Holothuriophilus trapeziformis Nauck, 1880 (Decapoda: Pinnotheridae) from the Pacific coast of Mexico: Taxonomic revision based on integrative taxonomy

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Background. *Holothuriophilus trapeziformis* Nauck, 1880 is a holothurian-dweller pinnotherid crab representing one of the two species of the genus distributed along the Pacific coast of Mexico and Chile, respectively. While the parasitic ecological interaction with its host is well established, the morphology of the male remains unknown, and DNA information for the species is not available. Furthermore, the only morphological trait separating both species of the genus is subjective and corresponds to the presence or absence of a gap between the fingers of the chelae. Our goal is to complete and clarify the taxonomic status of *H. trapeziformis* and describe the male morphology with the use of the integrative taxonomy, providing additional characters to differentiate this species.

Methods. We collected new biological material in the Pacific coast of Mexico including the topotypes. We also reviewed material from national collections to integrate morphology (based on a complete and detailed description and illustration of the species using light microscopy), ecological data (based on the identification of the host and the place where it was located within the host), and the mtCOI gene information (commonly known as DNA barcode) to differentiate *Holothuriophilus trapeziformis* from other related crabs.

Results. This species presents marked sexual dimorphism only in the primary sexual characters. For the first time we describe morphological variability of traditionally stable characters. In addition to the gap between the fingers of the chelae, *Holothuriophilus trapeziformis* differs from *H. pacificus* (Poeppig, 1836) by their ornamentation, the shape of the male abdomen, and the gonopod. COI distance divergence was >3% between both *Holothuriophilus* species forming a clear clade. DNA barcoding indicates only one taxon, with a maximum divergence of 2.2%. All the specimens have the same Barcode Index Number (BIN; BOLD: ADE9974). All the hosts for *H. trapeziformis* were identified as *Holothuria* (*Halodeima*) *inornata* Semper, 1868; the presence of the crab in the host's coelomic cavity was confirmed, and for the first time we found it within the intestine. The geographical distribution is the Pacific coast of Mexico. Based on the data presented here, the taxonomic status of *Holothuriophilus trapeziformis* is now complete.

9 ***Holothuriophilus trapeziformis* Nauck, 1880 (Decapoda: Pinnotheridae) from the Pacific**
10 **coast of Mexico: Taxonomic revision based on integrative taxonomy**

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33 **Abstract**

34   **Background.** *Holothuriophilus trapeziformis* Nauck, 1880 is a holothurian-dweller pinnotherid

35   crab representing one of the two species of the genus distributed along the Pacific coast of Mexico

36   and Chile, respectively. While the parasitic ecological interaction with its host is well established,

37   the morphology of the male remains unknown, and DNA information for the species is not

38   available. Furthermore, the only morphological trait separating both species of the genus is

39   subjective and corresponds to the presence or absence of a gap between the fingers of the chelae.

40   Our goal is to complete and clarify the taxonomic status of *H. trapeziformis* and describe the male

41   morphology with the use of the integrative taxonomy, providing additional characters to

42   differentiate this species.

43   **Methods.** We collected new biological material in the Pacific coast of Mexico including the

44   topotypes. We also reviewed material from national collections to integrate morphology (based on

45   a complete and detailed description and illustration of the species using light microscopy),

46   ecological data (based on the identification of the host and the place where it was located within

47   the host), and the mtCOI gene information (commonly known as DNA barcode) to differentiate

48   *Holothuriophilus trapeziformis* from other related crabs.

49   **Results.** This species presents marked sexual dimorphism only in the primary sexual characters.

50   For the first time we describe morphological variability of traditionally stable characters. In

51   addition to the gap between the fingers of the chelae, *Holothuriophilus trapeziformis* differs from

52   *H. pacificus* (Poeppig, 1836) by their ornamentation, the shape of the male abdomen, and the

53   gonopod. COI distance divergence was >3% between both *Holothuriophilus* species forming a

54   clear clade. DNA barcoding indicates only one taxon, with a maximum divergence of 2.2%. All

55   the specimens have the same Barcode Index Number (BIN; BOLD: ADE9974). All the hosts for

56   *H. trapeziformis* were identified as *Holothuria* (*Halodeima*) *inornata* Semper, 1868; the presence

57 of the crab in the host's coelomic cavity was confirmed, and for the first time we found it within

58 the intestine. The geographical distribution is the Pacific coast of Mexico. Based on the data

59 presented here, the taxonomic status of *Holothuriophilus trapeziformis* is now complete.

60 **Introduction.**

61 Pinnotherids (Crustacea: Pinnotheridae) are true decapod crabs, which show conspicuous sexual

62 dimorphism, notably different morphological stages of development and complex ecological

63 relationships with different invertebrates, however, they can also be found freeliving (Schmitt,

64 McCain & Davidson 1973; Ocampo et al. 2011; Becker & Türkay 2017). Worldwide, sixteen

65 species are known to be endobiontic with sea cucumbers (Ng & Manning 2003). Of these, only

66 two have been recorded on the Pacific coast of America and they are: *Holothuriophilus*

67 *trapeziformis* Nauck, 1880 (type locality in Mazatlan, Mexico, associated with the sea cucumber

68 *Holothuria* (*Halodeima*) *inornata* Semper,1868), and *H. pacificus* (Poeppig, 1836) (from

69 Talcahuano, Chile, associated with *Athyonidium chilensis* (Semper, 1868)) (Garth 1957; Manning

70 1993; Honey-Escandón & Solís-Marín 2018). *Holothuriophilus* (Nauck, 1880) is diagnosed by its

71 transversally elongated carapace, wider anterior to middle portion; its short, robust and compressed

72 walking legs, with the dorsal margin crested; and the third maxilliped with the ischiomerus

73 indistinguishably fused (Garth 1957; Manning1993; Ng & Manning 2003; Campos, Peláez-Zárate

74 & Solís-Marín 2012).

75 The taxonomic status of *H. trapeziformis* remains incomplete, because male morphology is

76 unknown and the available information from female illustrations shows some inconsistencies when

77 the carapace, Mxp3 shape, and setae patterns are compared (see Bürger 1985: 380–381, pl. 9, fig.

78 26; Ahyong & Ng 2007: 214, Figs. 20A, C; Campos, Peláez-Zárate & Solís-Marín 2012: 60, figs.

79 2B, C). Additionally, *Holothuriophilus trapeziformis* and *H. pacificus* only differ in a gap when

80 the fingers of the chelae closed in the latter (Campos, Peláez-Zárate & Solís-Marín 2012).

81 Nauck's (1880) description of *Holothuriophilus trapeziformis* was brief, a holotype was not

82 designed and the identity of the type host was misidentified. Moreover, the female syntypes

83 deteriorated over time (Bürger 1895; De Man 1887; Ng & Manning 2003). Later, Manning

84 (1993), Ng & Manning (2003), and Ahyong & Ng (2007) examined, described and illustrated the

85 syntype series to complete the diagnosis and designated a lectotype. However, there are

86 inconsistencies between their illustrations and the diagnostic characters are not informative with

87 the information available for *Holothuriophilus pacificus*.

88 For 84 years there were no new records of *H. trapeziformis* until Caso (1958, 1964, 1965)

89 reported four pinnotherids, determined as *Pinnixa barnharti* (not *Pinnixa barnharti* Rathbun,

90 1918), associated with *Holothuria inornata Semper* and *H. kefersteinii* (Selenka) (= *H. riojai*

91 Caso, 1964).

92 Thirty-four years later, Campos, Díaz & Gamboa-Contreras (1998) determined as

93 *Holothuriophilus* sp. the specimens of Caso (1964). More recently Campos, Peláez-Zárate &

94 Solís-Marín (2012) reviewed the genus and updated the species diagnosis. Finally, Honey-

95 Escandón & Solís-Marín (2018) confirmed the ecological association between *H. trapeziformis*

96 and *Holothuria inornata*, but the specimens of Caso (1958, 1965) associated to *Holothuria*

97 *kefersteinii* remain uncertain because they have not been included in any of these documents and

98 their location is unknown (F Solís-Marín, 2018, pers. comm.).

99 For *Holothuriophilus trapeziformis*, there is currently no data on any gene, while for *H.*

100 *pacificus*, there is information related to the COI sequence for one specimen recovered from the

101 shoreline in southern Chile (CFAD062-11; boldsystems.org). Within this context, sequencing of

102 approximately 650 bp region of the mitochondrial Cytocrome Oxidase 1 gene (COI) has been

103 promoted to conform a standardized DNA barcode system with the aim of being one more tool

104 for the identification of biological species with many applications in diverse fields of knowledge

105 (Hebert et al. 2003; Hajibabaei et al. 2007). Despite the fact that the acceptance to work with a

106 single molecular marker as a precise character to define a species was discussed (Will &

107 Rubinoff 2004), nowadays it is considered that the COI marker is the best for decapod

108 identification (Spielmann et al. 2019). The utility of DNA Barcoding (COI sequence) has been

109 useful to delimit species of pinnotherids (Ocampo et al. 2013; Perez-Miguel et al. 2019),

110 brachyuran larvae (Brandão, Freire & Bruton 2016) and other crustaceans (Costa et al. 2007;

111 Matzen da Silva et al. 2011).

112 Therefore, the goal of this study is to review the taxonomy of *Holothuriophilus trapeziformis* by

113 describing the male, examining the morphological variability in both sexes, updating the range of

114 distribution, and establishing a baseline of mitochondrial COI gene barcode. Finally, with this

115 information, we propose new diagnostic characters that will allow better identification of the two

116 species of *Holothuriophilus* known in the Pacific coast of America.

117 **Material & methods**

118 **Morphology**

119 Fifty-five crabs belonging to *Holothuriophilus trapeziformis* were extracted from the coelom and

120 intestine of the sea cucumber *Holothuria* (*Halodeima*) *inornata*. These were collected in 11

121 locations from the Pacific coast of Mexico (Fig. 1). These localities were grouped into two

122 arbitrary regions designated as the northern region (Sinaloa state; which include the type locality

123 Mazatlan) and the southern region (Guerrero and Oaxaca states). Hosts were manually collected

124 through skin and SCUBA diving at a maximum depth of 10 meters. The collected material was

125   labeled and fixed according to Elías-Gutiérrez et al. (2018) protocol for tissue preservation and

126   DNA analyses. Due to the size of the specimens and the thickness of the cuticle, we injected

127   ethanol into the body through the joints of the appendices with insulin syringes.

128   All biological material (Table S1) was classified and deposited in the Scientific Collection of

129   Marine Invertebrates of the Laboratorio de Sistemática de Invertebrados Marinos (LABSIM) at

130   Universidad del Mar (UMAR), Oaxaca, Mexico (OAX-CC-249-11). Hosts were identified with

131   specialized literature (Solís-Marín et al. 2009; Honey-Escandón & Solís-Marín 2018).

132   For the analysis of the taxonomic status of *Holothuriophilus trapeziformis* specialized literature

133   from Nauck (1880), Manning (1993), Ng & Manning (2003), Ahyong & Ng (2007), and

134   Campos, Peláez-Zárate & Solís-Marín (2012) was reviewed. Likewise, for *H. pacificus,* Poeppig

135   (1836), Nobili (1901), Rathbun (1918), and Garth (1957) were reviewed.

136   The species description follows the terminology of Manning (1993). We consider the gonopod

137   terminology as first gonopod and second gonopod of the male, according to Becker, Türkay &

138   Brandis (2012). The setae terminology is based on Garm & Watling (2013). Drawings were

139   made with the help of a camera lucida and then digitalized in a vector format. Pictures were

140   taken with a Nikon D5100 digital camera. Measurements are given in millimeters and latitude

141   and longitude were obtained from Google Earth^{TM}.

142   Because we were only able to obtain eight specimens (three males and five females) from the

143   northern region (Pinitos Beach in the type locality Mazatlan, Sinaloa, Mexico), in contrast to 47

144   (six males and 41 females) from localities in the southern region, and due to morphological

145   variability observed between these two regions, it was necessary to standardize the observations

146   by using specimens in the same stage of development. Eight millimeters carapace width was the

147   common size in both, the northern and southern regions, in males and adult ovigerous females.

148   This size-group was used to illustrate (Figs. 5 and 6) and discuss the observed morphological

149   variability. The specimen and the dissected appendages were mounted on a plastic clay base for

150   standardizing the observations, and to make the drawings. For the carapace contour, the samples

151   were mounted, so the dorsal view of the posterior margin line of the carapace was observed. For

152   the Mxp3, we extracted it from its base to obtain both endopod and exopod and to mount it with

153   the articles in the same perspective. The cutting edge of the fingers' chelae was cleaned of dirt to

154   see all the teeth. The first gonopod was extracted from its base, and the setae cleaned of dirt.

155   Abbreviations used in the text: CL, carapace length (taken as the middle line from the frontal

156   margin to the posterior margin of the carapace); CW, carapace width (measured in its medium-

157   anterior portion); Mxp2, second maxilliped; Mxp3, third maxilliped; WL, walking legs 1 to 4

158   (thoracopods 2–5).

159   **DNA extraction and PCR amplification**

160   Genomic DNA of individuals of *Holothuriophilus trapeziformis* was extracted from biological

161   material collected in the field and some individuals from the OAX-CC-249-11 regional

162   collection of the UMAR, using tissue from the walking legs, the chelae, or eggs from the

163   ovigerous females. Tissues were placed into 96-well microplates with a drop of 96% ethanol, and

164   DNA extraction was carried out following the standard glass fiber method consisting of a mix of

165   Proteinase K with an invertebrate lysis buffer according to Ivanova, De Waard & Hebert (2006).

166   Following the DNA extraction, the PCR mixture with a final volume of 12.5 μl contained 2 μl of

167   Hyclone ultrapure water (Thermo Fisher Scientific), 6.25 μl of 10% trehalose (previously

168   prepared: 5 g D-(+)- trehalose dihydrate (Fluka Analytical) in a total of 50 ml of molecular grade

169   ddH2O), 1.25 μl of 10X PCR Platinum Taq buffer (Invitrogen), 0.625 μl of 50 μmol/L MgCl2

170   (Invitrogen), 0.0625 μl of 10 μmol/L dNTP (KAPA Biosystems), 0.125 μl of each 10 μmol/L

171 primer, 0.06 µl of PlatinumTaq DNA polymerase (Invitrogen), and 2 µl of DNA template. All

172 specimens were amplified with the Zooplankton primers (ZplankF1_t1 and ZplankR1_t1, see

173 Prosser, Martínez-Arce & Elías-Gutiérrez 2013 for details). The reactions were cycled at 94°C

174 for 1 min, followed by five cycles of 94°C for 40 seconds, 45°C for 40 seconds and 72°C for 1

175 min, followed by 35 cycles of 94°C for 40 seconds, 51°C for 40 seconds and 72°C for 1 min,

176 with a final extension of 72°C for 5 min. PCR products were visualized on a pre-cast 2% agarose

177 gels (E-Gel© 96 Invitrogen), and the most intense positive products were selected for sequencing.

178 **Sequencing and DNA barcode**

179 Selected PCR products were sequenced using a modified (Hajibabaei et al. 2005) BigDye©

180 Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystem, Inc.), and then sequenced

181 bidirectionally on an ABI 3730XL automated capillary sequencer using M13F and M13R

182 sequence primers at the Biology Institute at the National Autonomous University of Mexico and

183 at the Eurofins Genomics Louisville Laboratory. Sequences were edited using CodonCode© v

184 3.0.1 (CodonCode Corporation, Dedham, MA, USA) and uploaded to BOLD. In some cases, the

185 original forward and reverse trace files uploaded to BOLD were checked again, consensus

186 assembly was generated, and edited manually with Sequencher© 4.1.4. (Gene Codes Corporation,

187 Ann Arbor, MI, USA), and then they were aligned using BioEdit© (Hall 1999).

188 **Likelihood tree and distance analysis**

189 COI sequences generated for *Holothuriophilus trapeziformis* in this study were compared with

190 COI sequences from other pinnotherids collected in the Eastern Pacific coast of America,

191 available in BOLD and/or GeneBank (Table S2). Sequence data, trace files, and primer details

192 for all *H. trapeziformis* specimens and for the outgroup species are available under the dataset

193 name "Holothuriophilus trapeziformis from Mexico" (DOI: dx.doi.org/10.5883/DS-

194   PINMX1HT) in the Barcode of Life Data System (barcodinglife.org). Additionally, *H.*

195   *trapeziformis* sequences were uploaded to GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

196   Accession numbers are noted in table S2.

197   We calculated the best-fitting evolution model of nucleotide substitution for distance based on

198   COI alignments for the Maximum Likelihood (ML) tree accordingly to the Akaike (AIC) and

199   Bayesian (BIC) criterions (Darriba et al. 2011) using jModelTest© 2.1.10 (Posada & Buckley

200   2004). The final tree was estimated with 1000 bootstrap replicates in MEGA© 7.0 (Tamura et al.

201   2013). With the compress/expand feature of MEGA we prepared the final tree. Also, we

202   estimated the interspecific COI genetic distances for the dataset using the Kimura-2 parameters

203   distance method in MEGA. Values greater than 3% were considered the threshold for the

204   delimitation of the species (Hebert et al. 2003).

205   Acronyms used in the text: BOLD, barcode of life database (boldsystems.org); BIN, barcode

206   index number (*sensu* Ratnasingham & Hebert 2013); BOLD-ID, Specimen ID in the Barcode of

207   Life Data System; CNE-ICML-UNAM, National Collection of Echinoderms of the Institute of

208   Marine Sciences and Limnology of the National Autonomous University of Mexico; DC-NHM,

209   Division of Crustacea, Natural History Museum, Smithsonian Institution; SMF-ZMG,

210   Senckenberg Museum für Naturkunde, Zoologisches Museum Göttingen University, Humboldt

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213   Collectors: AEV, Aidé Egremy Valdés; AGF, Andrea Glockner Fagetti; CCA, Carlos Cruz

214   Antonio; AHM, Adanely Hernández Muñoz; FBV, Francisco Benítez Villalobos; FCC, Fernando

215   Cortés Carrasco; HMC, Humberto Mesa Castillo; KFL, Karen Lizbeth Flores López; KMB,

216   Karen Mesa Buendía; RGF, Rebeca Granja Fernández; VCH, Valeria Chavez García.

217 For this study, we obtained a field collecting permit for non-commercial scientific research

218 purposes from Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación

219 (SAGARPA) and Comisión Nacional de Acuacultura y Pesca (CONAPESCA) (Collecting

220 permit: PPF/DGOPA-301/17).

221 **Results**

222 We analyzed the morphology of 55 specimens (Supplemental Table S1) from two coastal regions

223 (northern and southern) in the Mexican Pacific (Fig. 1). Notable variations were determined after

224 detailed morphological examination of the specimens. . In particular, the morphology of the

225 topotypes (northern region; Fig. 1A) shows variation in the general carapace shape outline and

226 general appearance which looks stouter and eroded in contrast to that of the southern region.

227 Nevertheless, in all the specimens we observed diagnostic characters of the species as the

228 subrectangular carapace shape, with its crested lateral margin, the compressed walking legs, and

229 the gap between the fingers of the chelae. Also, the male first and second gonopods plus the

230 DNA data, confirm that all material examined corresponds with *Holothuriophilus trapeziformis*.

231 **Systematics**

232 Infraorder Brachyura Latreille, 1802

233 Family Pinnotheridae De Haan, 1833

234 Genus *Holothuriophilus* Nauck, 1880

235 **Diagnosis** (modified from Manning 1993). Carapace broader than longer, widest on mid anterior

236 portion, transversely subcuadrangular, subrectangular, subovate or subtrapezoidal. Third

237 maxilliped with ischium and merus fused, no suture line; exopod with one flagellar segment;

238 palp with 3-segments; propodus conical, shorter than carpus, subspatuliform dactyl, articulated at

239 basal of propodus, extending beyond end of propodus. Dactylus of walking legs short, similar

240 and subequal. Abdomen of seven segments in both sexes.

241 ***Holothuriophilus trapeziformis* Nauck, 1880**

242 **(Figs. 2A–G, 3A–D, 4A–K, 5A–K, 6A–D)**

243 *Holothuriophilus trapeziformis* Nauck, 1880: 24, 66 [ovigerous female type]. —De Man 1887:

244 721–722 [female (CW = 13.8 mm, CL = 10.5 mm)]. — Manning 1993: 524-528, Fig. 3c

245 [resurrected to *Holothuriophilus*]. —Ng & Manning 2003: 903, 916-918, Fig. 7C–F [designated

246 female lectotype (CW = 7.7 mm, LC = 4.8 mm): SMF-ZMG 67/565a]. —Ahyong & Ng 2007:

247 213-214, Fig. 20 [redescribed and refigured]. —Campos, Peláez-Zárate & Solís-Marín 2012: 57–

248 62, Figs. 1A, B, 2A–D [female (CW = 9.1 mm, CL = 5.5 mm)].

249 *Pinnotheres trapeziformis*. — Bürger 1895: 380–381, plate 9, Fig. 26, plate 10, Fig. 25 [female

250 type (CW = 14 mm, CL = 10 mm), male (CW = 5 mm, CL = 8.5 mm)]. —Adensamer 1897: 107.

251 —Schmitt, McCain & Davidson 1973: 5, 13, 89 [list].

252 *Pinnoteres trapeziformis* Balss 1957: 1417 [not 1956 *fide* Schmitt, McCain & Davidson 1973].

253 *Pinnixa barnharti* (no Rathbun, 1918) Caso 1958: 329; 1965: 254–26.

254 *Holothuriophilus* sp. Campos, Díaz & Gamboa-Contreras 1998: 377, Fig. 1E.

255 **Material examined:** 56 specimens: 25 ovigerous females, 22 females, nine males (Table S1).

256 **General distribution:** Tropical Mexican Pacific.

257 **Previous records:** Mazatlan, Punta Tiburón (Sinaloa); Ixtapa (Guerrero).

258 **New records:** Pinitos Beach (Sinaloa); Nudista Beach, Zacatoso Beach, Caleta de Chón Beach

259 (Guerrero); Agua Blanca Beach, Coral Beach, Camarón Beach, Panteón Beach, Estacahuite

260 Beach, La Tijera Beach, San Agustín Beach, El Tejón Beach (Oaxaca).

261 **BIN:** BOLD:ADE9974

262   **Carapace size (mm):** See Supplemental Table S1.

263   **Diagnosis**. Carapace transversely subrectangular, suboval or subtrapezoidal. Crestated

264   anterolateral margin, a hepatic notch with a blunt tooth inside. Inner surface of merus and carpus

265   of chelipeds densely setose; ventral inner margin of the propodus with a row of short setae,

266   without a gap when the cutting edge of propodus and dactylus meet; cutting edge of the dactylus

267   with proximal denticles, a conspicuous medial tooth, and a distal convex or acute projection.

268   Dorsal surface of the merus of WL1, WL 3 and WL 4 with setae, WL2 without seta. Abdomen

269   with 6 somites plus free telson; on male, margin of somite 4 to 6 concave, telson subrounded.

270   First gonopod notably curved outward from its mid-distal portion**.**

271 **Description: Male (Fig 2A–C; UMAR-DECA-308; CW = 11 mm, CL = 7 mm):** Carapace,

272 transversely subtrapezoidal, wider than long, CW/CL ratio ca. 1.6, mid-anterior portion widest;

273 anterolateral margins slightly projected, crested, a hepatic notch with a blunt middle tooth (Figs.

274 3A, B; bold arrow); dorsal surface convex, smooth, undefined regions; mid-posterior and

275 posterolateral surface with microscopic pits with variable pilosity and size (Figs. 3A); inferior

276 lateral margin of the carapace with abundant plumose setae (Fig. 3A).

277 Front bilobed, scarcely visible in dorsal view, margin granulated, surface slightly pubescent (Fig.

278 3B).

279 Orbits small; eyes pigmented, same size as the orbits; ocular peduncle scarcely pubescent.

280 Antennules robust; peduncle with two segments, biflagellate, transversely folded inside fossae;

281 upper flagellum with two articles, second article the longest and sharpened distally with apical

282 setae (Fig. 4Ba); lower flagellum conic with four articles decreasing in size, the first three

283 articles with a transverse line of simple setae, fourth article with two transverse lines of simple

284 seta (Fig. 4Bb).

285 Antennae long, slender, with 12 articles, last article with short apical setae (Fig. 4A).

286 Pterygostomian region pubescence (Fig. 3B). Buccal frame trapezoidal, completely covered by

287 Mxp3. Mxp2 endopod with five articles, with setae (Fig. 4Ca), dactylus subrounded and shorter

288 than propodus (Fig. 4C); exopod with an article, wider distally, external surface with an elevated

289 ridge (Fig. 4Cb), flagellum with long apical setae (Fig. 4Cc), epipodite long, distal margin

290 rounded (Fig. 4Cd). Mxp3 ischiomerus fused without suture line, width/length ratio 0.7, mesial

291 margin convex with setae, medial margin with a conspicuous projection (Fig. 4Da); carpus

292 subconical, with short setae; propodus subconical (Fig. 4Dc); subspatuliform dactyl, widens

293 distally (Fig. 4D), exceeds the length of the propodus, external surface with short plumose setae,

294   external margin with long plumose setae; exopod with one article and a flagellum, external

295   margin and external surface with short simple setae, slender flagellum, with plumose long setae

296   (Fig. 4E).

297   Third sternal plate with anterior margin sinuous, anterolateral angles with crenulated margin

298   (Fig. 3C), surface scarcely pilose (Fig. 3C); fourth plate slightly globose, surface with

299   microscopic pits (Fig. 3C), anteroexternal angle curved outward, with margin crenulated (Fig.

300   3C).

301   Chelipeds subequal (Fig. 2A); external and lower surface of merus and lower margin of carpus

302   with plumose setae (Figs 2B); chelae wide with ventral margin microscopically granulated (Fig.

303   4F, 7C), dorsal margin slightly crested and bent inwards; fingers wider than longer, spooned with

304   acute tip subdistally (Fig. 4F), without a gap where the cutting edge of propodus and dactyl meet

305   (Fig. 7C). Movable finger shorter than fixed finger, crossed inward when closed. Movable

306   fingers of both chelae with cutting edge sinuous, three medial teeth (Fig. 4F) and a subdistal

307   convex projection (Fig. 4F). Fixed finger cutting edge of both chelae with nine teeth and

308   subdistal surface flatted (Fig. 4F), ventral margin with short setae.

309   Walking legs similar in both sides of the body, relative length WL3>WL2>WL1>WL4,

310   segments short, robust, compressed and dorsal margin crested, ventral surface with plumose

311   setae; dorsal margin of merus of the WL1, WL3, WL4 with plumose setae, and WL2 naked;

312   dactylus with acute tips, curved, and stout; length of the dactylus subequal to propodus of the

313   WL1−WL3, and WL4 shorter than propodus (Fig. 3A).

314   Abdomen symmetrical, subtriangular, six free somites and telson margin with short setae, lateral

315   margin from somites 4–6 slightly concave and narrowing, telson subrounded (Figs 3D).

316   First gonopod slender, margins sinuous, mid-distal portion notably curved outwards, surface with

317   abundant plumose setae (Fig. 3E). Second gonopod small, slightly bent inwards, tip pointing

318   upwards, distal margins convex with shallow notch (Fig. 3F).

319   **Female (Figs. 2D–F; UMAR-DECA-307; CW = 10.50, CL = 7):** Same as the male but with

320   less abundant setae in the pterygostomian region and ventral margin of the chelae; dorsal surface

321   of the merus of the WL1-4 and inner surface of the merus and carpus of the cheliped with long

322   and abundant setae. Carapace slightly more convex. Abdomen subovate. See variation section

323   for more details.

324   **Color in life:** Body beige or creamy white, dorsal surface of carapace and chelipeds carpus, and

325   on the external surface of the chelae with red patches. In fixed and preserved specimens this

326   pattern of color remains, or it could change from red to light or dark brown (Fig. 2A–F).

327   **Habitat:** Marine. Associated with the sea cucumber *Holothuria* (*Halodeima*) *inornata*, living in

328   its coelom and inside its intestine (Fig. 2G). This holothurian inhabits rocky-sand sea floor in

329   shallow waters (0–18 m).

330   **Variation:** Morphological variation observed between the 8 mm individuals from the two

331   regions (northern and southern) showed three general carapace shapes : subrectangular, suboval

332   and subtrapezoidal.

333   The subrectangular carapace (Figs. 5A, 6A) was observed in 33% of the males and in 11% of

334   females (three and five specimens, from the northern region). It has projected and straight margin

335   frontal lobes, and straight anterolateral margin with a deep hepatic notch, eroded, and extended

336   over the carapace (Fig 5A) in males but in females it is less conspicuous (Fig. 6A). Males have

337   truncated and scarcely projected anterolateral margins in which the anterior portion is concave

338   (Fig. 5A) compared to straight in the females (Fig. 4A).

339   The subovate carapace (Figs. 5E, I, 6D, G) was observed in 56% of the males and in 85% of

340   females (five and 40 specimens from the southern region). It has oblique and scarcely projected

341   frontal lobes; anterolateral margin continues smoothly to the lateral margin forming a convex

342   lobe (Figs. 5E, I), the hepatic notch in the males is deep, eroded and extended (Fig. 5E, I), while

343   in the females is shallow, slightly eroded, and less extended over the carapace (Figs. 6D, G).

344   The subtrapezoidal carapace (Fig. 2D, 7A) is present in 11% of the males and in 2% of females

345   (one and two specimens from the southern region). It has scarcely projected frontal lobes,

346   oblique and straight anterolateral margin ending lateral lobes.

347   Regardless of the carapace shape, the females have a more convex shell and margin of the frontal

348   lobes and the eyes are not visible in dorsal view, and only a slight hepatic notch can be seen

349   (Figs. 6A, D, G). This shape was more frequent in ovigerous females (ten specimens, 67%) than

350   in non-ovigerous (five specimens, 33%).

351   Despite the variation in the carapace shape of both sexes, all the ratios (v.g. CW/CL ratio, length

352   between the notch of the frontal lobes to the external orbital angle, length between the external

353   orbital angle to the anterolateral angle, ishiomerus width/length ratio of the Mxp3) were

354   constant.

355   Regardless of the shape of the carapace, the Mxp3 features show very subtle variations resulting

356   from mounting and drawing. However, the observed variations are: The ischiomerus inner

357   margin has a blunt or slightly acute projection (Figs. 5Ca, 5Ga, 5Ka, 6Ca, 6Fa, 6Ia). The dorsal

358   margin of the carpus from convex (Figs. 5Kb, 6Cb, 6Fb, 6Ib) to straight (Figs.5Cb, 5Gb).

359   Distally expanded subspatulated dactylus (Figs. 5Kd, 6Cd) or narrower suboblong one (Figs. 5

360   Cd, 5Gd, 6Fd, 6Id). However, in all cases there is a projected ridge on the internal surface of the

361 carpus with setae, and the distal margin of dactylus slightly overreaches the propodus (Figs. 5Cd,

362 Gd, Kd, 6Cd, Fd, Id).

363 The chelae fingers ornamentation is most variable and does not show a relationship with

364 carapace shape or size. In both sexes the cutting edge of the movable finger has two or three

365 proximal teeth (Figs. 5B, 5F, 5J, 6B, 6E, 6H). These teeth could be blunt or acute, but in the

366 females, they always are acute. The medial tooth in males could be simple (Figs. 5B, J) or

367 bicuspid (Fig. 5F), but in females it is always simple (Figs. 6B, E, H). The subdistal projection in

368 both sexes can be blunt (Figs. 5B, 5J, 6 B, 6H) or acute (Figs. 5F, 6E). The cutting edge of the

369 fixed finger is more variable between sexes. In males it has six to nine proximal teeth (Figs. 5B,

370 F, J), but in females it has four to thirteen (Figs. 6B, E, H). In males it has one middle tooth

371 which is always bicuspid (Figs. 5B, F, J) but in females could be bicuspid (Figs. 6B, E) or simple

372 (Fig. 6H). Only one specimen (DECA-1172) had different sized chelae and a different teething

373 pattern on the cutting edge of the fixed finger (Fig. 6J, K).

374 The first gonopod is related to the carapace shape. In caudal view it is similar between males of

375 the northern region with a subrectangular carapace and that of the southern region of Oaxaca

376 with suboavate and subtrapezoidal carapace. In these, the external and internal margins are

377 sinuous (Figs. 5D, L), the curvature degree is approximately 90° (Fig. 5D) and 75° (Fig. 5L).

378 The tip of the external margin is truncated (Figs. 5De-f, Le-f), and the ventral margin of the tip

379 has a blunt projection (Figs. 5De, Le).

380 Instead, other males from Guerrero, in the southern regions, with a subovate carapace, have the

381 external and internal margins of the first gonopod less sinuous and the curvature degree is

382 approximately 65° (Fig. 5H). The tip of the external margin is convex (Fig. 5He-f), and the

383 ventral margin of the tip has an acute projection (Fig. 5He).

384   **Remarks:** The taxonomic history of *Holothuriophilus trapeziformis* was synthetized by Campos,

385   Peláez-Zárate & Solís-Marín (2012), and they highlighted the fact that the specimen identified by

386   Bürger (1895) as a male based on the shape of the abdomen is actually a female. We observed

387   the same situation in several young individuals. The presence of pleopods in all of the abdominal

388   somites confirms that they are also females. In juvenile males the lateral margins of the abdomen

389   are straight instead of concave and the first and second gonopods are present. This finding allows

390   us to present the complete male morphology of *H. trapeziformis*.

391   All the biological material examined shows phenotypic variation, particularly between the

392   individuals from the type locality in Mazatlan (northern region) with respect to those from of

393   Guerrero and Oaxaca (southern region), but COI gene shows no differences.

394   With our detailed description of the male morphology, it is now possible to differentiate

395   *Holothuriophilus trapeziformis* from *H. pacificus* with certainty by the carapace shape and

396   CW/CL ratio. In *H. trapeziformis*, it can be subrectangular (Fig. 5A, 6A; CW/CL = ˜1.6), suboval

397   (Fig. 5E, I, 6D, G; CW/CL = ˜1.6) or subtrapezoidal (Fig. 1A, D, 2A, 7A; CW/CL = ˜1.6), while

398   in *H. pacificus* it is subcuadrangular (Fig. 7E; CW/CL = ˜1.2).

399   Nevertheless, the mxp3 does not present differences between both species, except for the

400   flagellum of the exopod that according to the illustrations is robust (Figs. 7B, J, K, 8A) in *H.*

401   *trapeziformis*, and slender in *H. pacificus* (Figs. 7F, 8D).

402   The abdomen of *Holothuriophilus trapeziformis* is subtriangular in males, with lateral margins

403   narrowing from the fourth to the sixth somite. The third somite has convex lateral margins. The

404   sixth somite has concave lateral margins, and the telson is subrounded and wider than long (Fig.

405   8B). In *H. pacificus*, it is triangular, the lateral margins are almost straight. The third and sixth

406 somite lateral margins are concave, and the telson is subtriangular and more extended than wide

407 (Fig. 8E).

408 In the case of adult ovigerous and non-ovigerous females of *Holothuriophilus trapeziformis*, the

409 abdomen is suboval and wider than long. The first somite has convex lateral margins. The

410 second somite has sinuous distal margins. The third somite has oblique and downward lateral

411 margins. The sixth somite has oblique and outward lateral margins, and the telson has a length to

412 width ratio ca. 0.2 (Fig. 7D). In contrast, in *H. pacificus* it is suboval and longer than wide. The

413 first somite has concave lateral margins. The second somite has almost straight distal margins.

414 The third somite has oblique and upwards lateral margins. The sixth somite has convex lateral

415 margins, and the telson has a length to width ratio ca. 0.3 (Fig. 7H).

416 The first gonopod of *Holothuriophilus trapeziformis* has a sinuous lateral margin with a larger

417 distal portion curved outwards, with abundant setae (Fig. 8C). In *H. pacificus* it is straight, with

418 the distal portion slightly curved outwards, and less setae (Fig. 8F).

419 **Distribution and ecological comments:** With this study we increased the previous known

420 distribution range that went from Punta Tiburón (Sinaloa state) to Playa Tejón (Oaxaca state).

421 We found crabs in the coelom cavity and near the cloaca of the host, as mentioned by Manning

422 (1993), Campos, Peláez-Zárate & Solís-Marín (2012), and Honey-Escandón & Solís-Marín

423 (2018). For the first time, we found the crab within the intestine (Fig. 1G).

424 *Holothuria* (*Halodeima*) *inornata* is distributed throughout the Tropical Eastern Pacific from the

425 Gulf of California, Mexico to Ecuador, and in the temperate island Lobos de Afuera, Peru

426 (Prieto-Rios et al. 2014; Honey-Escandón & Solís-Marín 2018). It also represents an important

427 fishery resource throughout its distribution range (Santos-Beltrán & Salazar-Silva 2011). There

428 are no records for *Holothuriophilus trapeziformis* outside the Pacific coast of Mexico.

DNA Barcodes

429

430 From the 56 crabs examined (Table S1), 51 were processed. The number of base pairs was

431 between 549 bp and 648 bp for 37 specimens with a sole Barcode Index Number (BIN;

432 Ratnasingham & Hebert 2013) in the BOLD database: ADE9974. Of those, 35 produced high

433 quality barcodes. The 14 crabs that could not be amplified correspond to old museum material

434 and recent collections that were not fixed according to the Elías-Gutiérrez et al. (2018) protocol.

435 A BLAST query in GenBank confirmed our sequences to belong to a brachyuran lineage.

436 **Maximum likelihood tree and genetic distance analysis**

437 The best nucleotide substitution model according to the AIC and BIC criterion was General Time

438 Reversible under a gamma distribution (GTR+G) model (Nei & Kumar 2000). The Maximum-

439 Likelihood (ML) distance method under the selected model delimited the 37 sequences of

440 *Holothuriophilus trapeziformis* from the dataset (DS-PINMX1HT) in a single cluster. The cluster

441 of *H. trapeziformis* is well separated from *H. pacificus* as shown in figure 9, with a 12 to 14%

442 divergence among all specimens. *Holothuriophilus* is also close to the *Calyptraeotheres* clade,

443 but far from other species (Fig. 9) with an interspecific divergence ranging from 12 to 19%. The

444 intraspecific divergences in *H. trapeziformis* ranged from 0 to 2.2%. This result is congruent

445 with the BOLD distance summary analyses, which shows an average distance of 0.73% and a

446 maximum of 2.27% for sequences with more than 500bp.

447 **Discussion**

448 A crucial problem for traditional taxonomy based solely on morphology, is the variability of the

449 phenotype of decapods. In pinnotherid taxonomy, a crucial goal is to provide a complete

450 description of the species with detailed illustrations of common and unusual structures for

451 comparative purposes (Derby & Antema 1980; Ahyong, Komai & Watanabe 2012; Salgado-

452 Barragán 2015). In that regard, characters previously not described like the antenna, the

453 antennule, the Mxp2, and the second male gonopod show no differences between all the material

454 examined, despite the variations noted above. These variations are greater when comparing

455 individuals from the northern region of Mexico (which include the topotype in Mazatlan, Sinaloa

456 state) to those from the southern region (Guerrero and Oaxaca states). However, COI data

457 analysis confirms all the material examined corresponds to the same species based on the

458 thresholds to delimitate species proposed by Hebert et al. (2003) and Lefébure et al. (2006).

459 Phenotype variation is the result of a plastic response to different environmental pressures

460 (Hurtado, Mateos & Santamaria 2010; Rossi & Mantelatto 2013) or due to recent or historical

461 processes that limit the flow of genes because of environmental barriers (Wares, Gaines &

462 Cunningham 2001; Avise 2009). Despite the fact that these processes are well documented, in

463 the case of brachyuran crabs, there is evidence showing that it does not occur in grapsids

464 (Cassone & Boulding 2006), ocypodids (Laurenzano, Mantelato & Schubart 2013), pinnotherids

465 (Ocampo et al. 2013), sesarmids (Zhou et al. 2015), and varunids (Zhang et al. 2017). However,

466 for pinnotherids, the several long-lasting growth phases require a specific host or a variety of

467 hosts to complete them (Bousquette 1980; Hamel, Ng & Mercier 1999; Ocampo et al. 2011) and

468 represent another drawback. Nevertheless, it allows them to maintain connectivity between

469 populations throughout their geographical distribution range (Haines, Edmunds & Pewsey 1994;

470 Hamel, Ng & Mercier 1999; Ocampo et al. 2012, 2013; Guilherme, Brustolin & de Bueno 2015;

471 Becker & Türkay 2017).

472 In the case of *Holothuriophilus trapeziformis*, it is considered a specific endobiontic parasite of

473 its host (Nauck 1880; Campos, Peláez-Zárate & Solís-Marín 2012), resulting in possibly limited

474 connectivity through larval dispersal. In addition to the above, the particular oceanographic

475   conditions known along the Pacific coast of Mexico and the distribution of the host (Hurtado et

476   al. 2007; Paz-García et al. 2012; Prieto-Rios et al. 2014; Gómez-Valdivia, Parés-Sierra & Flores-

477   Morales 2015; Honey-Escandón & Solís-Marín 2018), could explain the morphological

478   differences observed between the northern specimens compared to those of the south.

479   Currently, with the complete description of the male using the new characters described here, we

480   can conclude that *Holothuriophilus trapeziformis* is different from *H. pacificus*.

481   Regarding the DNA barcoding approach for the COI gene, in a difficult group to work with, we

482   were successful thanks to the injection of ethanol inside the body of the crabs through the joints

483   of the exoskeleton, and the use of semi-degenerate zooplankton primers (Prosser, Martínez-Arce

484   & Elías-Gutiérrez 2013) instead of Folmer's (Mantelatto et al. 2016). We obtained the

485   amplification of 72% of the specimens and 69% sequencing success.

486   The resulting maximum likelihood tree allowed us to confirm *Holothuriphilus trapeziformis* as a

487   separate species, indicating a divergence from 12 to 14% against the closest taxa, *H. pacificus*.

488   Also, our tree agrees with Palacios-Theil, Cuesta & Felder (2016) regarding the association of

489   the genus *Holothuriophilus* and *Calyptraeotheres*.

490   The taxonomic status of *Holothuriophilus trapeziformis* is now complete, based on the

491   morphology of both sexes, their distribution, specificity to a single host, and the DNA barcodes.

492   We believe that *Holothuriophilus trapeziformis* with its host reflects the restricted habitat in which

493   it lives and possibly the local environmental barriers in the Pacific coast of the American continent.

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728   Oaxaca, Mexico (UMAR-DECA-308): A, dorsal view (bold arrow indicates the internal blunt

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749   H, L, first gonopod in ventral view; e, gonopod tip in ventral view; f, gonopod tip, dorsal view

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756   (white arrow indicates the frontal notch, bold arrow indicates the hepatic notch); B, E, H, right

757   chela, external view (bold arrow indicates the proximal teeth, white arrow indicates the medial

758   tooth, dashed white arrow indicates the distal projection; dashed bold arrow indicates the medial

759   tooth); C, F, I, left Mxp3 endopod, external view (bold arrow indicates the ichiomerus

760   projection).

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762   ***pacificus* (Poeppig, 1836).** A–D, *H. trapeziformis* from Camarón Beach, Oaxaca, Mexico

763   (UMAR-DECA-1163): A, carapace; B, third maxilliped (bold arrow indicates the ischiomerus

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768   dorsal view of carapace; J, third maxilliped. K, *H. trapeziformis* from Guerrero, third maxilliped

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770   Scale of E= x3.5, F= x18.6, G= x4.6, H= x2.9 (*fide* Garth 1957).

771   **Figure 8. Comparison between males: *Holothuriophilus trapeziformis* Nauck, 1880 and *H.***

772   ***pacificus* (Poeppig, 1836).** A–C, from Panteón Beach, Oaxaca, Mexico; C, ventral view of the

773   first gonopod, Mexico (UMAR-DECA-308). D–F, from Talcahuano, Chile (drawing after Garth

774   1957: Figs. 11A, B, C). A, D, third maxilliped; a, dactylus; b, propodus; c, exopod flagellum. B,

775   F, abdomen. C, F, first gonopod. Scale of D= x21, E= x6, F= x25 (*fide* Garth 1957).

776   **Figure 9. Condensed unrooted Maximum likelihood tree based on mitochondrial**

777   **cytochrome c oxidase (COI) with the General Time Reversible with gamma distribution**

778   **(GTR+G) model.** Data: BOLD process ID, species name, associated BIN. Branch values

779   represent bootstrap probabilities (1000 permutations).

Figure 1

Sampling sites

A) northern region; type locality Mazatlan (*fide* Nauck, 1880); B-C) southern region.

Figure 2

Holothuriophilus trapeziformis Nauck, 1880

A–C, male from Panteón Beach, Oaxaca, Mexico (UMAR-DECA-308): A, dorsal view; B, ventral view; C, frontal view. D–F, female from Agua Blanca Beach, Oaxaca, Mexico (UMAR-DECA-307): D, dorsal view; E, ventral view; F, frontal view. G, male inside the gut of *Holothuria* (*Halodeima*) *inornata*, from Pinitos Beach, Sinaloa, Mexico.
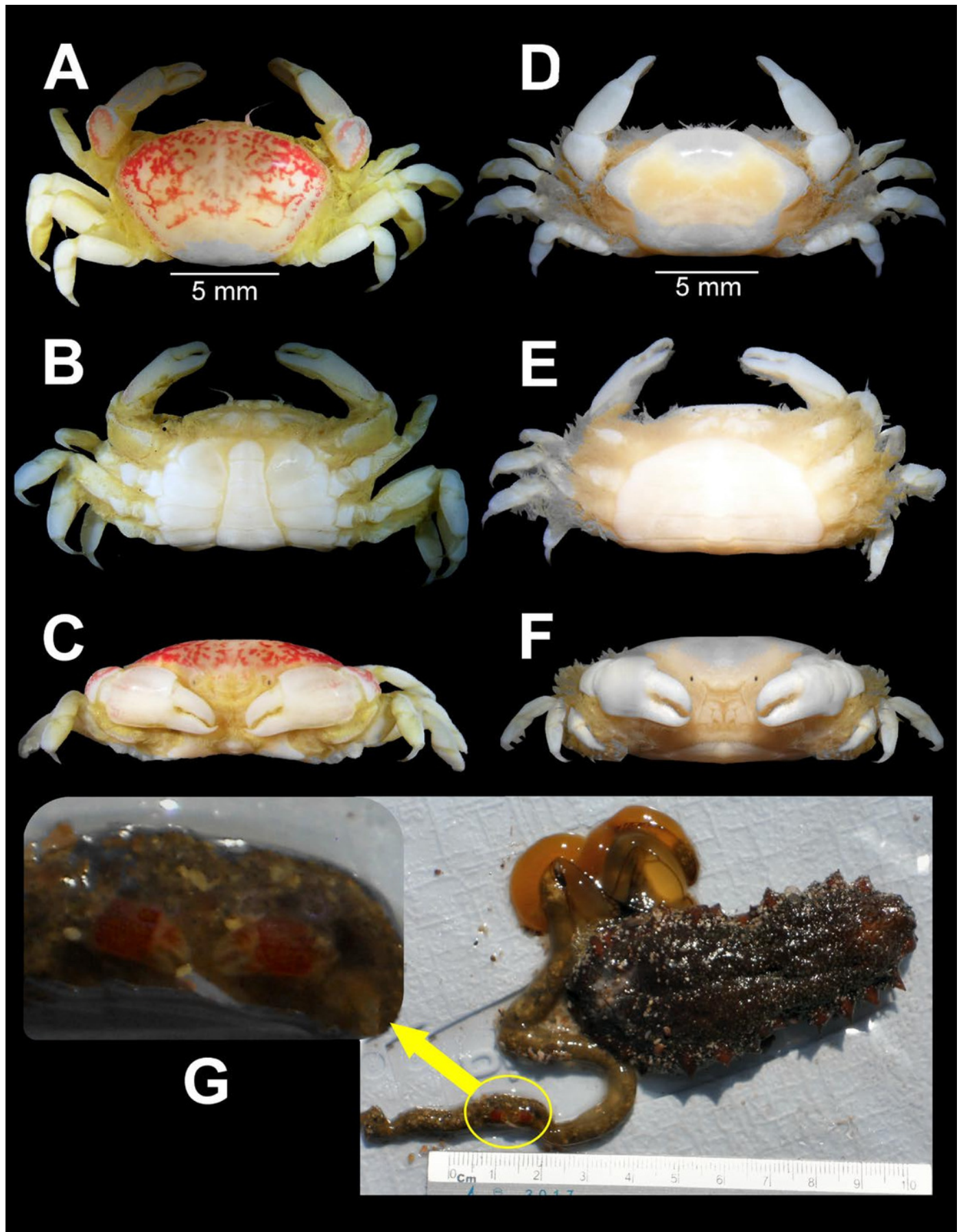
Figure 3

Holothuriophilus trapeziformis Nauck, 1880

A–D, male from Panteón Beach, Oaxaca, Mexico (UMAR-DECA-308): A, dorsal view (bold arrow indicates the internal blunt projection of the hepatic notch); B, frontal view (bold arrow indicates the hepatic notch); C, third-fourth sternal plate (bold arrow indicates the crenulated margin of the third plate, white arrow indicates the crenulated margin of the fourth plate); D, abdomen; E, abdominal view of the left first gonopod; F, ventral view of the left second gonopod (bold arrow indicates the distal notch); A, C, hollow circles indicating pits. Fine dots indicating pilosity. A–D, half of the illustration without ornamentation.
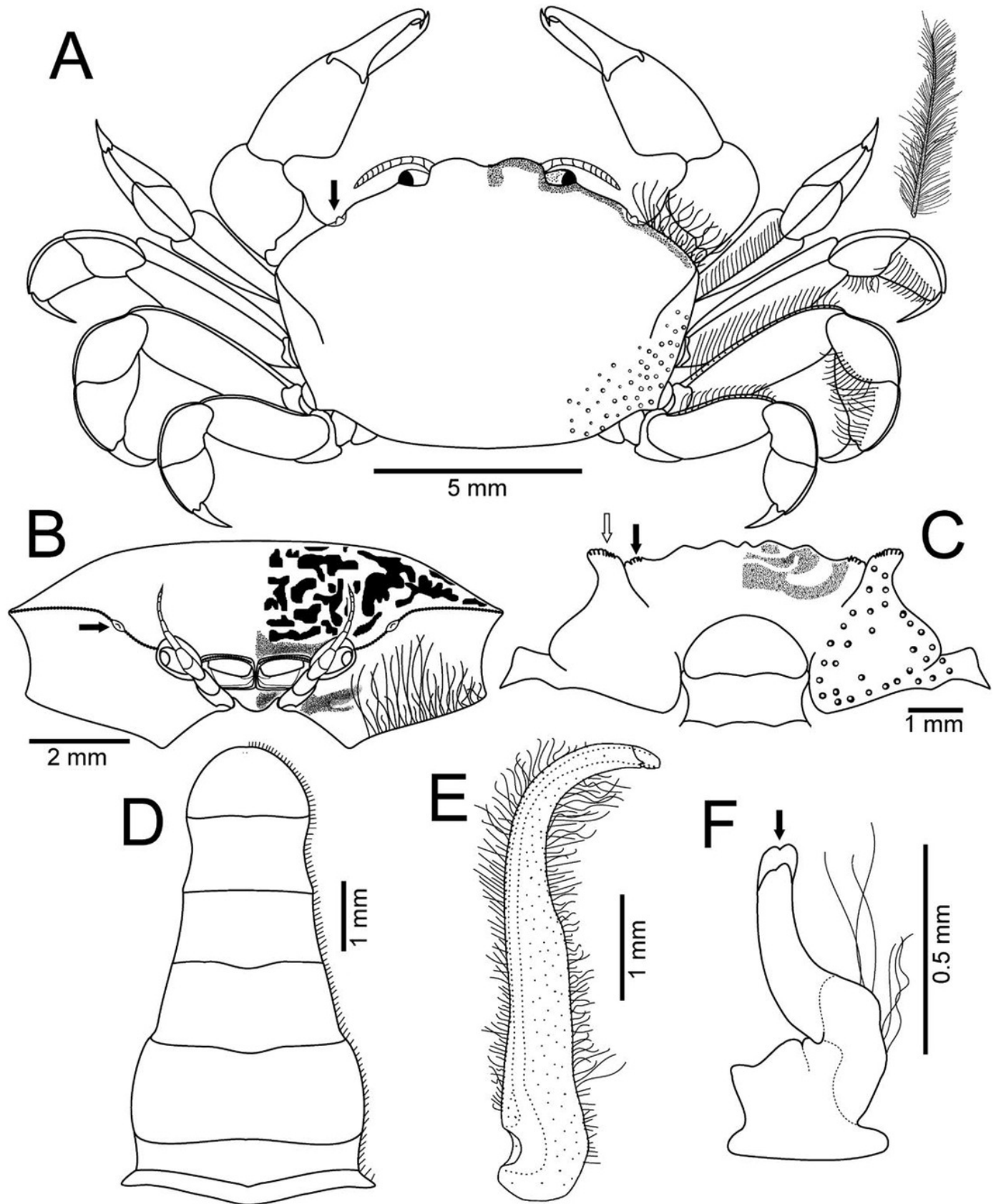
Figure 4

Holothuriophilus trapeziformis Nauck, 1880

A, antenna. B, antennule: a, superior palp; b, inferior palp; C, second maxilliped: a, endopod; b, exopod; c, exopod flagellum (bold arrow indicates the subrounded dactylus); D, third maxilliped (setae not illustrated): a, ischiomerus (white arrow indicates the conspicuous projection); b, carpus; c, propodus; d, dactylus (bold arrow indicates the distal widened dactylus). E, exopod of the third maxilliped. F, chela (upper bold arrow indicating mid-posterior teeth and the lower one the granulated inferior margin; dashed bold arrow indicating the lamella; white arrow indicates the subdistal projection).
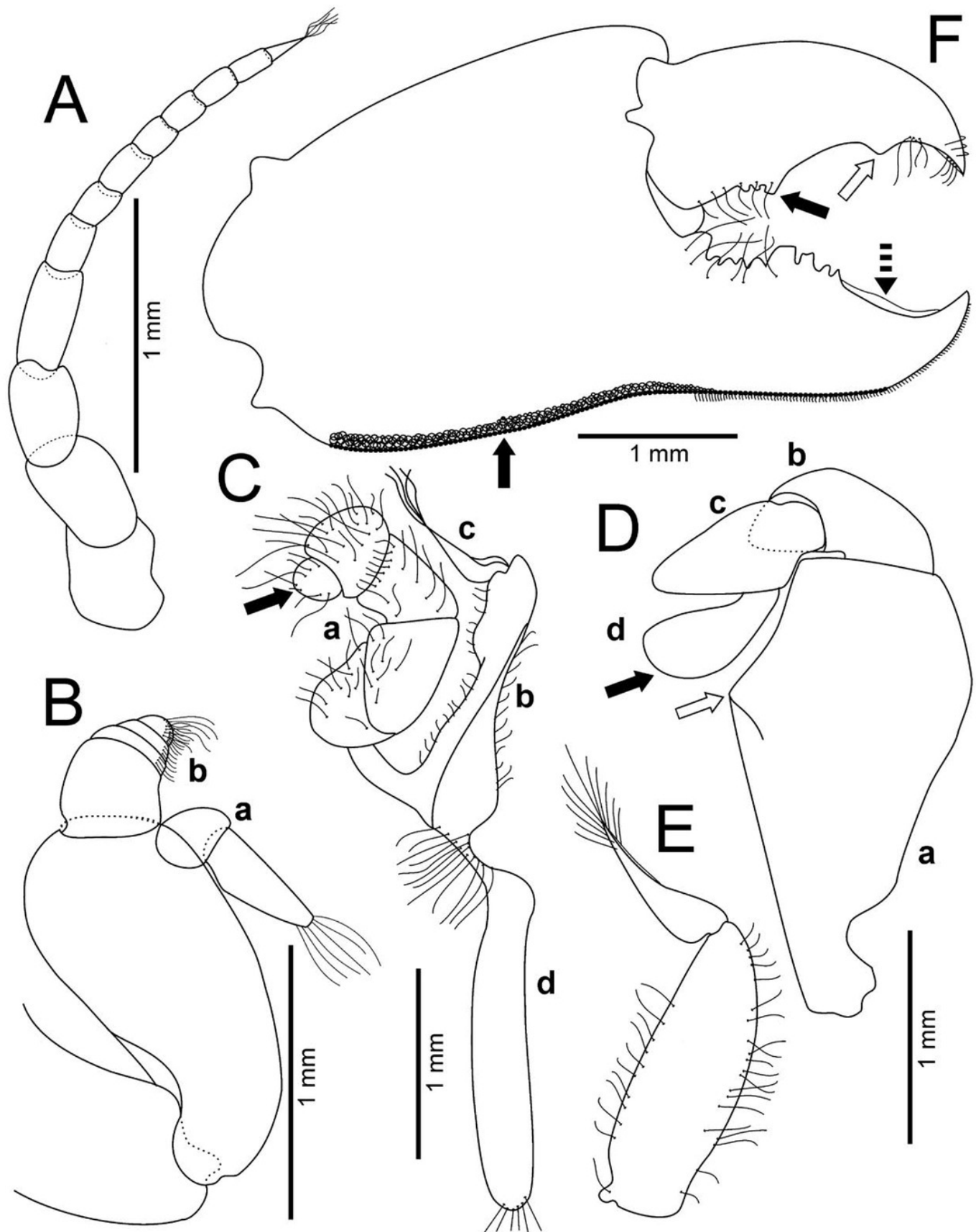
Figure 5

Comparison between males of *Holothuriophilus trapeziformis* Nauck, 1880 from the Pacific coast of Mexico

A–D, Sinaloa (DECA-1190; CW= 8 mm); E–H, Guerrero (DECA-1148; CW= 8 mm); I–L, Oaxaca (DECA-1270; CW= 8 mm). A, E, I, carapace outline (white arrow indicates the hepatic notch, bold arrow indicates the lateral lobes); B, F, J, right chela, external view (bold arrow indicates the proximal teeth, white arrow indicates the medial tooth, dashed white arrow indicates the distal projection; dashed bold arrow indicates the medial tooth); C, G, K, left Mxp3 endopod, external view (bold arrow indicates the ischiomerus projection); D, H, L, first gonopod in ventral view; e, gonopod tip in ventral view; f, gonopod tip, dorsal view (e–f, white arrow indicates the truncated or acute distal margin and bold arrow indicates the ventral process).
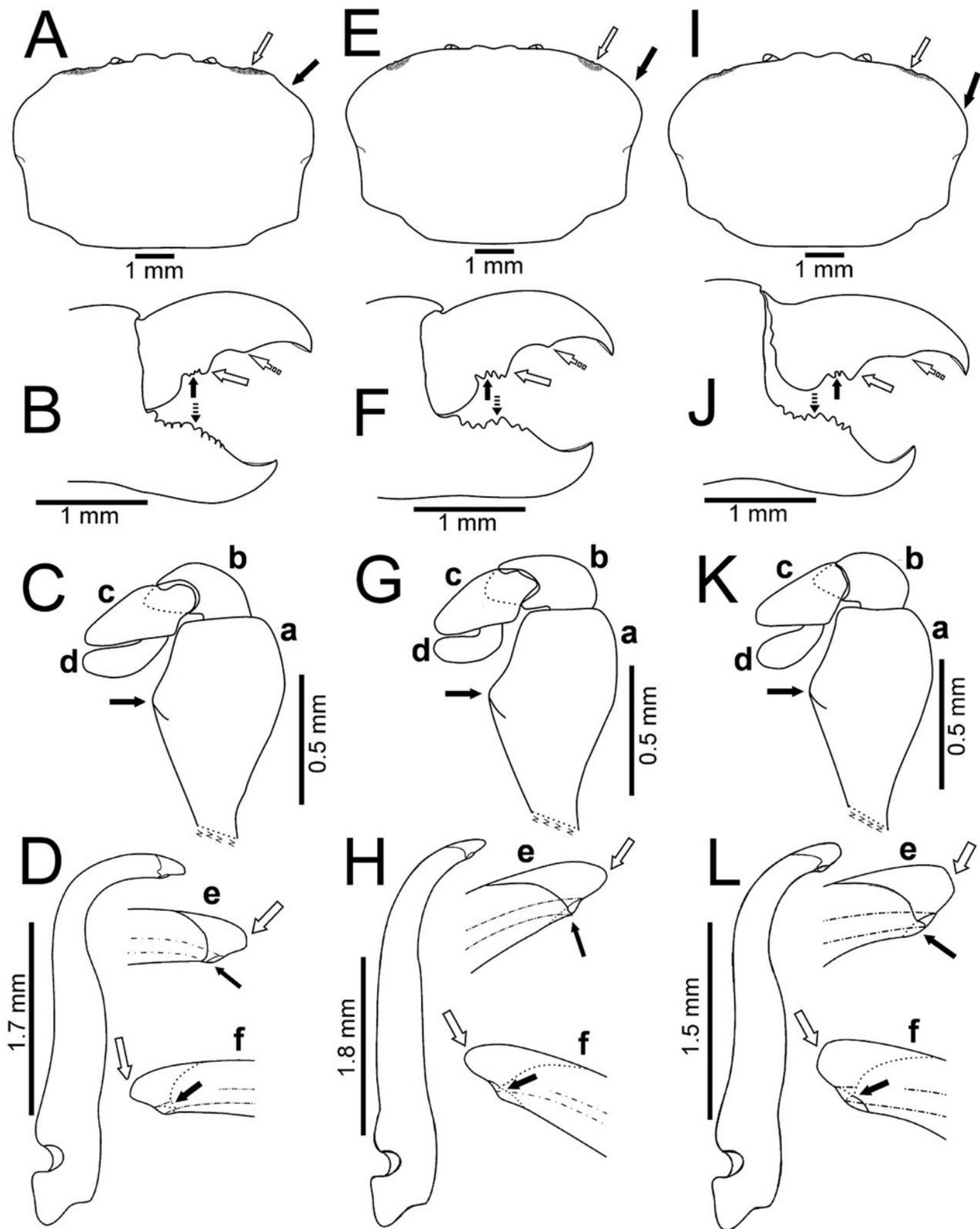
Figure 6

Comparison between ovigerous females of *Holothuriophilus trapeziformis* Nauck, 1880 from the Pacific coast of Mexico

A–C, Sinaloa (UMAR-DECA-1192; CW= 8 mm); D–F, Guerrero (DECA-1149; CW= 8 mm); G-I, Oaxaca (UMAR-DECA-1182; CW= 8 mm); J, K, chelae, external view, Oaxaca (UMAR-DECA-1172; CW= 9 mm). A, D, G, carapace outline (white arrow indicates the frontal notch, bold arrow indicates the hepatic notch); B, E, H, right chela, external view (bold arrow indicates the proximal teeth, white arrow indicates the medial tooth, dashed white arrow indicates the distal projection; dashed bold arrow indicates the medial tooth); C, F, I, left Mxp3 endopod, external view (bold arrow indicates the ichiomerus projection).
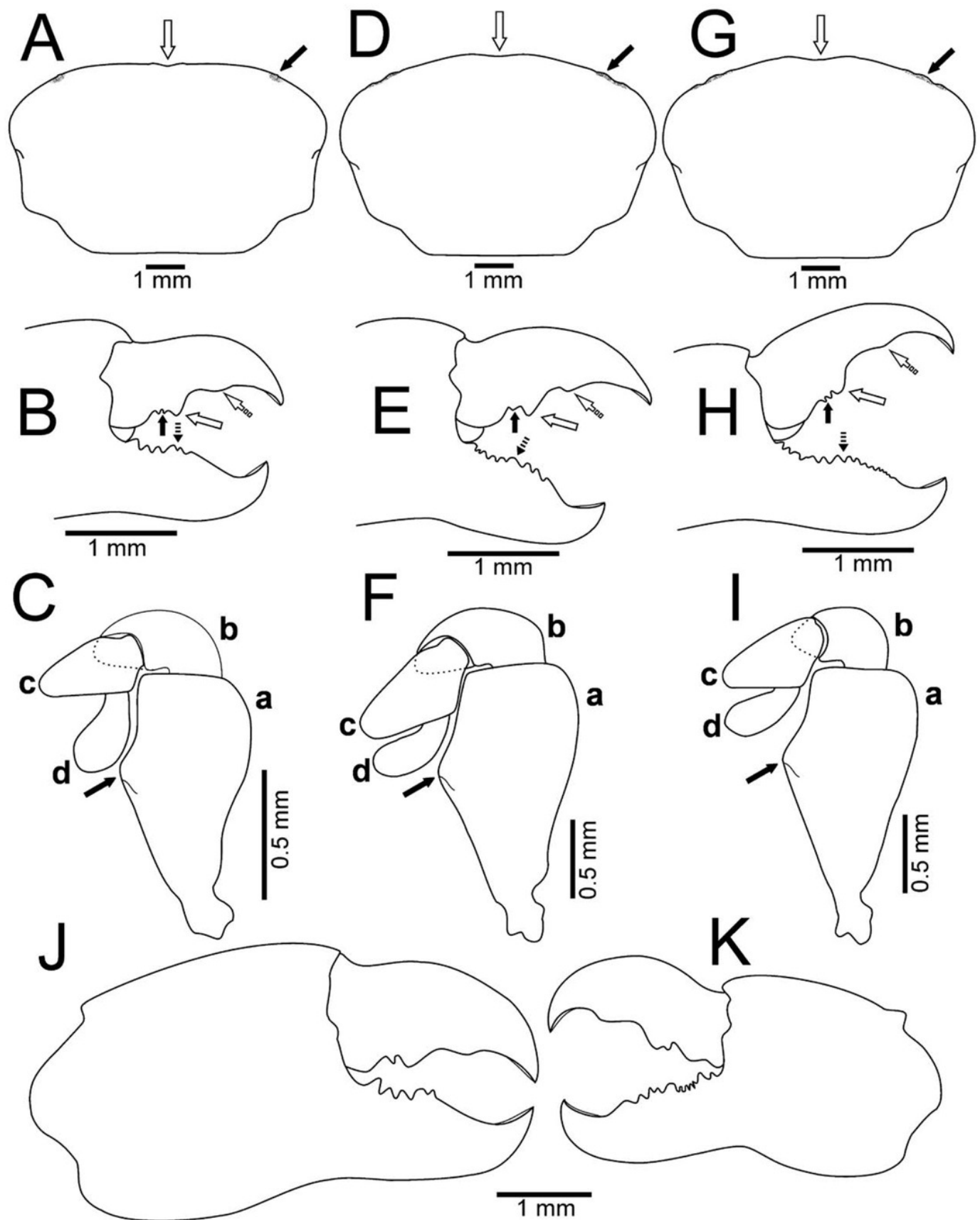
Figure 7

Comparison between females: *Holothuriophilus trapeziformis* Nauck, 1880 and *H. pacificus* (Poeppig, 1836)

A–D, *H. trapeziformis* from Camarón Beach, Oaxaca, Mexico (UMAR-DECA-1163): A, carapace; B, third maxilliped (bold arrow indicates the ischiomerus projection); C, chela (dashed bold arrow indicates the inferior granulated margin); D, ovigerous abdomen. E–H, *H. pacificus* from San Vicente, Chile (drawing after Garth 1957: figs. 10E, F, G, H as *Pinnaxodes silvestrii*): E, carapace; F, third maxilliped; G, chela; H, abdomen. I-J, lectotype of *H. trapeziformis* from Mazatlan, Mexico (drawing after Ahyong & Ng 2007: Fig. 20 A, C) I, dorsal view of carapace; J, third maxilliped. K, *H. trapeziformis* from Guerrero, third maxilliped of the adult female of (drawing after Campos, Peláez-Zárate, Solís-Marín 2012: Figs. 2B, C). Scale of E= x3.5, F= x18.6, G= x4.6, H= x2.9 (*fide* Garth 1957).
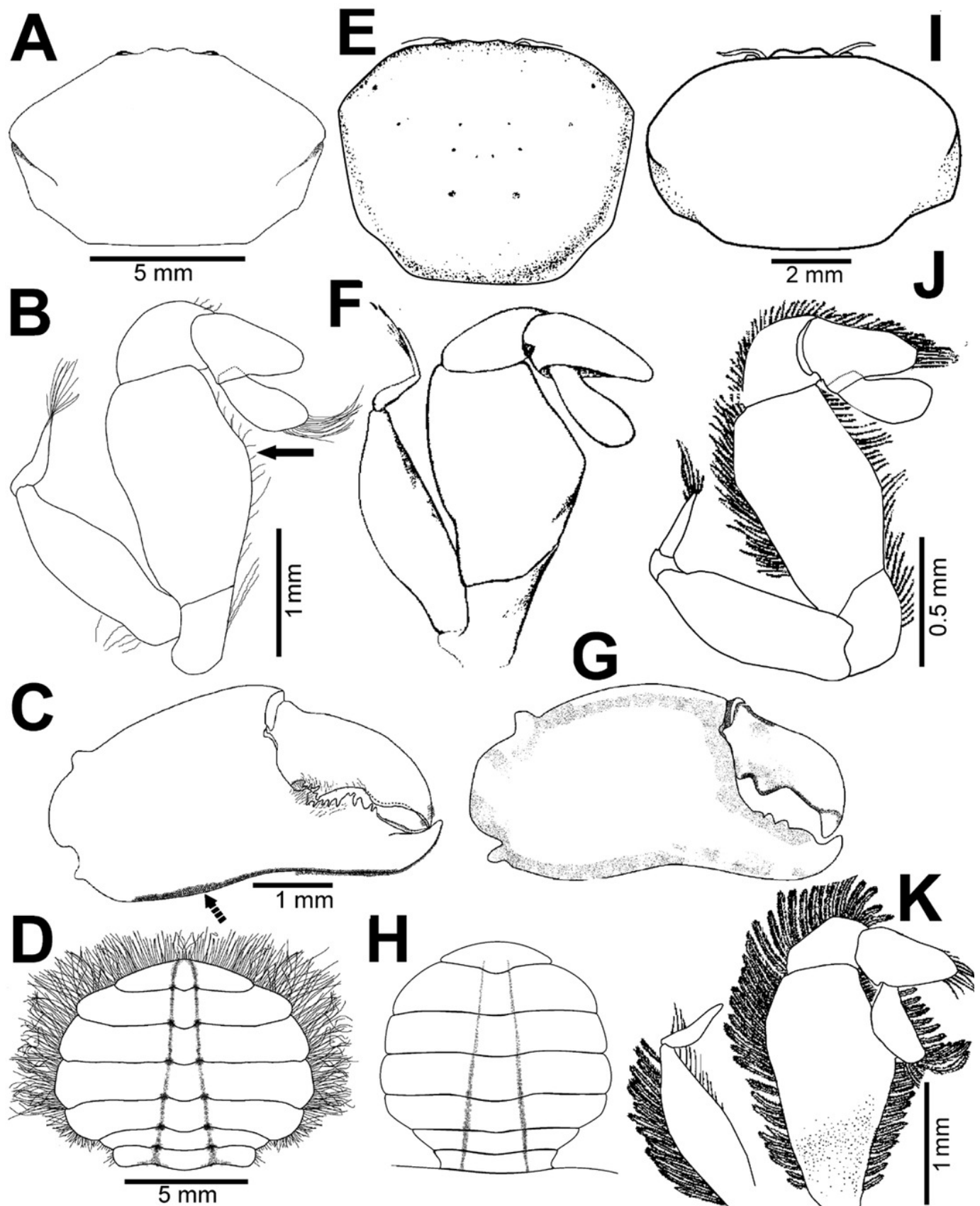
Figure 8

Comparison between males: *Holothuriophilus trapeziformis* Nauck, 1880 and *H. pacificus* (Poeppig, 1836)

A–C, from Panteón Beach, Oaxaca, Mexico; C, ventral view of the first gonopod, Mexico (UMAR-DECA-308). D–F, from Talcahuano, Chile (drawing after Garth 1957: Figs. 11A, B, C). A, D, third maxilliped; a, dactylus; b, propodus; c, exopod flagellum. B, F, abdomen. C, F, first gonopod. Scale of D= x21, E= x6, F= x25 (*fide* Garth 1957).
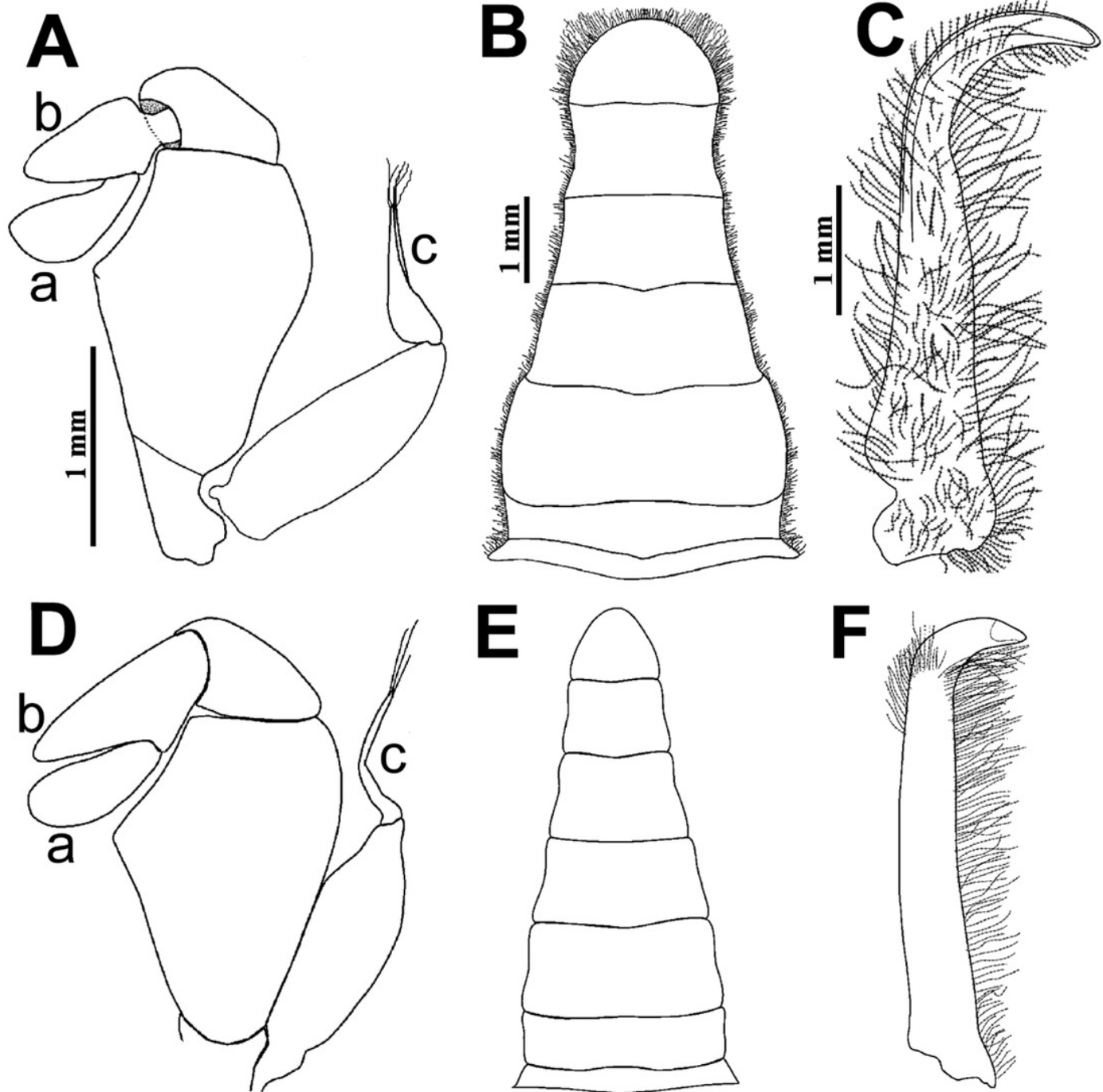
Figure 9

Condensed unrooted Maximum likelihood tree based on mitochondrial cytochrome c oxidase (COI) with the General Time Reversible with gamma distribution (GTR+G) model

Data: BOLD process ID, species name, associated BIN. Branch values represent bootstrap probabilities (1000 permutations).