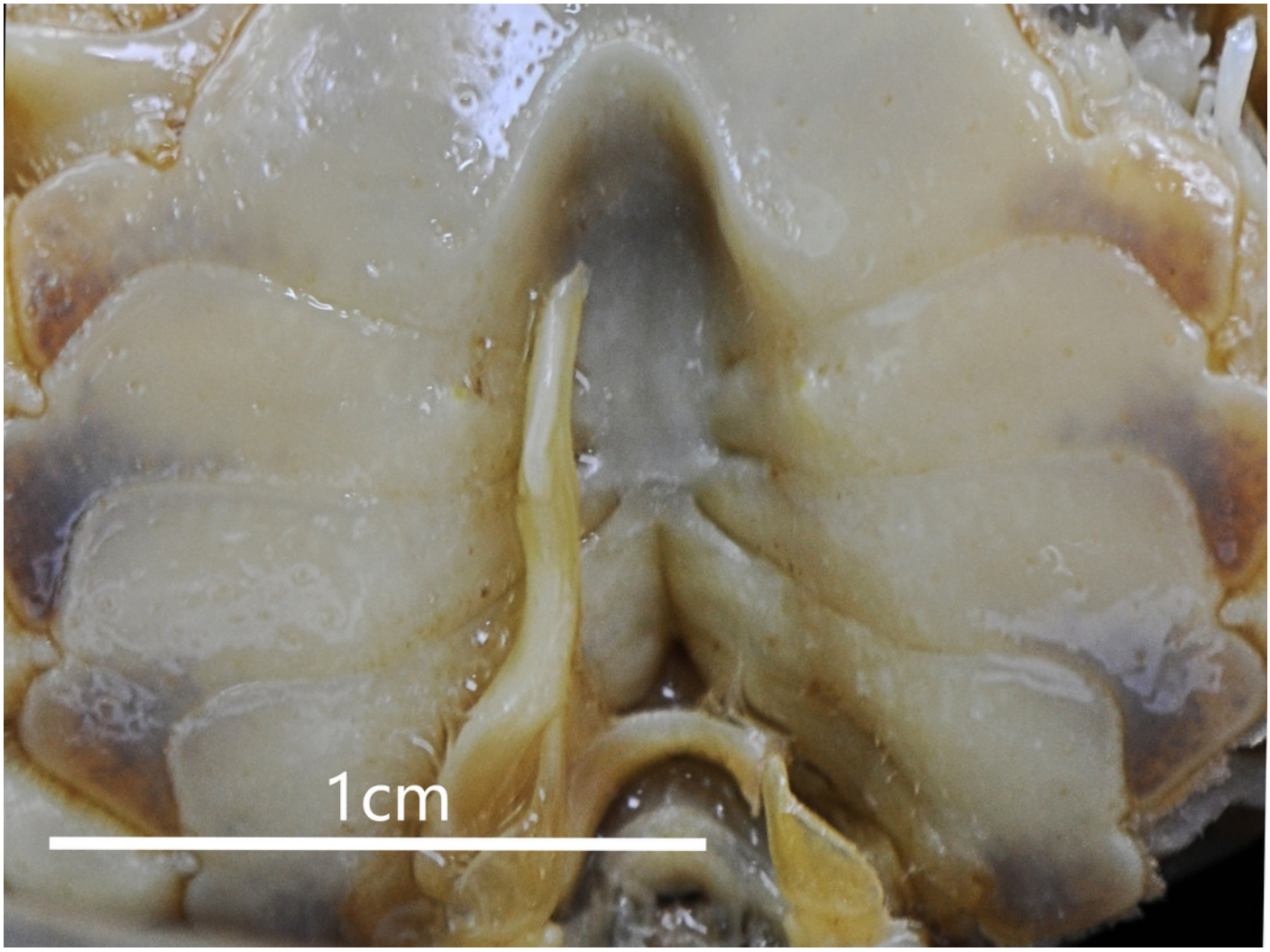

Figure 5

B.Chenzhouense sp.n. Holotype male (20.7 × 15.7 mm) (NCU MCP 643-1).

(A) ventral view of left G1; (B) ventral view of distal part of left G1; (C) dorsal view of distal part of left G1; (D) left G2. Scales: A, D = 1.0mm ; B, C = 0.5mm.

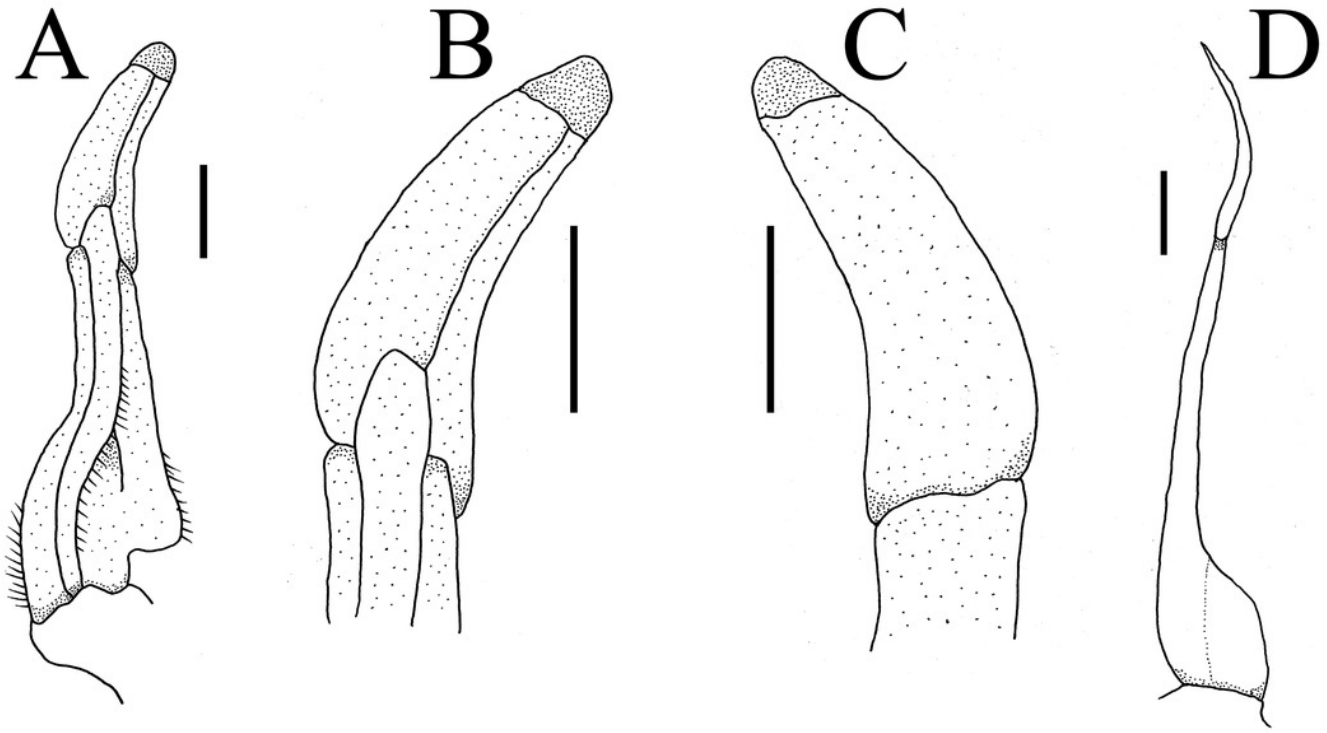

Figure 6

B.luxiense sp.n. Holotype male (18.72x15.69 mm) (NCU MCP 4200-Blx1).

Overall habitus. Photograph courtesy of Jie-Xin Zou, May 2019.

Figure 7

B.luxiense sp.n. Holotype male (18.72x15.69 mm) (NCU MCP 4200-Blx1).

(A) left third maxilliped; (B) outer view of chelipeds; (C) right fourth ambulatory leg.

Photograph courtesy of Jie-Xin Zou, May 2019.

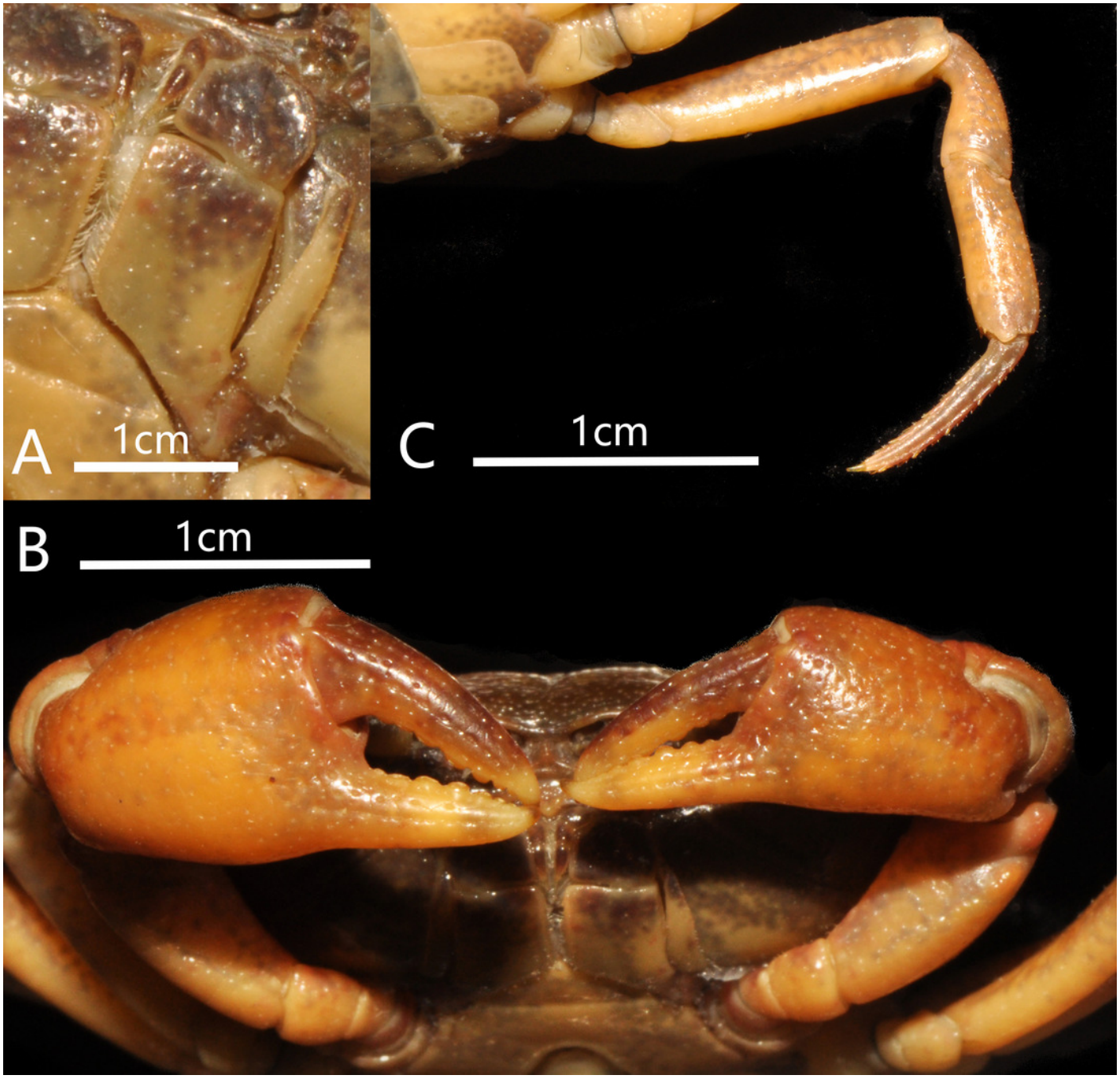

Figure 8

B.luxiense sp.n. Holotype male (18.72x15.69 mm) (NCU MCP 4200-Blx1).

Male sternum. Photograph courtesy of Jie-Xin Zou, May 2019.

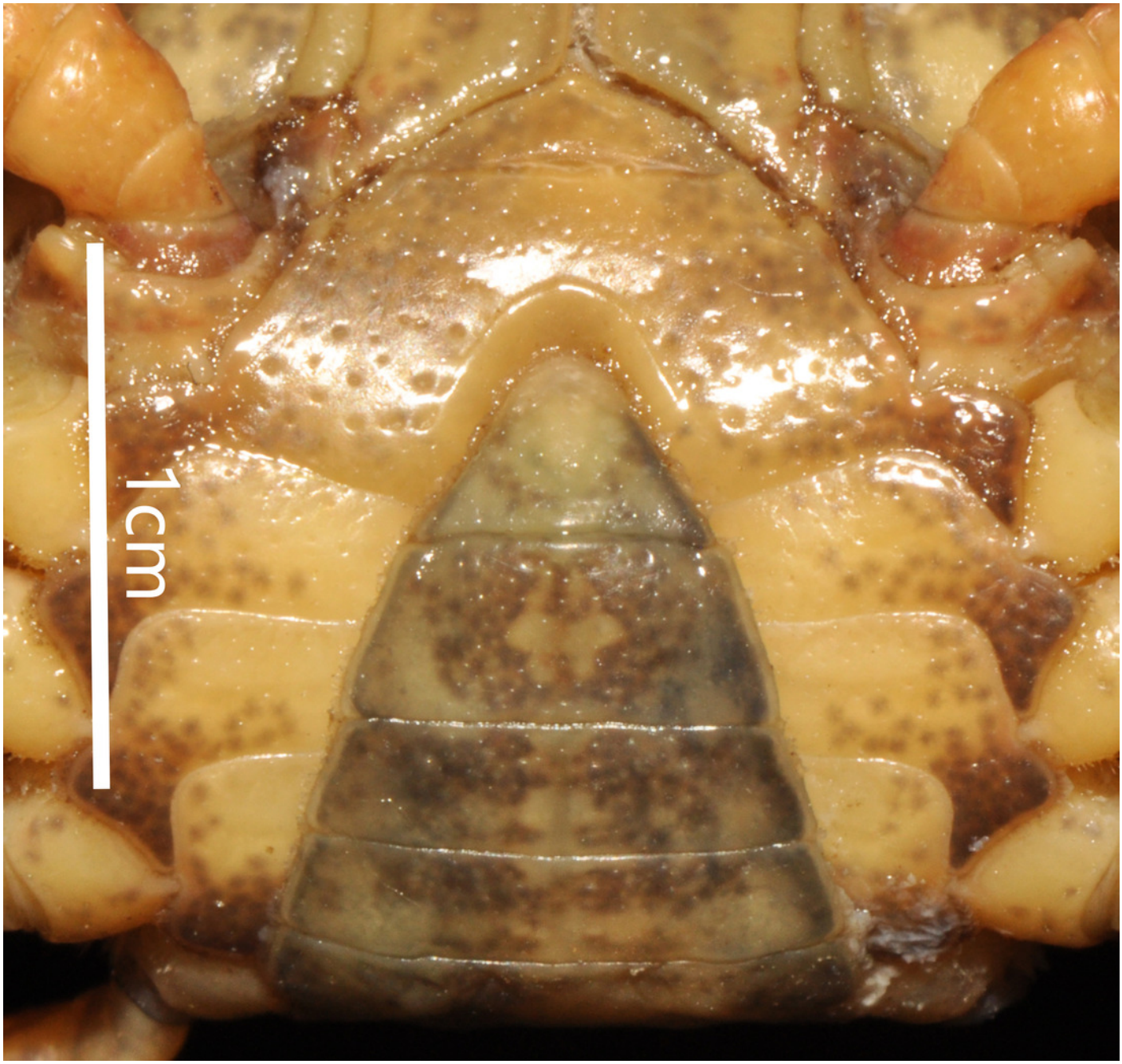

Figure 9

B.luxiense sp.n. Holotype male (18.72x15.69 mm) (NCU MCP 4200-Blx1).

Natural position of male G1 and median longitudinal suture of sternites 7,8. Photograph courtesy of Jie-Xin Zou, May 2019.

Figure 10

B.luxiense sp.n. Holotype male (18.72x15.69 mm) (NCU MCP 4200-Blx1).

(A) ventral view of left G1; (B) ventral view of distal part of left G1; (C) dorsal view of distal part of left G1; (D) left G2. Scales: A, D = 1.0mm ; B, C = 0.5mm.

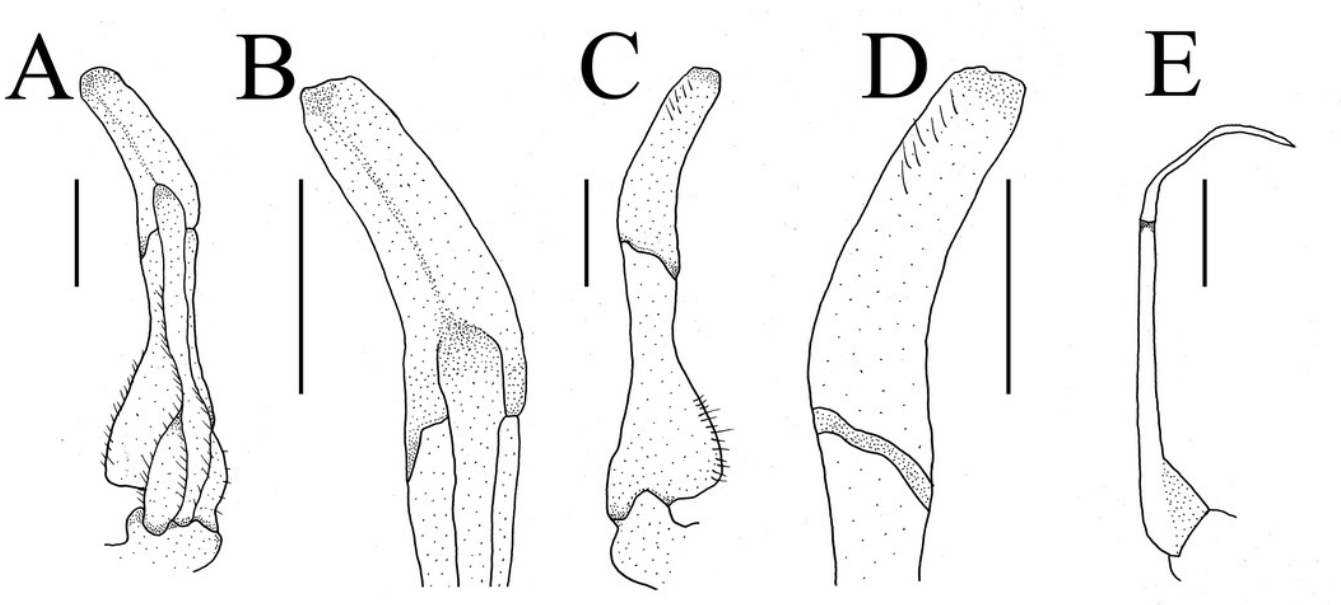

Figure 11

A limestone hill stream at the type locality.

Photo taken by Jie-Xin Zou, May 2019

Figure 12

Typical terrain of Luoxiao mountains.

Photo taken by Jie-Xin Zou, May 2019.

Figure 13

Phylogenetic tree of *Bottapotamon*

A maximum likelihood (ML) tree of the genus *Bottapotamon*from the Chinese coastal provinces, and outgroups, based on the combined mtDNA COI, 16S rRNA and nuclear histone H3 genes (length=1404bp). Support values(P≧50%) for ML, BI is represented at the nodes. Locality names in Table 1 are parenthesized behind specimens.

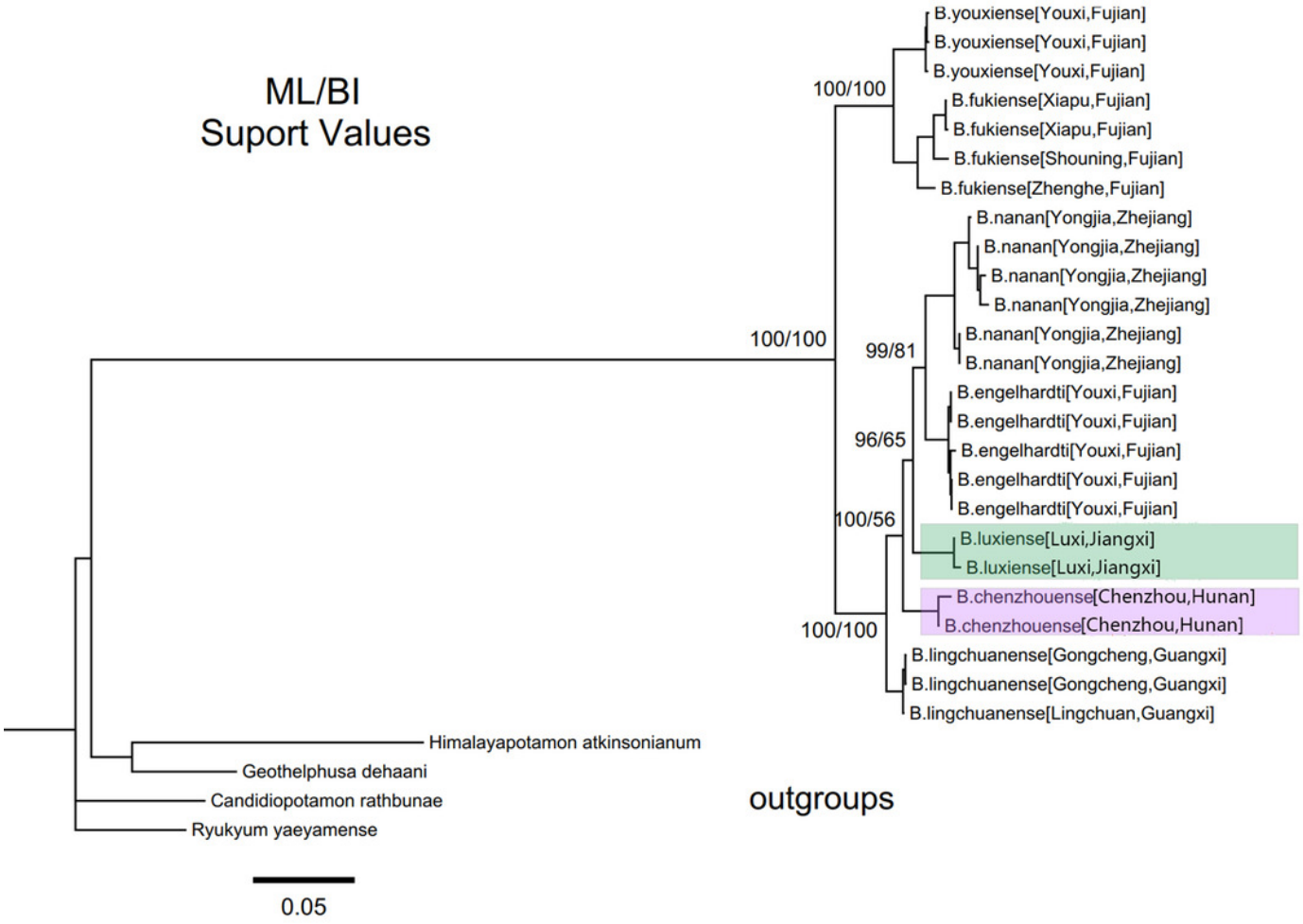

Figure 14

A chronogram of the genus *Bottapotamon* from the Chinese coastal provinces.

Based on the mtDNA COI, 16S rRNA genes. Calibration point 1 was set for the divergence time between subfamily Potamiscinae and subfamily Potaminae; Calibration point 2 was set for the glacial periods in Taiwan Strait; Formation time of Wuyi mountains was set for Calibration point 2. The divergence times estimated are shown in the main nodes. Lacality names in Table 1 are parenthesized behind specimen.

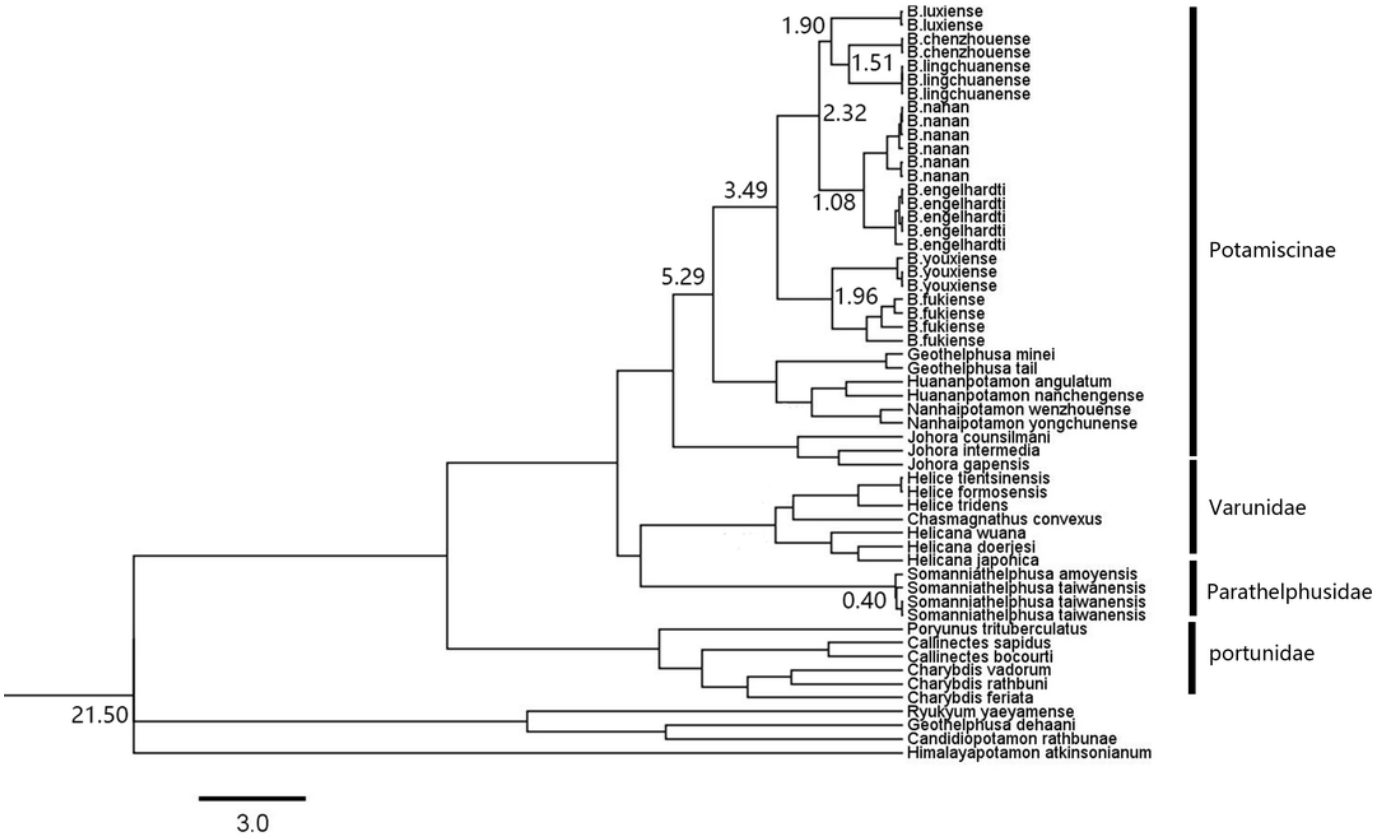

Figure 15

Collection sites for the genus *Bottapotamon* from the Chinese coastal provinces used in this study.

Mark the the main mountain. Natural geographical area of freshwater crab: II A (Central China region - Western mountain plateau subregion), II B (Central China region - Eastern hilly plains subregion), III B (South China region - Guangxi&Guangdong subregion). III C (South China region - Fujian&Guangdong&Jiangxi subregion), III E (South China region - TAIWAN subregion). The regional map comes from https://commons.wikimedia.org/wiki/Atlas_of_the_world and http://landsatlook.usgs.gov/;the map was edited with Adobe Photoshop CS6.