

Two new species and the molecular phylogeography of the freshwater crab genus *Bottapotamon* (Crustacea: Decapoda: Brachyura: Potamidae) (#36419)

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

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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* formed gradually.

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

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33 **Abstract**

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

51

52 **Introduction**

53 The genus *Bottapotamon* is a unique genus of freshwater crabs from the China mainland. In
54 1997, three species of the genus *Malayopotamon* (Bott R, 1967; Cheng YZ, JX Lin & XQ Luo,
55 1993; Dai AY et al., 1979) and one new species were identified as *Bottapotamon* on the basis of
56 its morphological characteristics, such as the form of carapace and first gonopod (G1) (Türkay &
57 Dai, 1997). Until the current study, the genus *Bottapotamon* contained *B. fukiense*, *B.*

58 *engelhardti*, *B. yonganense*, *B. lingchuanense* (Türkay & Dai, 1997), *B. youxiense* (Cheng et al.
59 2010) and *B. nanan* (Zhou, Zhu, and Naruse. 2008).

60 The relatively low fecundity and poor dispersal abilities of freshwater crabs (Daniels et al.,
61 2003; Yeo et al., 2008) mean that these crabs are easily isolated by barriers such as mountains or
62 seas. Geographically isolated populations then become genetically distinct and result in allopatric
63 speciation (Shih et al., 2006; Yeo et al., 2007). In mainland China, the distribution of the genus
64 *Bottapotamon* is restricted within the area of the Wuyi Mountain Range; *B. engelhardti*, *B.*
65 *yonganense*, *B. youxiense* and *B. nanan* are distributed east of the Wuyi Mountain Range, *B.*
66 *fukiense* occurs on both sides of the Wuyi Mountains (Fujian and Jiangxi Provinces), and only *B.*
67 *lingchuanense* has been isolated in the Nanling Mountain Range (Dai, 1997) (Fig. 1). The
68 geographic barrier separating the Wuyi Mountains from the Nanling Mountains is the Luoxiao
69 Mountain Range, which is the highest range in the area, exceeding 2120 m in height (Gong HL,
70 Zhuang WY, Liao WB, 2016). The terrain the genus *Bottapotamon* now inhabits is geologically
71 relatively stable and experienced little orogenic activity during the Cenozoic (Yi, 1996; Zhou and
72 Li, 2000). Therefore, we hypothesize that the distribution of the genus *Bottapotamon* in mainland
73 China was caused by the emergence of these mountains.

74 By organizing specimens deposited at the Department of Parasitology of the Medical
75 College of Nanchang University (NCU MCP) and newly collected specimens, the author
76 discovered two new species in Chenzhou City, Hunan Province, and Luxi County, Jiangxi
77 Province. This paper compares the morphological features of eight species including two new
78 species of the genus *Bottapotamon*, as well as 16S rRNA (Crandall et al. 1996), mtDNA COI
79 (Folmer et al. 1994) and nuclear histone H3 (Colgan et al. 1998) gene fragments were used to
80 support the establishment of new species in the genus *Bottapotamon*. The phylogenetic
81 relationship, distribution pattern and possible association with major geological and historical
82 events are also discussed.

83

84

85 **Materials & Methods**

86 **Specimen collection**

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

93 **Phylogenetic analyses and Divergence time estimation**

94 Genomic DNA was extracted from leg muscle tissue with an OMEGA EZNA™ Mollusc
95 DNA Kit. The 16S rRNA, mtDNA COI, and histone H3 regions were selected for amplification
96 by polymerase chain reaction (PCR) (Table 2). The amplification products were sent to the
97 Beijing Genomics Institute for bidirectional sequencing, and the sequencing results were spliced
98 manually to obtain the sequence data. DNA sequences of *B.yonganense* specimens collected

99 from the suburb of Sanming City, Fujian Province, China, could not be amplified due to poor
100 preservation.

101 After searching the National Center for Biotechnology Information (NCBI) database, we
102 finally selected the sequences of four individuals with the same primer sequences as the
103 outgroups (*Candidiopotamon rathbunae* (GenBank accession numbers: mtDNA COI-AB290649,
104 16S rRNA-AB208609, histone H3-AB290668), *Geothelphusa dehaani* (GenBank accession
105 numbers: mtDNA COI-AB290648, 16S rRNA-AB290630, histone H3-AB290667),
106 *Himalayapotamon atkinsonianum* (GenBank accession numbers: mtDNA COI-AB290651, 16S
107 rRNA-AB290632, histone H3-AB290670), and *Ryukyum yaeyamense* (GenBank accession
108 numbers: mtDNA COI-AB290650, 16S rRNA-AB290631, histone H3-AB290669)). After
109 comparing and selecting the conservative regions, each sequence was 1323 bp in length.
110 According to the Akaike information criterion (AIC), MrMTGui: ModelTest and MrModelTest
111 (phylogenetic analysis using parsimony (PAUP)) determined the best models was GTR+I+G;
112 MEGA 6.06 (Tamura et al. 2013) was used to establish a phylogenetic tree based on the
113 maximum likelihood (ML) (Trifinopoulos et al. 2016). The Bayesian inference (BI) tree was
114 established using MrBayes (Ronquist & Huelsenbeck 2003).

115 The divergence times of genus *Bottapotamon* were estimated from the combined 16S rRNA
116 and mtDNA COI sequences, based on the Bayesian evolutionary analysis sampling trees
117 (BEAST) program, and four calibration points were used. The Potamidae family has divided into
118 two major subfamilies, Potamiscinae and Potaminae, estimated to have a divergence time of
119 20.9-24.7 mya, which was set as calibration point 1 in our study (Shih et al. 2010). The
120 Parathelphusidae subfamily, *Somanniathelphusa taiwanensis*, which is distributed in Taiwan
121 Island and separated from *Somanniathelphusa amoyensis*, which is distributed in Fujian
122 Province, approximately 0.27-1.53 mya (Jia et al. 2018). The results are consistent with the
123 quaternary glacial period and interglacial period and agree with the separation of Taiwan Island
124 and Fujian Province; this time point was set as calibration point 2. In the geological area where
125 genus *Bottapotamon* is distributed, the Wuyi Mountains gradually formed by the compression of
126 the Pacific plate and the Indian plate in the Neogene-Quaternary (1.64-23.3 mya) (Li, 1984);
127 this time point was set as calibration point 3. A Yule speciation model was constructed for
128 speciation within the genus *Bottapotamon*. We used a GTR+G model with parameters obtained
129 from MrMTGui: ModelTest and MrModelTest (PAUP) for each gene. Seventeen independent
130 MCMC chains were run for 200,000,000 generations, and every 20,000 generations were
131 sampled. The convergence of the 17 combined chains was determined by the evolutionary stable
132 strategy (ESS) (>200 as recommended) for each parameter in Tracer after the appropriate burn-in
133 and cutoff (default of 10% of sampled trees). Trees in the 17 chains were combined using
134 LogCombiner (v. 1.6.1, distributed as part of the BEAST package) and were assessed using
135 TreeAnnotator (v. 1.6.1, distributed as part of the BEAST package). A chronogram was
136 constructed by FigTree.

137 **Nomenclatural note**

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

148

149 Results

150 Systematics

151

152 Potamidae Ortmann, 1896

153 *Bottapotamon* Türkay et Dai, 1997

154 *Bottapotamon chenzhouense* sp.n., *Bottapotamon luxiense* sp.n.

155

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

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

158

159 Materials examined

160 Holotype: 1 ♂ (25.72 × 15.69 mm) (NCU MCP 643), Huangcao Village, Chenzhou City,
161 Hunan Province, China, 25°39'24.60"N, 113°30'4.07"E, 141 m asl. Coll. Dingmei Luo, July 26th
162 2006. Paratypes: 1 ♀ (18.7 × 13.7 mm) (NCU MCP 643), the same data as the holotype.

163 Comparative materia

164 *B. fukiense* (Türkay & Dai, 1997): 4 ♂ (25.21 × 15.02 mm, 25.03 × 14.97 mm) (NCU MCP
165 4089), Xiapu Village, Ningde County, Fujian; (26.08 × 15.45 mm) (NCUMCP4156), Shangshan
166 Village, Zhenghe County, Fujian; (26.08 × 15.45 mm) (NCUMCP4090), Siqian Village,
167 Shouning County, Fujian; 1 ♀ (26.01 × 15.57 mm) (NCU MCP 4156), Shangshan Village,
168 Zhenghe County, Fujian. *B. engelhardti* (Türkay & Dai, 1997): 5 ♂ (23.01 × 14.03 mm, 24.68 ×
169 15.69 mm, 24.81 × 15.87 mm, 25.02 × 15.47 mm) (NCU MCP 4157), Tangsan Village, Youxi
170 County, Fujian; (25.21 × 15.16 mm) (NCU MCP 4091), Chimu Village, Youxi County, Fujian; 1
171 ♀ (26.01 × 16.35 mm) (NCU MCP 4091), Chimu Village, Youxi County, Fujian. *B. yonganense*
172 (Türkay & Dai, 1997): 1 ♂ (25.87 × 15.95 mm) (NCU MCP 4096), Sanming City, Fujian; *B.*
173 *lingchuanense* (Türkay & Dai, 1997), 3 ♂ (24.78 × 14.89 mm, 25.04 × 15.06 mm) (NCU
174 MCP4076), Yuanpu Village, Gongcheng County, Guangxi Zhuang Autonomous Region; (25.25
175 × 15.11 mm) (NCU MCP 3281), Bindong Village, Lingchuan County, Guangxi Zhuang
176 Autonomous Region; 3 ♀ (25.48 × 14.92 mm, 25.14 × 15.09 mm, 25.78 × 14.79 mm), (NCU

177 MCP 3281), Bindong Village, Lingchuan County, Guangxi Zhuang Autonomous Region. *B.*
 178 *youxiense* (Cheng et al., 2010): (24.91 × 15.72 mm) (NCU MCP 4092), (25.11 × 15.16 mm)
 179 (NCU MCP 4158), (25.34 × 15.52 mm) (NCU MCP 4059), Xiwei Village, Youxi County,
 180 Fujian; 1♀ (26.04 × 14.92 mm) (NCU MCP 4059), Xiwei Village, Youxi County. *B. nanan*
 181 (Zhou et al., 2008): 6♂ (26.26 × 15.72 mm, 26.03 × 15.55 mm) (NCU MCP4090), Siqian
 182 Village, Shouning County, Fujian; (26.15 × 15.43 mm, 26.16 × 15.32 mm) (NCU MCP4038),
 183 Yongjia County, Zhejiang; (26.25 × 15.36 mm, 26.06 × 15.52 mm) (NCU MCP4039), Yongjia
 184 County, Zhejiang; 1♀ (26.11 × 15.12 mm) (NCU MCP4039), Yongjia County, Zhejiang.

185 **Diagnosis**

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

195 **Description**

196 Carapace approximately about 1.6 times broader than long, dorsal surface gently convex,
 197 surface slightly pitted. Cervical groove shallow, indistinct. H-shaped groove between the gastric
 198 region and cardiac region shallow but distinct. Postfrontal lobe blunt, separated medially by a Y-
 199 shaped groove extending to frontal region; postorbital crest indistinct, postorbital region slight
 200 concave. Frontal region deflexed downwards. Dorsal orbital margin ridged, external orbital angle
 201 triangular outer margin smooth; Anterolateral margin cristate, epibranchial tooth pointed,
 202 indistinct, clearly demarcated from external orbital tooth (Fig. 2).

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

206 The male sternum is relatively flat and has granular small pits. The first section is triangular
 207 and the second to fourth sections are fused. The interruption between sternite sutures is
 208 intermediate in depth and wide. The median longitudinal sutures of sternites 7/8 are shorter; the
 209 tubercle of abdominal lock is on the medial side of the fifth male ventral nail (Fig. 4).

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

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

216 G1 slender, a fold covering the surface of the entire subterminal article with a transparent
 217 distal region. tip of terminal segment slightly reaching beyond sternal press-button in situ,

218 subterminal segment about 1.3 times as long as terminal segment. G1 slightly curved
219 ~~ventrolaterally~~; distal part of G1 terminal segment distinctly broader than proximal part. G2
220 subterminal segment about 2.3 times as long as terminal segment (Fig. 5A, 6A).

221 **Remarks**

222 The new species fits well within the morphological definition of the hitherto monotypic
223 *Bottapotamon* (Türkay & Dai, 1997; Cheng et al., 2010; Zhou et al., 2008): G1 is slender, tip of
224 terminal segment reaching suture between thoracic sternites 4, 5 in situ; terminal segment
225 slightly elongated inward (Table. 3). Nonetheless, the new species can be distinguished from
226 comparative specimens, by the Carapace surface gently convex, cervical groove indistinct; H-
227 shaped groove shallow but distinct. epibranchial tooth pointed and indistinct, third maxilliped
228 without flagellum; chelipeds carpus with sharp spine on inner distal angle; G1 is sturdy and blunt
229 (Table. 3).

230 **Etymology**

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

232 **Distribution**

233 *B. chenzhouense* sp. n. was found under stones in a mountain stream in Huangcao village,
234 Chenzhou city, Hunan Province, China.

235

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

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

238

239 **Materials examined**

240 Holotype: 1 ♂ (18.72×15.69 mm) (NCU MCP 4200), Yixiantian Wugongshan Mountain,
241 Luxi county, Pingxiang city, Jiangxi Province, China, 27°28'56.16"N, 114°10'27.51"E, 1331 m
242 asl. Coll. Jiexin Zou, May 6th 2019. Paratypes: 1 ♂ (19.22 × 16.38 mm) (NCU MCP 4200).
243 Others: 12 ♀ (16.7 × 15.7 mm, 15.41×15.36 mm, 14.23×12.98 mm, 15.63×14.52 mm,
244 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,
245 16.02×15.43 mm, 15.89×15.01 mm, 13.13×12.46 mm) (NCU MCP 4200), 12 ♂ (15.66×13.89
246 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,
247 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,
248 15.26×14.36 mm) (NCU MCP 4200), same data as holotype.

249 **Comparative materia**

250 Same as *Bottapotamon chenzhouense* sp. n.

251 **Diagnosis**

252 Carapace about 1.4 times broader than long, dorsal surface gently convex longitudinally and
253 transversely; cervical groove distinct, H-shaped groove between gastric and cardiac regions
254 distinct. Male pleon triangular, sixth somite width 2.3 times length; telson triangular, tip
255 rounded, with proximal width 1.6 times length. G1 long, tip of terminal segment reaching suture
256 between thoracic sternites 4, 5 in situ; subterminal segment 1.2 times as long as terminal
257 segment; terminal segment slightly elongated inward, distal part of terminal segment elongated

258 with ventrally directed semicircular lobe. Female vulvae partially exposed anteriorly to the
259 thoracic sternites 5, 6 in situ, ovate, deep, posteromesial margin with a law raised rim, opened
260 inward.

261 Description

262 Carapace nearly ellipse, about 1.4 times broader than long, dorsal surface gently convex,
263 surface slightly pitted. Cervical groove distinct. H-shaped groove between the gastric region and
264 cardiac region shallow but distinct. Postfrontal lobe blunt; postorbital crest indistinct, postorbital
265 region slight concave. Frontal region deflexed downwards. Dorsal orbital margin ridged, external
266 orbital angle triangular, outer margin smooth. Anterolateral margin cristate, epibranchial tooth
267 pointed (*Fig. 7*).

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

271 Thoracic sternum pitted; sternites 1, 2 completely fused to form triangular structure; sternites
272 2, 3 separated by continuous suture; boundary between sternites 3, 4 present, indistinct. Sterno-
273 pleonal cavity broad, shallow, with narrow median interruption in sutures 4/5, 5/6, 6/7 ; median
274 line between sternites 7, 8 moderately long (*Fig. 9*).

275 The male sternum is relatively flat and has granular small pits. The first section is triangular
276 and the second to fourth sections are fused. The interruption between sternite sutures is medium
277 in depth and wide. The median longitudinal sutures of sternites 7/8 are shorter; the tubercle of
278 abdominal lock is on the medial side of the fifth male ventral nail (*Fig. 6B*).

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

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

284 G1 is sturdy and blunt, tip of terminal segment slightly reaching beyond sternal press-button
285 in situ, subterminal segment about 1.4 times as long as terminal segment. G1 slightly curved
286 ventrolaterally; distal part of G1 terminal segment distinctly broader than proximal part. G2
287 subterminal segment about 2.2 times as long as terminal segment (*Fig. 5B. 6B*).

288 Remarks

289 The new species fits well within the morphological definition of the hitherto monotypic
290 *Bottapotamon* (*Türkay & Dai, 1997; Cheng et al., 2010; Zhou et al., 2008*): ~~cervical groove~~
291 ~~indistinct, H-shaped groove between gastric and cardiac regions distinct, G1 long, tip of~~
292 ~~terminal segment reaching suture between thoracic sternites 4, 5 in situ; terminal segment~~
293 ~~slightly elongated inward (Table. 3).~~ Nonetheless, the new species can be distinguished from
294 comparative specimens, by the Carapace surface gently convex, cervical groove shallow and
295 indistinct; H-shaped groove shallow but distinct. epibranchial tooth pointed and indistinct, third
296 maxilliped without flagellum; chelipeds carpus with sharp spine on inner distal angle, with
297 spinule at base; G1, a fold covering the surface of the entire subterminal article with a transparent
298 distal region (*Table. 3*).

299 Etymology

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

302 **Living color**

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

306 **Distribution**

307 *B. luxiense* sp. n. was found under stones in a mountain stream in Yixiantian Wugongshan
308 Mountain, Luxi county, Pingxiang city, Jiangxi Province, China (Fig. 10).

309 **Ecology**

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

316

317 **Phylogenetic analyses and Divergence time estimation**

318 The combined mtDNA COI, 16S rRNA and nuclear histone H3 phylogenetic trees were
319 constructed by ML analysis, and the corresponding support values were calculated by ML and BI
320 analyses, both of which had high support values. The results showed that the genus
321 *Bottapotamon* is monophyletic, and confirmed that *B. chenzhouense* sp. n. and *B. luxiense* sp. n.
322 are new species of genus *Bottapotamon* and supported the relationship of the genus
323 *Bottapotamon* (Fig. 11). *B. engelhardti*, *B. yonganense* and *B. nanan*, which are mostly
324 distributed in the Wuyi Mountain Range, form a clade; *B. luxiense* sp. n. forms a sister clade to
325 the clade of *B. engelhardti*, *B. yonganense* and *B. nanan*. The next sister clade is composed of *B.*
326 *chenzhouense* sp. n., which is distributed in the Luoxiao Mountain Range, and the furthest sister
327 clade is composed of *B. lingchuanense*, which is distributed in the Nanling Mountain Range. *B.*
328 *fukiense* and *B. youxiense* are also distributed in the Wuyi Mountain Range, but they do not
329 assemble with *B. engelhardti*, *B. yonganense* and *B. nanan*.

330 The divergence time estimation results are consistent with the four calibration points. The
331 genus *Bottapotamon* diverged approximately 3.49-1.08 mya, *B. fukiense* and *B. youxiense*
332 diverged 1.96 mya (95% confidence interval =2.65-1.31 mya), *B. luxiense* diverged 1.90 mya
333 (95% confidence interval =2.05-1.09 mya), *B. lingchuanense* and *B. chenzhouense* sp. n.
334 diverged 1.51 mya (95% confidence interval =1.6-0.7 mya); *B. engelhardti* and *B. nanan*
335 diverged 1.08 mya (95% confidence interval =1.76-0.80 mya). (Fig. 12)

336

337 **Discussion**

338 In mainland China, the genus *Bottapotamon* is primarily distributed in the Wuyi Mountain
339 Range area; *B. luxiense* sp. n., *B. youxiense*, *B. nanan*, *B. engelhardti* and *B. yonganense* are

340 restricted within an area east of the Wuyi Mountain Range (Fig. 1). There is no record of any of
341 these five species in Jiangxi, despite extensive surveys of this area by the authors and their
342 colleagues over many years (Dai, 1999; Shi, 2012). The altitude of the Wuyi Mountain Range is
343 clearly high enough to prevent these species from reaching Jiangxi. *B. fukiense* occurs on both
344 sides of the Wuyi Mountain Range (Fujian and Jiangxi Provinces) and is able to disperse across
345 these mountains. The divergence time of *B. fukiense* and *B. youxiense* is 1.96 mya (95%
346 confidence interval =2.65-1.31 mya) (Fig. 12), which agrees well with records of the Pacific
347 plate and Indian plate extrusion in the Neogene-Quaternary (1.64-23.3 mya) (Li, 1984).
348 Therefore, these geological events may explain the distribution pattern of the genus
349 *Bottapotamon* in the Wuyi Mountain Range. The ancestor of the genus *Bottapotamon* originated
350 in an area close to the Wuyi Mountains, as the Wuyi Mountain Formation and smaller-scale
351 mountain deformations resulted in sufficient geographic barriers to isolate populations; thus, the
352 two species-groups were separated by the Wuyi Mountains.

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

373

374 Conclusions

375 *B. chenzhouense* sp. n. and *B. luxiense* sp. n., two new species from the Luoxiao Mountains
376 were reported in this paper. These two new species compensated for the geographical gap in the
377 genus *Bottapotamon*, and confirm the independence and intra- and interspecific relationships of

378 genus *Bottapotamon*. Combined with estimates of divergence times, this paper suggests that the
379 genus *Bottapotamon* was formed at 3.49-1.08 mya. Molecular evidence further supports the
380 scientific hypothesis of the authors that genus *Bottapotamon* originated on both sides of the
381 Wuyi Mountains and Luoxiao Mountains. In the geological area where the genus *Bottapotamon*
382 is distributed, the Wuyi Mountains gradually formed offshore and inland of southeastern China
383 by the compression of the Pacific plate and the Indian plate in the Neogene-Quaternary, and the
384 Luoxiao Mountains formed continuously in the north-south direction because of neotectonic
385 movement. Thus, the geographical distribution patterns of the genus *Bottapotamon* formed
386 gradually.

387

388

389 **ADDITIONAL INFORMATION AND DECLARATIONS**

390 **Acknowledgements**

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393 Shu-xin Xu for for their help and advice on the manuscript. We would also like to thank
394 Professor Xian-min Zhou for his guidance in this study.

395 **Data Availability**

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

399

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Table 1 (on next page)

Specimens and GenBank accession numbers of genus *Bottapotamon*.

1

	Localities	Museum catalogue No.	Haplotypes	COI Accession No.	16S Accession No.	H3 Accession No.
<i>Bottapotamon fukiense</i>	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
<i>Bottapotamon youxiense</i>	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
<i>Bottapotamon engelhardti</i>	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
<i>Bottapotamon nanan</i>	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

<i>Bottapotamon lingchuanense</i>	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
<i>Bottapotamon chenzouense</i> sp.n.	Zixing County, Chenzhou City, Hunan	NCU MCP643	Bcz1	MK920079	MK795646	MK952574
		NCU MCP643	Bcz2	MK920080	MK795647	MK952575
<i>Bottapotamon luxiense</i> sp.n.	Yixiantian Wugongshan Mountain, Luxi County, Pingxiang City, Jiangxi	NCU MCP4200	Blx1	MK993542	MK981408	MK993544
		NCU MCP4200	Blx2	MK993543	MK981409	MK993545
<i>Bottapotamon yonganense</i>	Sanming City, Fujian	NCUMCP4096		Lack of sequence		

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	16S-1471	CCTGTTTANCAAAAACAT	550bp	Crandall and Fitzpatrick, 1996.
rRNA	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.

	<i>B. fukiense</i>	<i>B. yonganense</i>	<i>B. engelhardti</i>	<i>B. nanan</i>	<i>B. youxiense</i>	<i>B. lingchuanense</i>	<i>B. chenzhouense</i> sp. n	<i>B. luxiense</i> sp. n.
Carapace	Flat, cervical groove indistinct	Swollen, cervical groove distinct	Swollen, cervical groove indistinct	Swollen, cervical groove distinct.	Swollen, cervical groove indistinct	Swollen, cervical groove indistinct	Swollen, cervical groove indistinct	Swollen, cervical groove distinct.
Exorbital angle	Blunt	Triangle	Blunt	Blunt	Triangle	Triangle	Triangle	Triangle
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 abdomen 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 abdomen telson	Width to length ratio 1.5	Width to length ratio 1.3	Width to length ratio 1.3	Width to length ratio 1.4	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	Stout, straight	Distal Segment tabular arcuate	Slender, distal dorsal lobe convex	Slender, distinct longitudinal groove	Slender, distal segment spacious and strong	Slender, terminal Segment tortuous slightly	slender, ventral flap with transparent protrusion	Sturdy and blunt

Figure 1

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

Mark the the main mountain. 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.

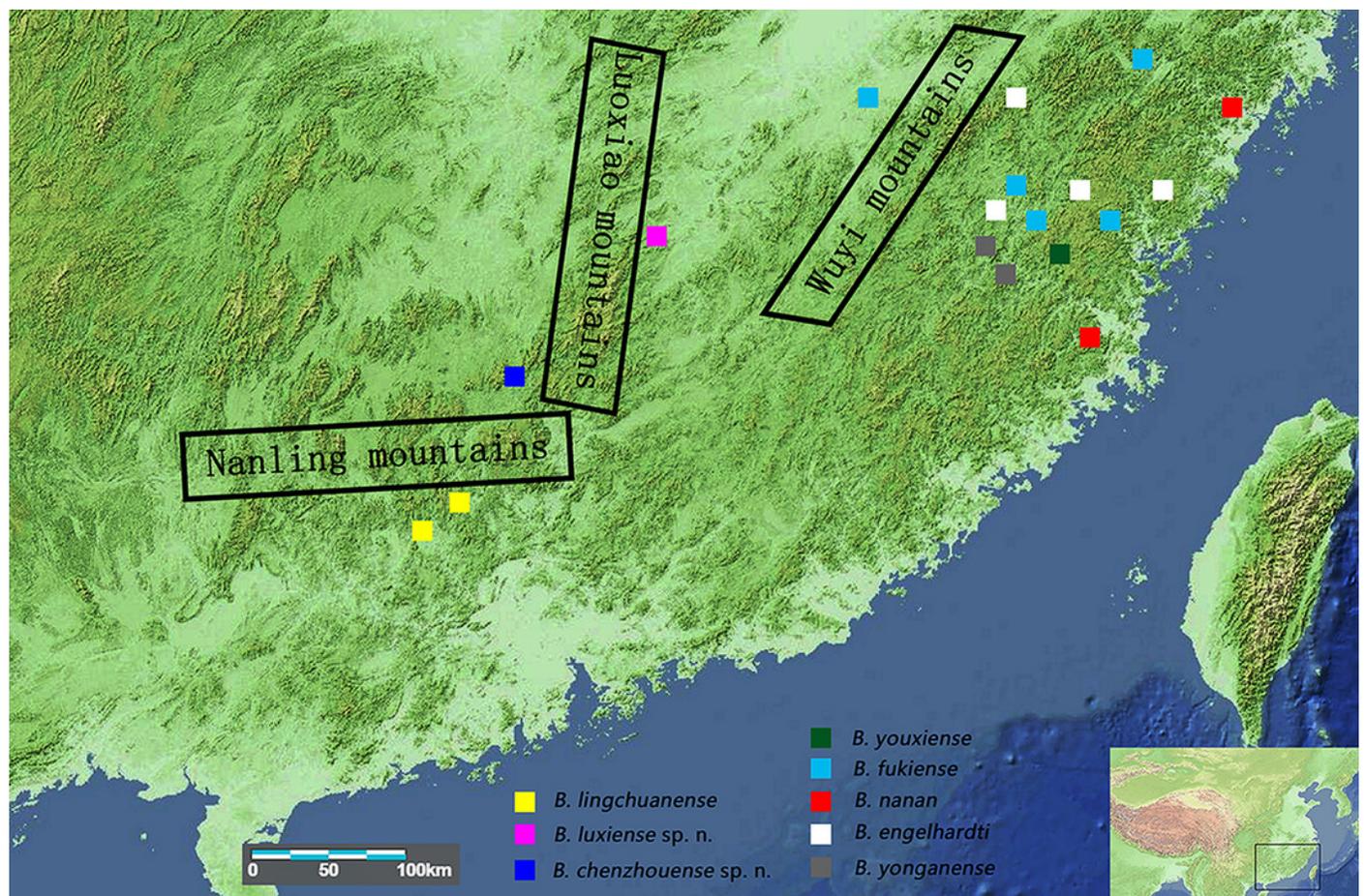


Figure 2

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.

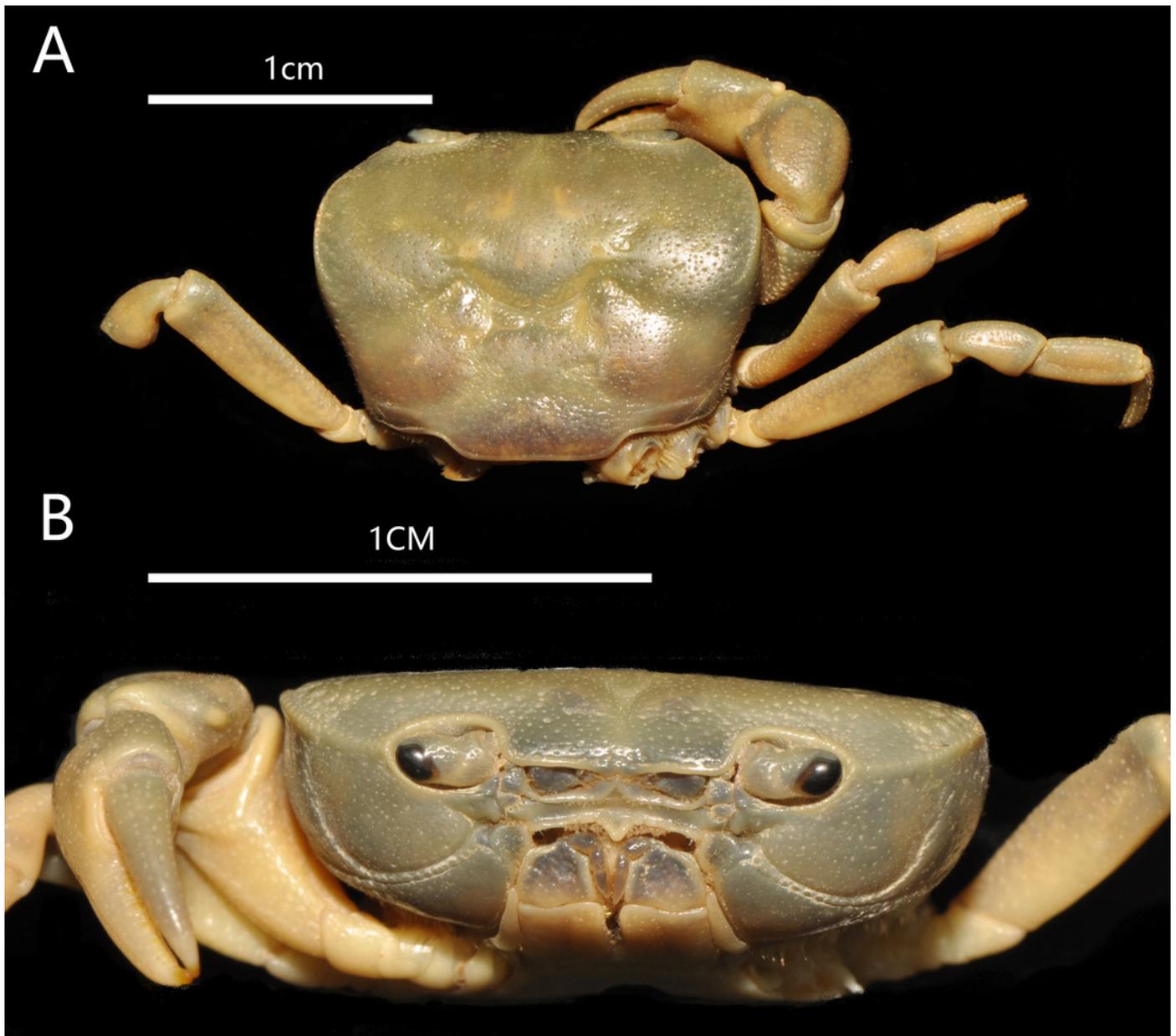


Figure 3

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

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

Photograph courtesy of Jie-Xin Zou, November 2018.

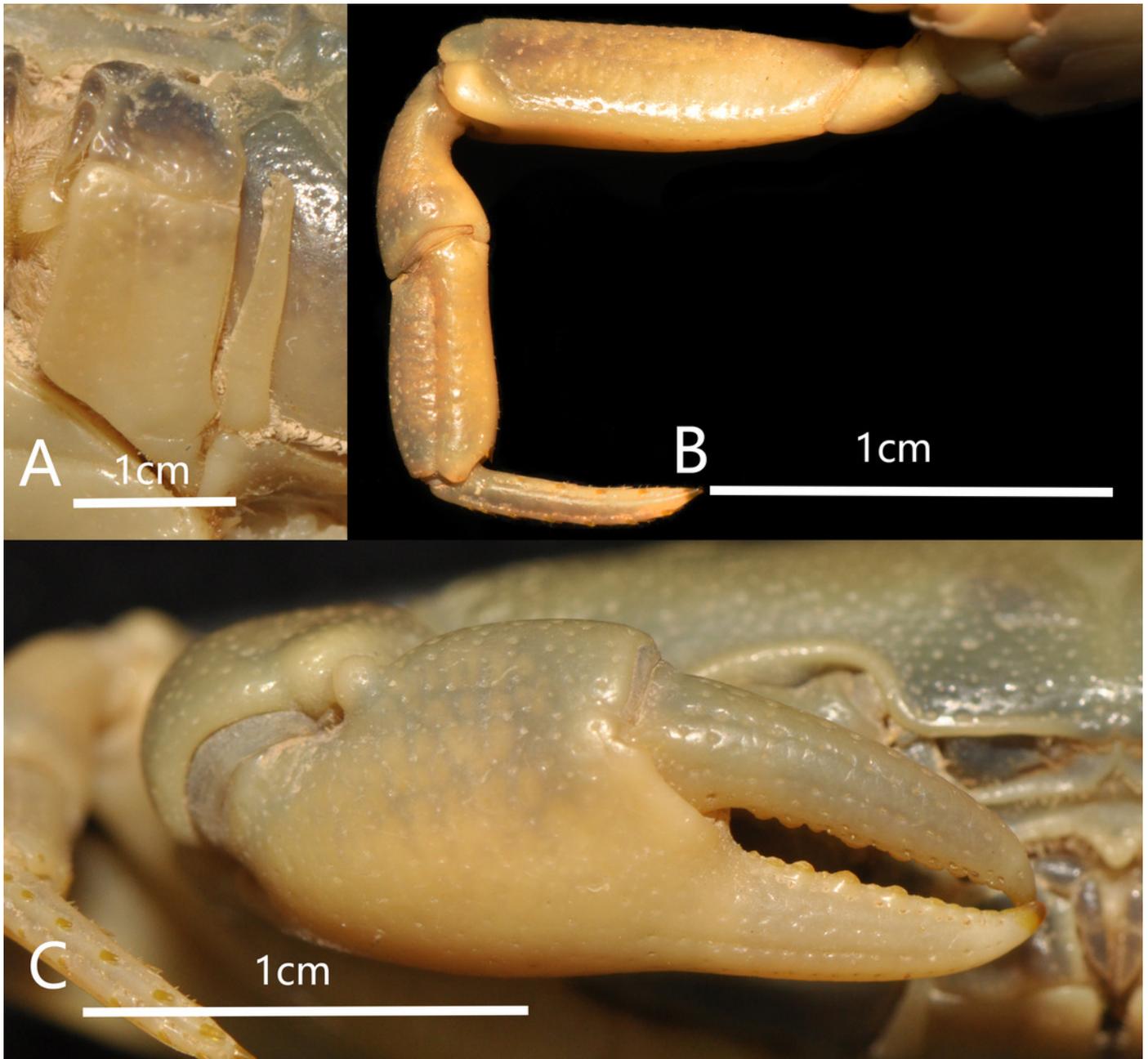


Figure 4

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 longitudinal suture of sternites 7, 8. Photograph courtesy of Jie-Xin Zou, November 2018.

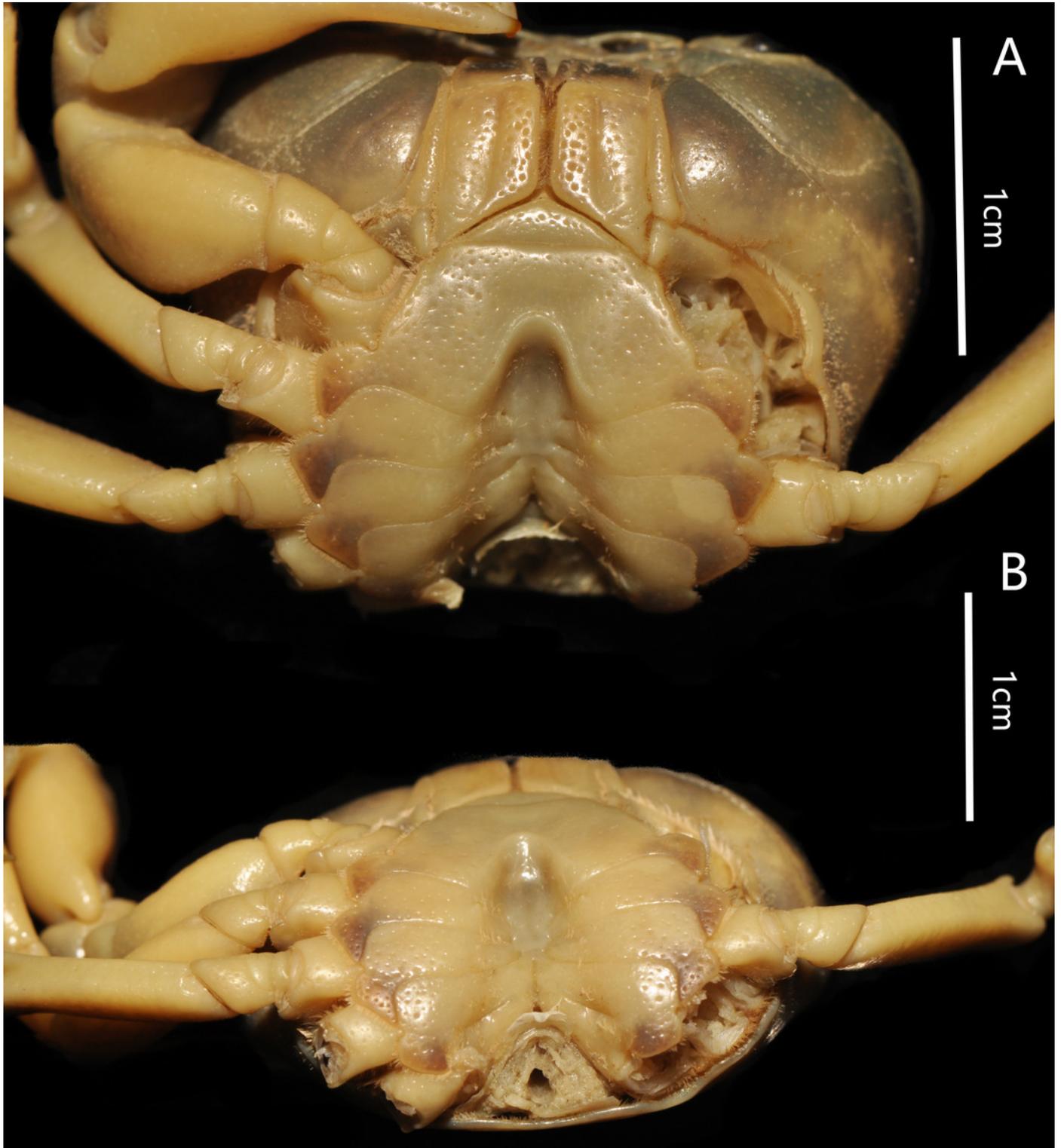


Figure 5

Natural position of male G1 and median longitudinal suture of sternites 7,8

~~*Bottapotamon*~~.

(A) *B. chenzhouense* sp. n. Holotype male (20.7 × 15.7 mm) (NCU MCP 643-1); (B) *B. luxiense* sp. n. Holotype male (18.72 × 15.69 mm) (NCU MCP 4200-Blx1).

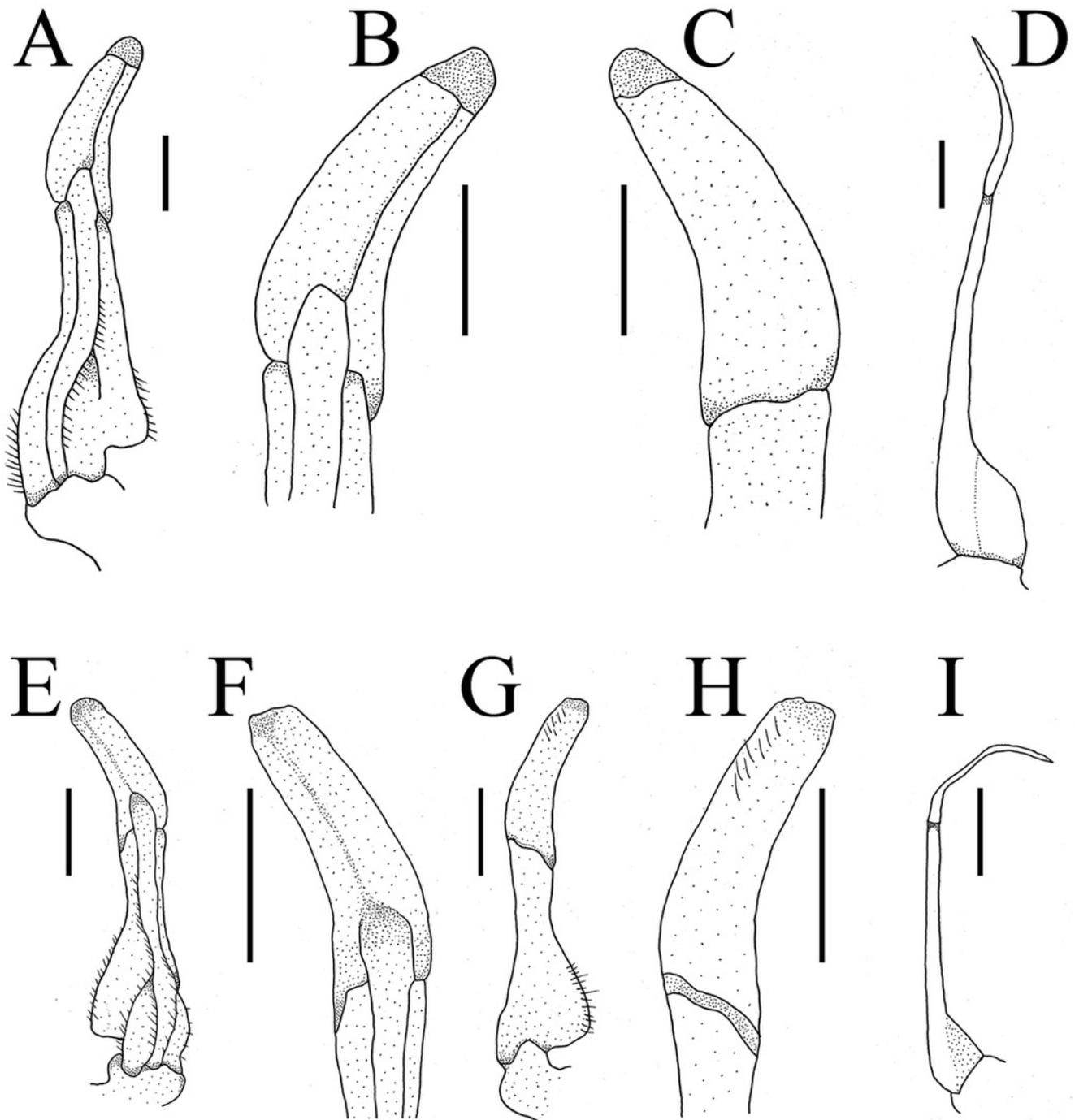


Figure 6

Gonopods. (A-I)

(A-D) *B. chenzhouense* sp .n. Holotype male (20.7 × 15.7 mm) (NCU MCP 643-1); (E-I) *B. luxiense* sp. n. Holotype male (18.72 × 15.69 mm) (NCU MCP 4200-Blx1).

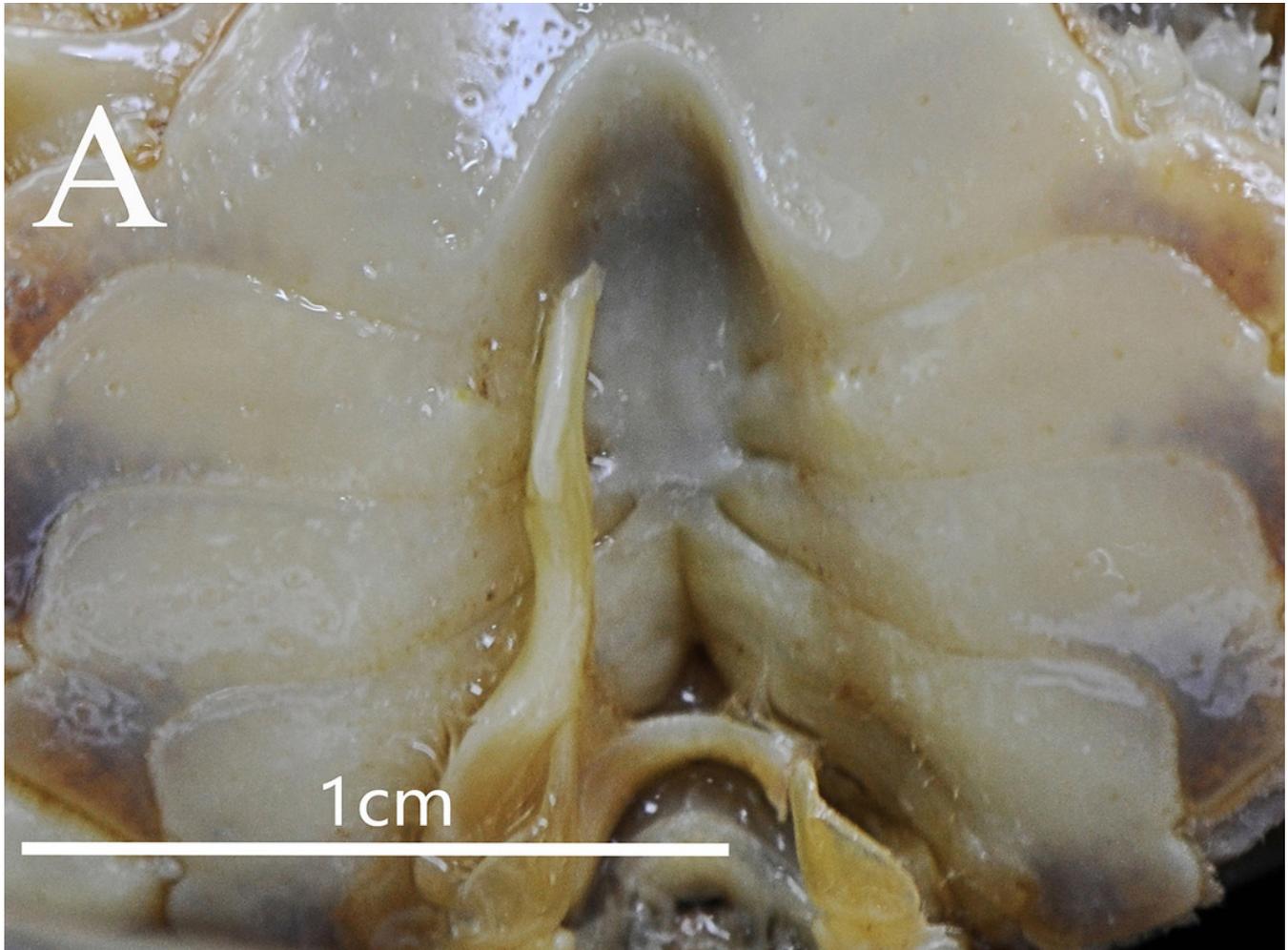


Figure 7

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 8

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.

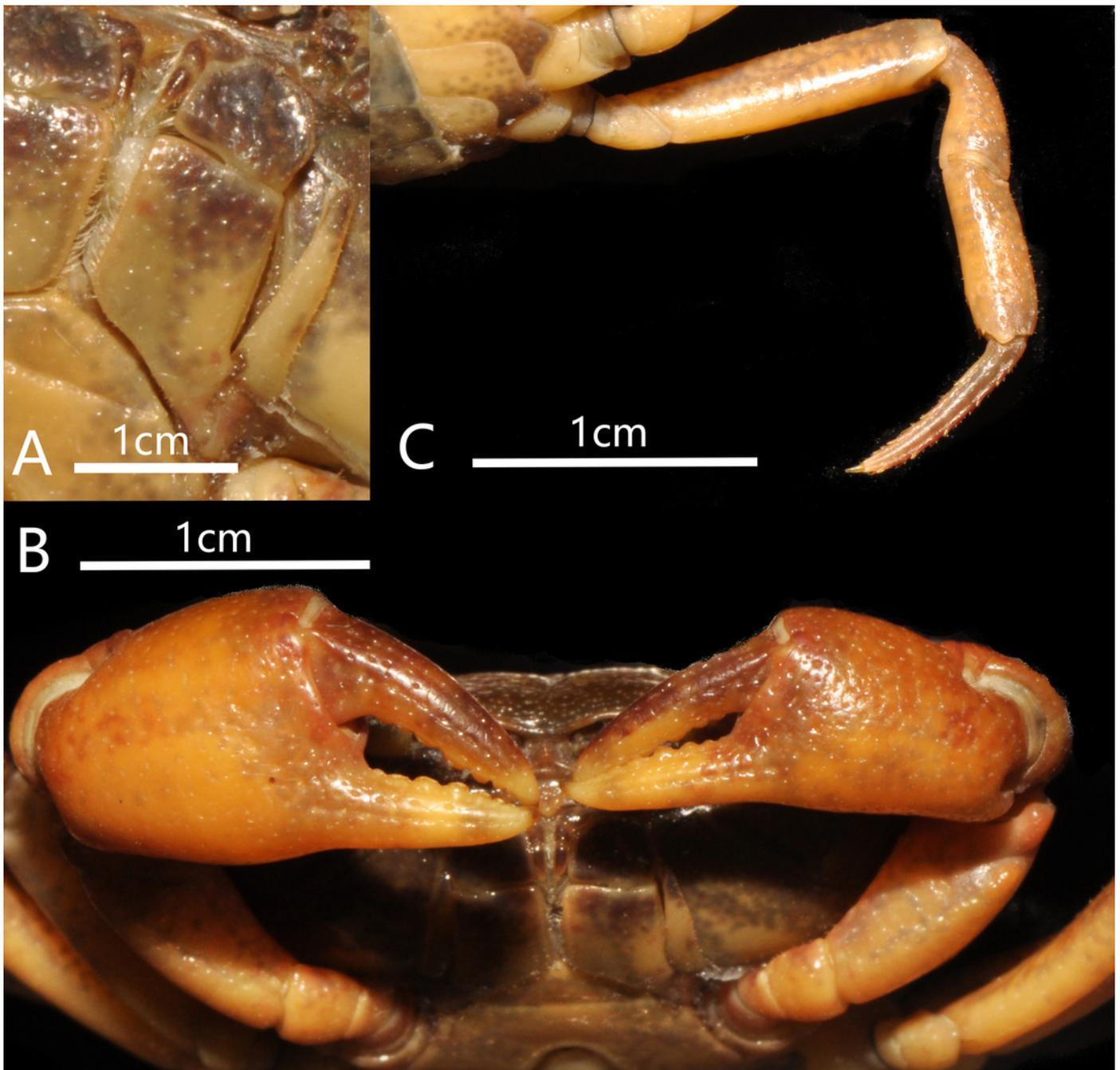


Figure 9

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

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

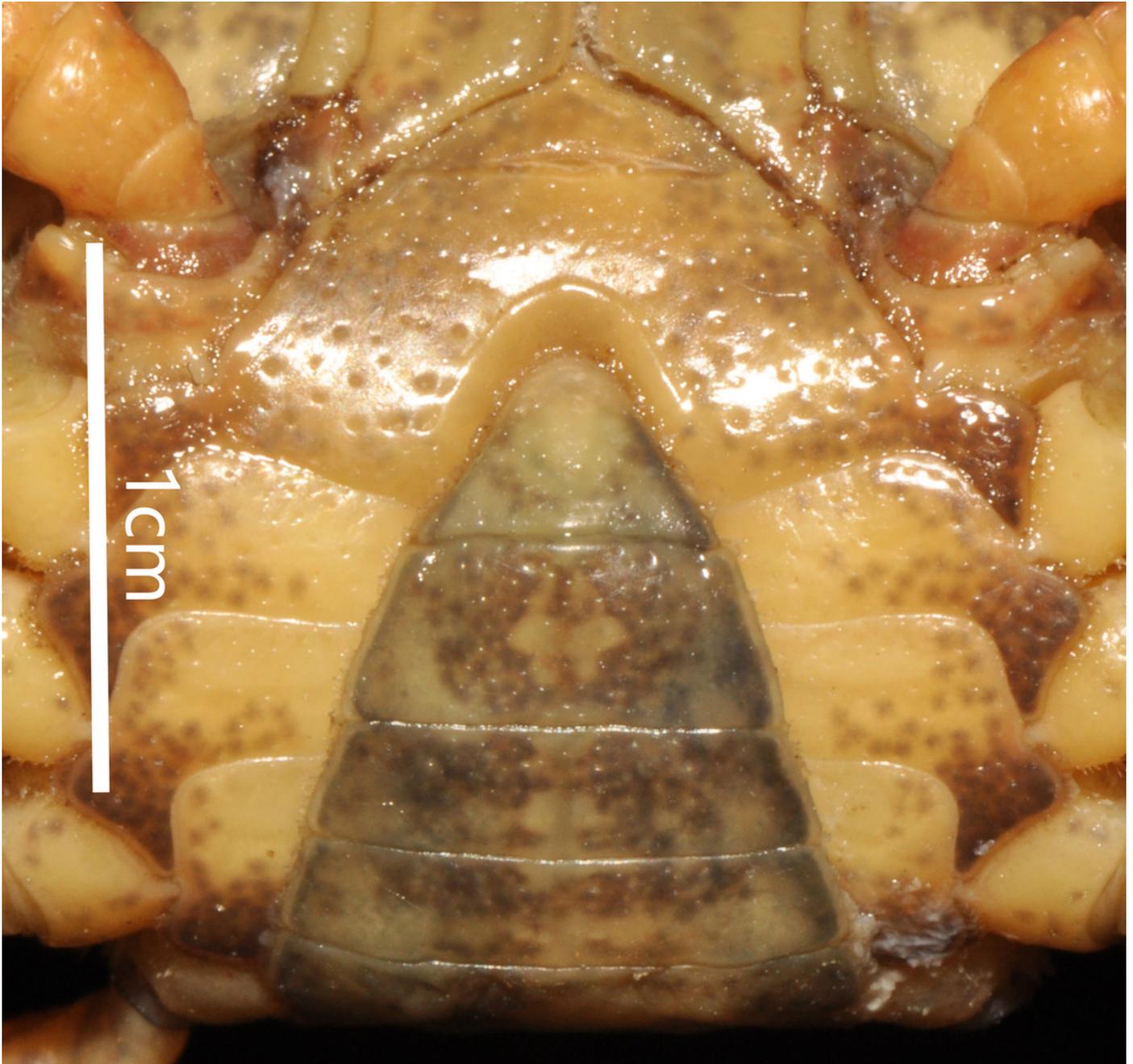


Figure 10

The type locality of *B. luxiense* sp. n.

Photo taken by Jie-Xin Zou, May 2019



Figure 11

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 ($P \geq 50\%$) for ML, BI is represented at the nodes. Locality names in Table 1 are parenthesized behind specimens.

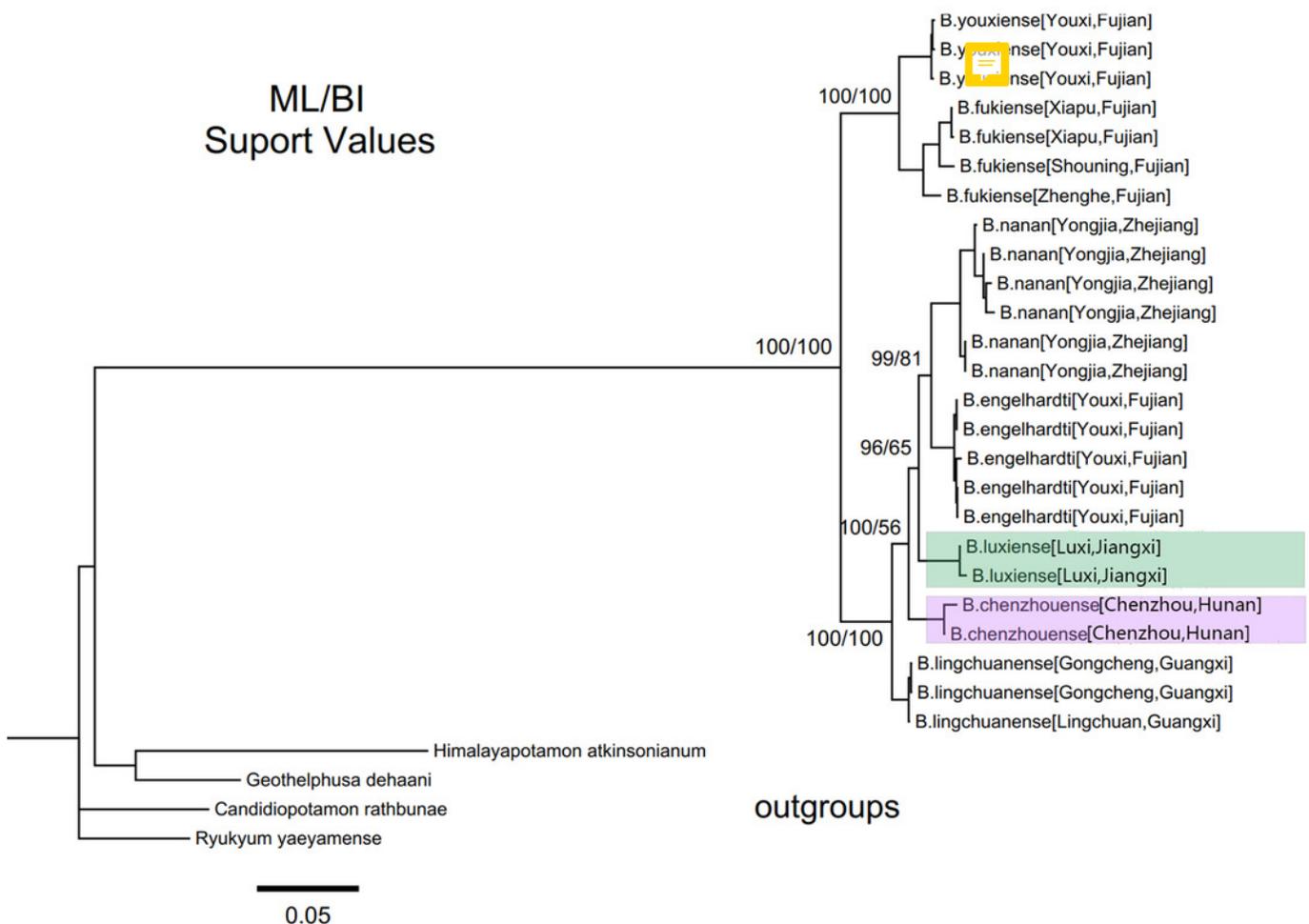


Figure 12

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 3. The divergence times estimated are shown in the main nodes.

