Revision of *Holothuriophilus trapeziformis* Nauck, 1880 (Decapoda: Pinnotheridae) from the Pacific coast of Mexico, based on integrative taxonomy (#57986)

First revision

Guidance from your Editor

Please submit by **23 Jul 2021** for the benefit of the authors .

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Custom checks
Make sure you include the custom checks shown below, in your review.

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

Files

Download and review all files from the materials page.

1 Tracked changes manuscript(s)
1 Rebuttal letter(s)
9 Figure file(s)
1 Raw data file(s)
1 Other file(s)

Custom checks

DNA data checks

- Have you checked the authors data deposition statement?
- Can you access the deposited data?
- Has the data been deposited correctly?
- Is the deposition information noted in the manuscript?

Field study

- Have you checked the authors field study permits?
- Are the field study permits appropriate?

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

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

Fernando Cortés-Carrasco [1], **Manuel Elías-Gutiérrez** [Corresp., 2], **María del Socorro García-Madrigal** [3]

[1] Aquatic Ecology and Systematics, El Colegio de la Frontera Sur, Chetumal Unit, Chetumal, Quintana Roo, Mexico

[2] Aquatic Ecology and Systematics, El Colegio de la Frontera Sur, Chetumal, Quintana Roo, Mexico

[3] Campus Puerto Ángel, Universidad del Mar, Puerto Ángel, Oaxaca, Mexico

Corresponding Author: Manuel Elías-Gutiérrez
Email address: melias@ecosur.mx

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 America. 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 existing morphological separation of both species of the genus is subjective since it is based on the interdactilar gape condition of the pincers finger. Our goal is to complete and clarify the taxonomic status of H. trapeziformis and describe the male morphology, providing more stable characters to differentiate this species. This goal will be accomplished with the use of the integrative taxonomy.

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

Results. This species presents marked sexual dimorphism only in the primary sexual characters. Morphological variation is high on this species, but 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). We confirmed that H. trapeziformis is a different species from his closest congener, H. pacificus. We observed additional characters to the previously known: the ornamentation of the pincers fingers, the shape of the male abdomen, and its first gonopod. Intra-specific COI distance was >3% of divergence, and the species forms a clear, unique clade compared with other family members. 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, but it was also found within the intestine. The location of the species beyond the previously established area also allowed us to extend its distribution range along the Pacific coast of Mexico. With the data recovered from our research, the taxonomic status of Holothuriophilus trapeziformis is now complete.

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

Fernando Cortés-Carrasco[1], Manuel Elías-Gutiérrez[1*], María del Socorro García-Madrigal[2]

[1]Aquatic Ecology and Systematics. El Colegio de la Frontera Sur. Chetumal Unit, Quintana Roo, Mexico.
[2] Campus Puerto Ángel, Universidad del Mar, Puerto Ángel, Oaxaca, México.

*Corresponding author:
Manuel Elías-Gutiérrez
melias@ecosur.mx

Abstract

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 America. 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 existing morphological separation of both species of the genus is subjective since it is based on the interdactilar gape condition of the pincers finger. Our goal is to complete and clarify the taxonomic status of *H. trapeziformis* and describe the male morphology, providing more stable characters to differentiate this species. This goal will be accomplished with the use of the integrative taxonomy.

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

Results. This species presents marked sexual dimorphism only in the primary sexual characters. Morphological variation is high on this species, but 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). We confirmed that *H. trapeziformis* is a different species from his closest congener, *H. pacificus*. *We observed additional characters to the* previously known: the ornamentation of the pincers fingers, the shape of the male abdomen, and its first gonopod. Intra-specific COI distance was >3% of divergence, and the species forms a clear, unique clade compared with other family members. 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, but it was also found within the intestine. The location of the species beyond the previously established area also allowed us to extend its distribution range along the Pacific coast of Mexico. With the data recovered from our research, the taxonomic status of *Holothuriophilus trapeziformis* is now complete.

Introduction.

Pinnotherids (Crustacea: Pinnotheridae) are true decapod crabs, which show a conspicuous sexual dimorphism, notably different morphological stages of development and complex ecological relationships with different invertebrates, but can also be found in free life (Schmitt, McCain & Davidson 1973; Ocampo et al. 2011; Becker & Türkay 2017). Worldwide, sixteen species are known to be endobiontic with sea cucumbers (Ng & Manning 2003), of which two species assigned to the genus *Holothuriophilus* Nauck, 1880, are distributed in the Pacific coast of America (Manning 1993). *H. trapeziformis* Nauck, 1880 with a type locality in Mazatlan, Mexico, is associated with the sea cucumber *Holothuria* (*Halodeima*) inornata Semper, 1868. *H. pacificus* (Poeppig, 1836) from Talcahuano, Chile (Manning 1993) is associated with a different sea cucumber, *Athyonidium chilensis* (Semper) (Garth 1957; Honey-Escandón & Solís-Marín 2018). This genus is diagnosed by its transversally elongated carapace, wider anterior to middle portion; its short, robust and compressed walking legs, with the dorsal margin cristate; and the third maxilliped with the ischiomerus indistinguishably fused (Garth 1957; Manning1993; Ng & Manning 2003; Campos, Peláez-Zárate & Solís-Marín 2012).

However, the taxonomic status of *H. trapeziformis* remains confuse, because male morphology is unknown and the available information from female illustrations shows some inconsistencies when the carapace, Mxp3 shape, and setae patterns are compared (see Bürger 1985: 380–381, pl. 9, fig.

26; Ahyong & Ng 2007: 214, Figs. 20A, C; Campos, Peláez-Zárate & Solís-Marín 2012: 60, figs. 2B, C). Due to this situation the differentiation of *Holothuriophilus trapeziformis* from *H. pacificus* is based on a single morphological character; the former species has a narrow opening when the pincers fingers are closed, but in the latter species the fingers gap is conspicuous (Campos, Peláez-Zárate & Solís-Marín 2012).

Despite Nauck's (1880) effort to provide a detailed description of the *Holothuriophilus trapeziformis*, the preservation quality of the sample, and the data from the reviewed material made it impractical to designate a type specimen, make a complete description, and determine the identity of the host with certainty. Moreover, the female syntypes deteriorated over time and the male was unknown (Bürger 1895; De Man 1887; Ng & Manning 2003). Later, Manning (1993), Ng & Manning (2003), and Ahyong & Ng (2007) examined the syntype series to complete the diagnosis and designated a lectotype which was described and illustrated; however, there are inconsistencies in their illustrations and the diagnostic characters are not informative when considering the information available for *Holothuriophilus pacificus*. In addition, for 84 years, *H. trapeziformis* was not collected until Caso (1958, 1964, 1965) gathered four pinnotherids, determined as *Pinnixa barnharti* (not *Pinnixa barnharti* Rathbun, 1918), associated with *Holothuria inornata* Semper, 1868 and *H. kefersteinii* (Selenka) (= *H. riojai* Caso, 1964). Thirty-four years later, one of Caso's specimens was determined as *Holothuriophilus* sp. by Campos, Díaz & Gamboa-Contreras (1998). More recently Campos, Peláez-Zárate & Solís-Marín (2012) updated the species diagnosis and made a review of the genus. Finally, Honey-Escandón & Solís-Marín (2018) confirmed the ecological association between *H. trapeziformis* and *Holothuria inornata*, but Caso's (1958, 1965) records of *Holothuria kefersteinii* as a host remains uncertain because the field collection data does not correspond with the material

reviewed by Honey-Escandón & Solís-Marín (2018), and the location of these pinnotherids and their holothurian hosts is unknown (F. Solís-Marín, 2018, pers. comm.).

For *Holothuriophilus trapeziformis,* there is currently no data on any gene. In contrast, for *H. pacificus*, there is information related to the COI gene sequence for one specimen recovered from the shoreline in southern Chile (CFAD062-11; boldsystems.org). Within this context, sequencing of approximately 650 bp region of the mitochondrial Cytocrome Oxidase 1 gene (COI) has been promoted to conform a standardized DNA barcode system with the aim of being one more tool for the identification of biological species with many applications in diverse fields of knowledge (Hebert et al. 2003; Hajibabaei et al. 2007). In spite of the difficulty to work with COI regarding the debate about the acceptance of one molecular marker as an accurate character to define a species (Will & Rubinoff 2004), it has been considered the best marker for identification in other decapods (Spielmann et al. 2019). The utility of the DNA Barcoding (COI sequence) has been useful to delimit other pinnotherids (Ocampo et al. 2013; Perez-Miguel et al. 2019), brachyuran larvae (Brandão, Freire & Bruton 2016), and other crustacean taxa (Costa et al. 2007; Matzen da Silva et al. 2011).

Considering that integrative taxonomy based on morphological and molecular data is increasingly useful to define and delimit biological species with greater certainty, the goal of this study is, therefore, to define the taxonomic status of *Holothuriophilus trapeziformis* by completing the information on the species with the description of the male, revising the morphological variability in both sexes, updating the range of distribution, and establishing a baseline of mitochondrial COI gene barcode. Finally, this information will provide new diagnostic characters that will allow a clearer separation of both species of *Holothuriophilus* from the Pacific coast of America.

Material & methods

Morphology

Fifty-two crabs belonging to the *Holothuriophilus trapeziformis* were extracted from the coelom and intestine of the sea cucumber *Holothuria inornata.* Hosts were manually collected through skin and SCUBA diving at a maximum depth of 10 meters in Sinaloa, Guerrero, and Oaxaca, Mexico (Fig. 1). The collected material was labeled and fixed according to the Elías-Gutiérrez et al. (2018) protocol for tissue preservation and DNA analyses. Due to the size of the specimens and the thickness of their cuticle, we injected ethanol into the body and joints of the appendices with insulin syringes.

All biological material (Table S1) was classified and deposited in the Scientific Collection of Marine Invertebrates of the Laboratorio de Sistemática de Invertebrados Marinos (LABSIM) from Universidad del Mar (UMAR), Oaxaca, Mexico (OAX-CC-249-11). Hosts were identified with specialized literature (Solís-Marín et al. 2009; Honey-Escandón & Solís-Marín 2018).

For the analysis of the taxonomic status of *Holothuriophilus trapeziformis* specialized literature from Nauck (1880), Manning (1993), Ng & Manning (2003), Ahyong & Ng (2007), and Campos, Peláez-Zárate & Solís-Marín (2012) was reviewed. Likewise, for *H. pacificus,* Poeppig (1836), Nobili (1901), Rathbun (1918), and Garth (1957) were reviewed.

For this study, we got a field permit for collections with non-commercial scientific research purposes by Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA) and Comisión Nacional de Acuacultura y Pesca (CONAPESCA) (Collecting permit: PPF/DGOPA-301/17).

The species description follows the terminology of Ahyong & Ng (2007). The setae terminology is based on Garm & Watling (2013). Drawings were made with the help of a lucid camera and

149 then digitalized in a vector format. Pictures were taken with a Nikon D5100 digital camera.
150 Measurements are given in millimeters and the latitude and longitude of the collections were
151 obtained from Google Earth™.
152 Because we were only able to obtain nine specimens (three males and six females) from the type
153 locality, in contrast to 47 (six males and 41 females) from the southern region, and due to
154 morphological variability observed, it was necessary to standardize the observations by using
155 specimens in the same stage of development. The shared stage of development between the three
156 regions (Sinaloa, Guerrero, and Oaxaca) corresponded to males and ovigerous females with a
157 carapace width measurement equal to eight millimeters. To standardize the observations, the
158 specimen and the dissected pieces were mounted on a plastic clay base to make the drawings.
159 For the carapace contour, the samples were mounted, so the dorsal view of the posterior margin
160 line of the carapace was observed. For the Mxp3, an attempt was made to extract it from its base
161 to obtain both endopod and exopod and to mount it with the articles in the same perspective. The
162 cutting edge of the fingers' chelae was cleaned of dirt to see all the teeth, the first gonopod was
163 extracted from its base, and the setae cleaned of dirt.
164 Abbreviations used in the text: CL, carapace length (taken as the middle line from the frontal
165 margin to the posterior margin of the carapace); CW, carapace width (measured in its medium-
166 anterior portion); Mxp2, second maxilliped; Mxp3, third maxilliped; P2–5, walking legs 1 to 4.
167 Acronyms used in the text: BOLD, barcode of life database (boldsystems.org); BIN, barcode
168 index number (*sensu* Ratnasingham & Hebert, 2013); BOLD-ID, Specimen ID in the Barcode of
169 Life Data System; CNE-ICML-UNAM, National Collection of Echinoderms of the Institute of
170 Marine Sciences and Limnology of the National Autonomous University of Mexico; DC-NHM,
171 Division of Crustacea, Natural History Museum, Smithsonian Institution; SMF-ZMG,

Senckenberg Museum für Naturkunde, Zoologisches Museum Göttingen University, Humboldt Universität, Berlin; UABC, Autonomous University of Baja California, Mexico; UMAR, Universidad del Mar campus Puerto Angel, Oaxaca, Mexico.

Collectors: AEV, Aidé Egremy Valdés; AGF, Andrea Glockner Fagetti; CCA, Carlos Cruz Antonio; AHM, Adanely Hernández Muñoz; FBV, Francisco Benítez Villalobos; FCC, Fernando Cortés Carrasco; HMC, Humberto Mesa Castillo; KFL, Karen Lizbeth Flores López; KMB, Karen Mesa Buendía; RGF, Rebeca Granja Fernández; VCH, Valeria Chavez García.

DNA extraction and PCR amplification

Genomic DNA of individuals of *Holothuriophilus trapeziformis* was extracted from biological material collected in the field and some individuals from the OAX-CC-249-11 regional collection of the Universidad del Mar, using tissue from the walking legs, the chelae, or eggs from the ovigerous females. Tissues were placed into 96-well microplates with a drop of 96% ethanol, and DNA extraction was carried out following the standard glass fiber method consisting of a mix of Proteinase K with an invertebrate lysis buffer according to Ivanova, De Waard & Hebert (2006). Following the DNA extraction, the PCR mixture with a final volume of 12.5 µl, contained 2 µl of Hyclone ultrapure water (Thermo Fisher Scientific), 6.25 µl of 10% trehalose (previously prepared: 5 g D-(+)- trehalose dihydrate (Fluka Analytical) in a total of 50 ml of molecular grade ddH2O), 1.25 µl of 10X PCR Platinum Taq buffer (Invitrogen), 0.625 µl of 50 µmol/L MgCl2 (Invitrogen), 0.0625 µl of 10 µmol/L dNTP (KAPA Biosystems), 0.125 µl of each 10 µmol/L primer, 0.06 µl of PlatinumTaq DNA polymerase (Invitrogen), and 2 µl of DNA template. All specimens were amplified with the Zooplankton primers (ZplankF1_t1 and ZplankR1_t1, see Prosser, Martínez-Arce & Alías-Gutiérrez 2013 for details). The reactions were cycled at 94°C for 1 min, followed by five cycles of 94°C for 40 seconds, 45°C for 40

seconds and 72°C for 1 min, followed by 35 cycles of 94°C for 40 seconds, 51°C for 40 seconds and 72°C for 1 min, with a final extension of 72°C for 5 min. PCR products were visualized on a pre-cast 2% agarose gels (E-Gel© 96 Invitrogen), and the most intense positive products were selected for sequencing.

Sequencing and DNA barcode

Selected PCR products were sequenced using a modified (Hajibabaei et al. 2005) BigDye© Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystem, Inc.), and then sequenced bidirectionally on an ABI 3730XL automated capillary sequencer using M13F and M13R sequence primers at the Biology Institute at the National Autonomous University of Mexico and at the Eurofins Genomics Louisville Laboratory (USA). Sequences were edited using CodonCode© v 3.0.1 (CodonCode Corporation, Dedham, MA, USA) and uploaded to BOLD. In some cases, the original forward and reverse tracers uploaded to BOLD were checked again. Consensus assembly was generated, and edited manually with Sequencher© 4.1.4. (Gene Codes Corporation, Ann Arbor, MI, USA), and then they were aligned using BioEdit© (Hall 1999).

Likelihood tree and distance analysis

COI sequences generated for *Holothuriophilus trapeziformis* in this study were compared with COI sequences from other pinnotherids collected in the Eastern Pacific coast of America, available in BOLD and/or GeneBank (Table S2). Sequence data, trace files, and primer details for all *H. trapeziformis* specimens and for the other species are available under the dataset name PINMX1HT ("Htrapeziformis from Mexico") in the Barcode of Life Data System (barcodinglife.org). Additionally, *H. trapeziformis* sequences were uploaded to GenBank (https://www.ncbi.nlm.nih.gov/). The accession numbers are noted in the table S2.

To construct the tree, the best-fitting evolution model of nucleotide substitution for distance based on COI alignments was established on the Maximum Likelihood (ML) for 24 different nucleotide substitution models, selected according to the Akaike (AIC) and Bayesian (BIC) criterion (Darriba et al. 2011), and tested using jModelTest© 2.1.10 (Posada & Buckley 2004). The final tree was obtained with nodal support for the resulting branches estimated with 1000 bootstrap replicates in MEGA 7.0 (Tamura et al. 2013). It was simplified with the compress/expand feature of MEGA. Also, interspecific COI genetic distances for the dataset were estimated using the Kimura-2 parameters distance method in MEGA. Values greater than 3% were considered the threshold for the delimitation of the species (Hebert et al. 2003).

Results

The morphology of 56 specimens from three coastal regions in the Mexican Pacific was analyzed, including the type locality. A detailed morphological revision of the specimens allowed us to determine notable variations, mostly on the carapace general shape, features of the first male gonopod, and in the pincers chelae ornamentation. ~~Northern type locality m~~orphology shows a notable variation in the general carapace outline shape and general appearance which looks more stout and eroded in contrast to that of the southern specimens. However, all specimens show features that define *Holothuriophilus trapeziformis* according to Ng & Manning (2003) and Campos, Peláez-Zárate & Solís-Marín (2012). In addition, previously undescribed structures like the Mxp2 and male second gonopod plus the genetic data resolution, confirm that all the revised material corresponds with the *H. trapeziformis*. Complete morphology description of the male and the discussion of character variations in both sexes are annotated in the Systematics section and DNA barcoding analyses are annotated after that.

Systematics

Infraorder Brachyura Latreille, 1802

Family Pinnotheridae De Haan, 1833

Genus *Holothuriophilus* Nauck, 1880

Holothuriophilus. — Manning, 1993: 225.

Diagnosis (modified from Manning 1993). Carapace broader than long, widest on mid anterior portion, transversely subcuadrangular, subrectangular, subovate or subtrapezoidal. Third maxilliped with ischium and merus indistinguishable fused; exopod with one segmented flagellum; endopod palp 3-segmented; propodus shorter than carpus, conical; subspatulate

dactylus articulated basally on propodus, extending beyond end of propodus. Dactyli of walking legs similar and subequal, short. Abdomen of seven segments in both sexes.

***Holothuriophilus trapeziformis* Nauck, 1880**

(Figs. 2A–G, 3A–D, 4A–K, 5A–K, 6A–D)

Holothuriophilus trapeziformis Nauck, 1880: 24, 66 [ovigerous female type]. —De Man 1887: 721–722 [female (CW = 13.8 mm, CL = 10.5 mm)]. —Ng & Manning 2003: 903, 916-918, Fig. 7C–F [female lectotype (CW = 7.7 mm, LC = 4.8 mm): SMF-ZMG 67/565a]. —Ahyong & Ng 2007: 213-214, Fig. 20. —Campos, Peláez-Zárate & Solís-Marín 2012: 57–62, Figs. 1A, B, 2A–D [female (CW = 9.1 mm, CL = 5.5 mm)].

Pinnotheres trapeziformis Bürger 1895: 380–381, plate 9, Fig. 26, plate 10, Fig. 25 [female type (CW = 14 mm, CL = 10 mm), male (CW = 5 mm, CL = 8.5 mm)]. —Adensamer 1897: 107. —Schmitt, McCain & Davidson 1973: 5, 13, 89 [list].

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

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

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

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

General distribution: Tropical Eastern Pacific (Mexico).

Previous records: Mazatlán, Punta Tiburón (Sinaloa); Ixtapa (Guerrero).

New records: Playa Pinitos (Sinaloa); Playa Nudista, Playa Zacatoso, Playa Caleta de Chon (Guerrero); Playa Agua Blanca, Playa Coral, Playa Camaron, Playa Panteón, Playa Estacahuite, Playa La Tijera, Bahía San Agustín, Playa El Tejón (Oaxaca).

BIN: ADE9974

Size range (mm): Males: CW = 5.5–11, CL = 3.2-7; females: CW = 5.1–11, CL = 3–7; ovigerous females: CW = 7.3–13, CL = 5–8.

Diagnosis. Carapace general shape transversely subrectangular, suboval or subtrapezoidal. Cristated anterolateral margin with a hepatic notch and a vanished blunt tooth inside the notch. Chelipeds merus and carpus inner surface densely setose; propodus ventral inner margin with a row of conspicuous short setae; cutting edge of propodus and dactylus almost meeting when closed, interdactilar gape narrow; dactylus cutting edge with proximal denticles, with a conspicuous medial tooth, and with a distal convex or acute projection. Merus dorsal surface of W1, 3 and 4 with setae, W2 without seta. Abdomen with 6 somites plus free telson; on male, margin of somite 4 to 6 concave, telson subrounded. Male first gonopod notably curved outward from its mid-distal portion**.**

Description: Male (Fig 2A–C; UMAR-DECA-308; CW = 11 mm, CL = 7 mm): Carapace, transversely subtrapezoidal, wider than long, CW/CL ratio ca. 1.6, mid-anterior portion wider; anterolateral margins slightly projected, cristated, a hepatic notch with a blunt middle tooth (Figs. 3A, B; bold arrow); dorsal surface convex, smooth, without defined regions; mid-posterior and posterolateral surface with microscopic pits of variable size and pilosity (Figs. 3A; hollow circles and dots); inferior lateral margin with abundant plumose setae (Fig. 3A; simple lines represent the enlarged schematic setae).

Front bilobed, scarcely visible in dorsal view, margin granulated, surface slightly pubescent (Fig. 3B; dots).

Orbits small, completely filled by eyes; eyes pigmented; ocular peduncle scarcely pubescent. Antennules robust; peduncle 2-segmented, biflagellate, transversely folded into ~~the~~ fossae; superior flagellum 2-articles, second article ~~the~~ longe~~st~~, tapering distally, with ~~six~~ apical setae

(Fig. 4Ba); inferior flagellum conic, with four articles decreasing in size, article one to three with ~~a~~ transverse line of simple setae, fourth article with two transverse lines of simple seta (Fig. 4Bb).

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

Pterygostomian region pubescence (Fig. 3B; fine dots). Buccal frame trapezoidal, completely covered by ~~the~~ Mxp3. Mxp2 endopod 5-articles, with setae (Fig. 4Ca), dactylus subrounded and shorter than propodus (Fig. 4C; black arrow); exopod 1-article, wider distally, external surface with an elevated ridge (Fig. 4Cb), flagellum with long apical setae (Fig. 4Cc), epipodite long, distal margin rounded (Fig. 4Cd). Mxp3 ischiomerus fused without suture line, width/length ratio = 0.7, ~~external~~ margin convex with setae, ~~internal~~ margin with a medial conspicuous projection (Fig. 4Da; white arrow); carpus subconial, external margin with short setae; propodus subconical (Fig. 4Dc); dactylus subspatuliform, wider distally (Fig. 4D; black arrow), slightly overreaching propodus, external surface with short plumose setae, external margin with long plumose setae; exopod 1-article, external margin and external surface with short simple setae, flagellum slender, with plumose long setae (Fig. 4E).

Sternal third plate with anterior margin sinuous, anterolateral angles with crenu-denticulated margin (Fig. 3C; black arrow), surface scarcely pilose (Fig. 3C; dots); fourth plate slightly globose, surface with microscopic pits (Fig. 3C; hollow circles), ~~distal external~~ angle curved outward, margin crenu-denticulated (Fig. 3C; white arrow).

Chelipeds subequal~~s~~ (Figs. 2A–C); merus external surface and carpus anterior margin with plumose setae; ~~chelae~~ width and length subequal, ventral margin microscopically granulated (Fig. 4F bold arrow, 7C; dashed arrow), dorsal margin slightly cristate and bent inwards; fingers wider than long, ~~length equal~~, spoon-tipped, tip acute (Fig. 4F), interdactylar gap narrow (vg.

Fig. 7C); movable finger shorter than fixed finger, crossed inward when ~~the pincer is~~ closed, cutting edge sinuous, with three medial teeth (Fig. 4F; bold arrow) and a mid-distal convex projection (Fig. 4F; white arrow); fixed finger cutting edge with nine teeth, faint lamella over the smooth portion of the cutting edge (Fig. 4F; dashed arrow), ventral inner surface with short setae. Walking legs similar, relative length W3>W2>W1>W4, segments short, robust, compressed, dorsal margin cristate, ventral surface with plumose setae; merus dorsal margin on W1, W3, W4 with plumose setae, on W2 ~~without setae~~; dactylus curved, stout, tips acute; W1–W3, dactylus subequal ~~than~~ propodus, ~~of W4~~ shorter than its propodus (Fig. 3A).

Abdomen symmetrical, subtriangular, six free somites plus ~~a~~ telson, margin with short setae, lateral margin from ~~segments~~ 4–6 slightly concave and narrowing, telson subrounded (Figs. 3D). In juvenile males the lateral margins are straight instead of concave, but the gonopods are present.

First gonopod slender, margins sinuous, mid-distal portion notably curved outwards, surface with abundant plumose setae (Fig. 3E). Second gonopod small, ~~flagellum curved outwards,~~ slightly bent inwards, tip pointing upwards, margins convex with ~~a~~ shallow notch (Fig. 3F; black arrow).

Female (Figs. 2D–F; UMAR-DECA-307; CW = 10.50, CL = 7): Same as the male but with less abundant seta in the pterygostomian region and in the ventral surface of the propodus chelae, setae of dorsal surface of ~~the~~ merus walking legs and inner surface of the merus and carpus chelae more abundant and long. Carapace slightly more convex. Abdomen subovate. See "variation" section for more details.

Color in life: Body beige or creamy white, dorsal surface of carapace and chelipeds carpus, and on the external surface of the chelae with red patches. In fixed and preserved specimens this pattern of color remain or it could change from red to light or dark brown (Fig. 2A–F).

Habitat: Marine. Associated with the sea cucumber *Holothuria* (*Halodeima*) *inornata*, living in its coelom and inside its intestine (Fig. 2G). This holothurian inhabits rocky-sand bottoms in shallow waters (0–18 m).

Variation: The ~~revised~~ material showed three general outlines on the carapace shape. Between males, a transversally subrectangular carapace shape was observed in 33% (three specimens from Sinaloa) of the revised material. A subovate shape was observed in 56% (five specimens) of the material and comes from Guerrero and Oaxaca, and a subtrapezoidal shape in 11% (one specimen from Oaxaca). In females, the subrectangular shape was observed in 11% (five specimens from Sinaloa) of the material. The subovate shape in 85% (40 specimens from Guerrero and Oaxaca), and the subtrapezoidal shape in 2% (two specimens from Oaxaca).

The subrectangular shape (Figs. 5A, 6A) is defined by a straight and notably projected margin of the frontal lobes. There is a straight anterior margin in which the hepatic notch in males is notably deeper, eroded, and extended over the carapace (Fig 5A, white arrow). In females it is less conspicuous (Fig. 6A, black arrow). The males have truncated and scarcely projected lateral lobes with the anterior portion concave (Fig. 5A, black arrow), however in the female it is straight. In contrast the subovate shape (Figs. 5E, I, 6D, G) is defined by an entire even margin which is outlined by the slightly oblique and scarcely projected frontal lobes. The convex anterolateral margin continues smoothly to the lateral margin forming a notably convex lobe (Figs. 5E, I, black arrows) in which the hepatic notch in the males is deep, eroded and extended (Fig. 5E, I, white arrow). In females it is shallow, slightly eroded, and less extended over the carapace (Figs. 6D, G, black arrow).

The subtrapezoidal shape is defined by the scarcely projected margin of the frontal lobes, which continues evenly and smoothly to the straight anterolateral margin forming notably projected

lateral lobes (Fig. 2D, 7A). In all the females, the margin of the frontal lobes and the eyes are not visible in dorsal view and only a slight notch can be seen (Figs. 6A, D, G, white arrows), because the frontal-dorsal surface is more convex than in males. However, if the carapace is placed so that the posterior margin line of the carapace cannot be seen, then the general carapace outline looks like that of the males from Guerrero or Oaxaca (v.g. Figs. 5E, I).

In frontal view, the convexity of the frontal-dorsal surface allows a pair of inflated and only drawn lobes on the surface to be seen. The remarkably convex frontal-dorsal surface ~~which~~ obscures the frontal margin and the eyes in dorsal view ~~was observed~~ in 16 specimens (15 females, one male). This shape was more frequent in ovigerous females (10 specimens, 67%) than in non-ovigerous ones (five specimens, 33%). The less convex shape was observed in 39 specimens (31 females, eight males).

Despite the variation in the shape of the carapace in both sexes, in all cases the CW/CL ratio is the same. Additionally, the length measured from the notch of the margin of the frontal lobes to the external orbital angle and the external orbital angle to the posterior angle of the hepatic notch is the same.

Regarding the Mxp3, the ischiomerus external margin appears notably convex on its mid-distal portion or slightly even throughout its length. Its inner margin could have a concave or sinuous mid-distal portion. Nevertheless, the inner margin always has a blunt or slightly acute projection (Figs. 5Ca, Ga, Ka, 6Ca, Fa, Ia; black arrow), but its width/length ratio is constant in all the outlines' variations. The carpus is conical and, due to the drawing's perspective, the main variation in its appearance is the length and the convexity or straightness of the dorsal margin, (Figs. 5Cb, Gb, Kb, 6Cb, Fb, Ib). Regardless of its appearance, a projected ridge on the internal surface has a conspicuous tuft of setae. The propodus also looks variable in its width/length ratio.

385 It has an acute or rounded distal margin due to how the piece is mounted. Despite that, its
386 proximal ventral margin always forms a straight angle where the dactylus is jointed (Figs. 5Cc,
387 Gc, Kc, 6Cc, Fc, Ic). Finally, the dactylus shows two closely related outlines, one subspatulated
388 and the other suboblong. The first one has a more expanded distal portion instead of a narrow
389 shape as in the latter. Nevertheless, its distal margin always overreaches the propodus slightly
390 (Figs. 5Cd, Gd, Kd, 6Cd, Fd, Id).
391 We observed a variation in the ornamentation of the chelae fingers. Between males, the cutting
392 edge of the movable finger has two or three proximal blunt or acute teeth (Figs. 5B, F, J, black
393 arrows). The medial tooth is simple (Figs. 5B, J, white arrow) or bicuspid (Fig. 5F, white arrow),
394 and the subdistal projection is acute (Figs. 5B, F, white dashed arrow) or blunt (Fig. 5J, white
395 dashed arrow). The fixed finger has six to nine blunt (Fig. 5B) or acute (Fig. 5F, J) teeth, and the
396 middle or more conspicuous tooth is always bicuspid (Figs. 5B, F, J, black dashed arrow).
397 Between females, the movable finger shows two to three acute teeth (Fig. 6B, E, H, black arrow).
398 The medial tooth can be acute (Figs, 6B, E, white arrow) or blunt (Fig. 6H, white arrow), and
399 there is a blunt subdistal projection (Figs. 6B, E, H, white dashed arrow). The fixed finger has
400 four to thirteen teeth, with a bicuspid blunt medial tooth (Fig. 6B, E, black dashed arrow) or it is
401 simple, acute (Fig. 6H, black dashed arrow). Only one specimen (DECA-1172) had a different
402 chelae size and a different teething pattern on the cutting edge of the fixed finger (Fig. 6J, K).
403 The first gonopod of the males shows variation in the degree of curvature and in the proportion
404 of the distal section that is curved, as well in the general outline shape of the gonopod tip.
405 However, it may be similar in different stages of development. In this sense, the general
406 appearance in the abdominal view of males from Sinaloa and Oaxaca is more similar because the
407 external and internal margins are sinuous (Figs. 5D, L). The curvature degree is approximately

90° (Fig. 5D) and 75° (Fig. 5L) respectively. The tip of the external margin is truncated (Figs. 5De-f, Le-f; white arrow), and the ventral margin of the tip has a blunt projection (Figs. 5De, Le; black arrow). In males from Guerrero, the external and internal margins are less sinuous and the curvature degree is approximately 65° (Fig. 5H). The tip of the external margin is convex (Fig. 5He-f; white arrow), and the ventral margin of the tip has a pointed projection (Fig. 5He; black arrow).

Also, in sternal view, the ventral process shape of the internal margin tip is variable. In males from Sinaloa, it is obtuse (Fig. 5Df; black arrow), while those from Guerrero had a convex one (Fig. 5Hf; black arrow). Those from Oaxaca had it oblique (Fig. 5Lf; black arrow), but this may also vary between the different sizes of the crabs.

Remarks: The taxonomical history of *Holothuriophilus trapeziformis* was synthetized by Campos, Peláez-Zárate & Solís-Marín (2012) and they highlight the fact that the specimen identified by Bürger (1895) as a male, based on the shape of the abdomen, is actually a female. We observed the same in several young individuals with abdomens showing a similar shape to that of juvenile males, however, the presence of pleopods in all the abdominal somites confirms that they are females. This finding allowed us to present the complete male morphology of *H. trapeziformis*.

All the biological material examined shows phenotypic variation, particularly between the individuals from the type locality in Mazatlan with respect to those from of Guerrero and Oaxaca, but COI gene shows no differences. With our detailed description of the male morphology it is now possible to differentiate Holothuriophilus trapeziformis from H. pacificus with certainty. The carapace can be subrectangular (Fig. 5A, 6A), suboval (Fig. 5E, I, 6D, G) or

subtrapezoidal (Fig. 1A, D, 2A, 7A) in the former, but it is always subcuadrangular in the latter (Fig. 7E).

Holothuriophilus trapeziformis has the Mxp3 dactylus with its distal portion notably expanded, the external distal margin slightly truncated, and the flagellum of the exopod is ~~long and~~ robust (Figs. 7B, J, K, 8A). In contrast, *H. pacificus* has a rounded distal margin and the flagellum of the exopod is ~~long and~~ slender (Figs. 7F, 8D).

The first gonopod of *Holothuriophilus trapeziformis* has a more sinuous lateral margin, with a distal portion larger, curved outwards with abundant setae (Fig. 8C). In *H. pacificus*, it is straight with just the distal portion slightly curved outwards and with less abundant setae (Fig. 8F).

The abdomen of *Holothuriophilus trapeziformis*, in males, is subtriangular with lateral margins narrowing from the fourth to the sixth somite, the third somite has notably convex lateral margins, the sixth somite has notably concave lateral margins, and the telson is subrounded and wider than long (Fig. 8B). In *H. pacificus*, it is triangular, the lateral margins are almost straight, the third and sixth somite lateral margins are concave, and the telson is subtriangular and more extended than wide (Fig. 8E).

In the case of *Holothuriophilus trapeziformis*, adult ovigerous and non-ovigerous females, the abdomen is suboval and broader than long, the first somite has convex lateral margins, the second somite has sinuous distal margins, the third somite has oblique and downward lateral margins, the sixth somite has oblique and outward lateral margins, and the telson has a length to width ratio ca. 0.2 (Fig. 7D). In contrast, *H. pacificus* have it suboval and more extended than wide, the first somite has concave lateral margins, the second somite has almost straight distal margins, the third somite has oblique and upwards lateral margins, the sixth somite has convex lateral margins, and the telson has a length to width ratio ca. 0.3 (Fig. 7H).

Distribution and ecological comments: currently, we increased the previously known distribution range from Punta Tiburón, Sinaloa to Playa Tejón, Oaxaca. We found crabs in the coelom cavity and near the cloaca of the host, as mentioned by Manning (1993), Campos, Peláez-Zárate & Solís-Marín (2012), and Honey-Escandón & Solís-Marín (2018). By the first time, we found the crab within the intestine (Fig. 1G).

Holothuria (*Halodeima*) *inornata* is distributed throughout the Tropical Eastern Pacific from the Gulf of California, Mexico to Ecuador, and in the temperate island Lobos de Afuera, Peru (Prieto-Rios et al. 2014; Honey-Escandón & Solís-Marín 2018). It also represents an important fishery resource throughout its distribution range (Santos-Beltrán & Salazar-Silva 2011). Nevertheless, there are no records for *Holothuriophilus trapeziformis* outside the Pacific coast of Mexico.

DNA Barcodes

From the 56 examined crabs (Table S1), 51 were processed. The number of base pairs was between 549 bp and 648 bp for 37 specimens with a sole Barcode Index Number (BIN; Ratnasingham & Hebert 2013) in the BOLD database: ADE9974. Of those, 35 produced a high quality trace file (chromatogram). The 14 crabs that could not be amplified correspond to old museum material and recent collections that were not fixed according to the Elías-Gutiérrez et al. (2018) protocol. A BLAST query in GenBank confirmed our sequences to belong to a brachyuran lineage.

Maximum likelihood tree and genetic distance analysis

The best nucleotide substitution model according to the AIC and BIC criterion was General Time Reversible under a gamma distribution (GTR+G) model (Nei & Kumar 2000). The Maximum-Likelihood (ML) distance method under the selected model delimited the 37 sequences of

476 *Holothuriophilus trapeziformis* from the dataset (DS-PINMX1HT) in a single cluster. The cluster
477 of *H. trapeziformis* is well separated from *H. pacificus* in the maximum likelihood tree (ML), as
478 shown in figure 9, with a 12 to 14% divergence among all specimens. *Holothuriophilus* is also
479 close to the *Calyptraeotheres* clade, but far from other species (Fig. 9) with an interspecific
480 divergence ranging from 12 to 19%. The intraspecific divergences in *H. trapeziformes* ranged
481 from 0 to 2.2%. This result is congruent with the BOLD distance summary analyses, which show
482 an average distance of 0.73% and a maximum of 2.27% for sequences with more than 500 bp.

483 **Discussion**

484 A major problem for traditional taxonomy, based solely on morphology, is the variability of the
485 phenotype of decapods. In pinnotherid taxonomy, a crucial goal is to provide a complete
486 description of the species with detailed illustrations of common and unusual structures for
487 comparative purposes (Derby & Antema 1980; Ahyong, Komai & Watanabe 2012; Salgado-
488 Barragán 2015). In that regard, characters previously not described like the antenna, the
489 antennule, the Mxp2, and the second male gonopod show no differences between all the
490 examined material despite the variations noted above. These variations are greater when
491 comparing individuals from the northern region (the topotype in Mazatlán, Sinaloa) to those
492 from the southern region (Guerrero and Oaxaca). However, COI data analysis confirmed that our
493 examined specimens correspond to a single species.

494 Phenotype variation is the result of a plastic response to different environmental pressures
495 (Hurtado, Mateos & Santamaria 2010; Rossi & Mantelatto 2013) or due to recent or historical
496 processes that limit the flow of genes because of environmental barriers (Wares, Gaines &
497 Cunningham 2001; Avise 2009). Despite the fact that these processes are well documented, in
498 the case of brachyuran crabs, there is evidence showing that this does not occur in grapsids

(Cassone & Boulding 2006), ocypodids (Laurenzano, Mantelato & Schubart 2013), pinnotherids (Ocampo et al. 2013), sesarmids (Zhou et al. 2015), and varunids (Zhang et al. 2017). However, for pinnotherids, the several long-lasting growth phases require specific or various hosts to complete them (Bousquette 1980; Hamel, Ng & Mercier 1999; Ocampo et al. 2011) and represent a drawback. Nevertheless, it allows them to maintain connectivity between populations throughout their geographical distribution range (Haines, Edmunds & Pewsey 1994; Hamel, Ng & Mercier 1999; Ocampo et al. 2012, 2013; Guilherme, Brustolin & de Bueno 2015; Becker & Türkay 2017).

In the case of *H. trapeziformis*, is considered a specific endobiotic parasite of its host (Nauck 1880; Campos, Peláez-Zárate & Solís-Marín 2012), resulting in possibly more limited connectivity through larval dispersal. In addition to the above, the particular oceanographic conditions known along the Pacific coast of Mexico and the distribution of the host (Hurtado et al. 2007; Paz-García et al. 2012; Prieto-Rios et al. 2014; Gómez-Valdivia, Parés-Sierra & Flores-Morales 2015; Honey-Escandón & Solís-Marín 2018) could explain the morphological differences observed between the northern species concerning those of the south.

Currently, with the complete description of the male, we can conclude that *Holothuriophilus trapeziformis* is different from *H. pacificus* using the different characters described here

Regarding the DNA barcoding approach, the injection of ethanol inside the body of the crabs through the joints of the exoskeleton, and the use of semi-degenerate zooplankton primers (Prosser, Martínez-Arce & Elías-Gutíerrez 2013) instead of Folmer's, we got a success in a difficult group to work with COI gene (Mantellato et al. 2016). We obtained the amplification of 72% of the specimens and 69% sequencing success.

The resulting maximum likelihood tree allowed us to confirm *Holothuriophilus trapeziformis* as a separate species, indicating a divergence from 12 to 14% against the closest taxa, *H. pacificus*. Also, our tree agrees with Palacios-Theil, Cuesta & Felder (2016) regarding the association of the genus *Holothuriophilus* and *Calyptraeotheres*.

We can assert that the taxonomic status of *Holothuriophilus trapeziformis* is now complete, based on the morphology of both sexes, their distribution, specificity of a single host, and according to the DNA barcodes.

We believe that *Holothuriophilus trapeziformis* with its host reflects the restricted habitat in which it lives and possibly the local environmental barriers.

Acknowledgements

We are grateful to the Chetumal Node of the Mexican Barcode of Life (MEXBOLD) for their support for the genetic analysis, in particular Alma Estrella García-Morales who assisted with the DNA process of the biological samples. To J. Rolando Bastida-Zavala for providing access to the collection material of Laboratorio de Sistemática de Invertebrados Marinos (LABSIM) from Universidad del Mar. To Fernando Álvarez-Noguera and José Luis Villalobos-Hiriart for providing access to the collection material of the Colección Nacional de Crustáceos (CNCR) del Instituto de Biología de la Universidad Nacional Autónoma de México. To Virgilio Antonio Pérez and staff from Buceo Huatulco for their support during the field work. To Carmen Caelen for the English language proofreading and editing.

References

Adensamer T. 1897. Revision der Pinnotheriden in der Sammlung des K. K. Naturhistorischen Hofmuseums in Wien. *Annalen des K.K. Naturhistorischen Hofmuseums* 12:105–110.

Ahyong ST, Ng PKL. 2007. The pinnotherid type material of Semper (1880), Nauck (1880) and Bürger (1895) (Crustacea: Decapoda: Brachyura). *Raffles Bulletin of Zoology* Supplement 16:191–226.

Ahyong ST, Komai T, Watanabe T. 2012. First *Viridotheres* Manning, 1996, from Japan, with a key to the species (Decapoda, Brachyura, Pinnotheridae). In: Komatsu H, Okuno J, Fukuoka K, eds. *Studies on Eumalacostraca*: A *Homage to Masatsune Takeda, Crustaceana Monographs* 17:35–47 https://doi.org/10.1163/9789004202894_003

Avise JC. 2009. Phylogeography: retrospect and prospect. *Journal of Biogeography* 36:3–15 https://doi.org/10.1111/j.1365-2699.2008.02032.x

Balss H. 1957. Decapoda. In: Bronns GH, ed. *Klassen und Ordnungen des Tierreichs*. Fünfter Band 5, 1 Abteilung, 7 Buch, 12 Lieferung. pp. 1505–1672.

Becker C, Türkay M. 2017. Host specificity and feeding in European pea crabs (Brachyura, Pinnotheridae). *Crustaceana* 90(7–10):819–844 https://doi.org/10.1163/15685403-00003661

Brandão M, Freire AS, Burton RS. 2016. Estimating diversity of crabs (Decapoda: Brachyura) in a no-take marine protected area of the SW Atlantic coast through DNA barcoding of larva. *Systematics and Biodiversity* 14(3):288–302 https://doi.org/10.1080/14772000.2016.1140245

Bousquette GD. 1980. The larval development of *Pinnixa longipes* (Lockington, 1877) (Brachyura: Pinnotheridae) reared in the laboratory. *Biological Bulletin* 159:592–605 https://doi.org/10.2307/1540825

Bürger O. 1895. Ein Beitrag zur kenntniss der Pinnotherinen. *Zoologische Jahrbücher, Abtheilung für Systematik, Geographie und Biologie der Thiere* 8:361–390.

Campos E, Díaz V, Gamboa-Contreras JA. 1998. Notes on distribution and taxonomy of five poorly known species of pinnotherid crabs from the eastern Pacific (Crustacea: Brachyura: Pinnotheridae). *Proceedings of the Biological Society of Washington* 111:372–381.

Campos E, Peláez-Zárate V, Solís-Marín FA. 2012. Rediscovery, host and systematics of *Holothuriophilus trapeziformis* Nauck, 1880 (Crustacea, Brachyura, Pinnotheridae). *Zootaxa* 3528:57–62 https://doi.org/10.11646/zootaxa.3528.1.4

Caso ME. 1958. Contribución al conocimiento de los holoturoideos de México. III. Algunas especies de holoturoideos litorales de la costa Pacífica de México. *Anales del Instituto de Biología*, *Universidad Nacional Autónoma de México* 28:309–338.

Caso ME. 1964. Contribución al conocimiento de los holoturoideos de México. Descripción de una n. sp. de *Holothuria* de un nuevo subgénero (*Paraholothuria* N. SG.). *Anales del Instituto de Biología*, *Universidad Nacional Autónoma de México* **3**4(1–2):367–380.

Caso ME. 1965. Estudio sobre Equinodermos de México. Contribución al conocimiento de los holoturoideos de Zihuatanejo y de la Isla de Ixtapa (primera parte). *Anales del Instituto de Biología*, *Universidad Nacional Autónoma de México* 36:253–291.

Cassone JB, Boulding GE. 2006. Genetic structure and phylogeography of the lined shore crab, *Pachygrapsus crassipes*, along the northeastern and western Pacific coast. *Marine Biology* 149:213–226 https://doi.org/10.1007/s00227-005-0197-9

Costa FO, De Waard JR, Boutillier J, Ratnasingham S, Dooh RT, Hajibabaei M, Hebert PDN. 2007. Biological identification through DNA barcodes: the case of the Crustacea. *Canadian Journal of Fisheries and Aquatic Science* 64:272–295 https://doi.org/10.1139/f07-008

588 **Darriba D, Taboada GL, Doallo R, Posada D.** 2011. jModelTest 2: more models, new
589 heuristics and parallel computing. *Nature Methods* 9(8):772
590 https://doi.org/10.1038/nmeth.2109
591 **Derby CD, Antema J.** 1980. Induced host odor attraction in the pea crab *Pinnotheres*
592 *maculatus*. *Biological Bulletin* 158:26–33 https://doi.org/10.2307/1540755
593 **De Man JG.** 1887. Uebersicht der Indo-pacifischen Arten der Gattung Sesarma Say, nebst einer
594 Kritik der von W. Hess und E. Nauck in den Jahren 1865 und 1880 beschriebenen
595 Decapoden. *Zoologische Jahrbücher. Abteilung für Systematik, Geographie und Biologie*
596 *der Tier* 2:639–722.
597 **Elías-Gutiérrez M, Valdez-Moreno M, Topan J, Young MR, Cohulo-Colli JA.** 2018.
598 Improved protocols to accelerate the assembly of DNA barcode reference libraries for
599 freshwater zooplankton. *Ecology and Evolution* 8:3002–3018
600 https://doi.org/10.1002/ece3.3742
601 **Garth SJ.** 1957. Reports of the Lund University Chile Expedition 1948–49, 29, The crustacea
602 decapoda brachyura of Chile. *Lunds Universitets Årsskrift*. N.F. Avd. 2. Bd. 53. Nr. **7:**1–
603 134.
604 **Garm A, Watling L.** 2013. The crustacean integument: setae, setules, and other ornamentation.
605 In: Watling, L. & Thiel, M. (Eds.) *Functional morphology and diversity. The natural*
606 *history of crustacean series*, 1. Oxford University Press, Oxford, pp:167–198
607 https://doi.org/10.1093/acprof:osobl/9780195398038.003.0006
608 **Gómez-Valdivia F, Parés-Sierra A, Flores-Morales AL.** 2015. The Mexican Coastal Current:
609 A subsurface seasonal bridge that connects the tropical and subtropical Northeastern
610 Pacific. *Continental Shelf Research* 110:100–107 https://doi.org/10.1016/j.csr.2015.10.010

611 **Guilherme PDB, Brustolin MC, de Bueno ML.** 2015. Distribution of ectosimbiont crabs and
612 their sand dollar host in a subtropical estuarine sandflat. *International Journal of Tropical*
613 *Biology*, *Revista de Biología Tropical* 63(2):209–220.
614 **Haines CMC, Edmunds M, Pewsey AR.** 1994. The pea crab, *Pinnotheres pisum* (Linnaeus
615 1767), and its association with the common mussel, *Mytilus edulis* (Linnaeus, 1758), in the
616 Solent (U.K.). *Journal of Shellfish Research* 13:5–10.
617 **Hajibabaei M, Singer GAC, Hebert PDN, Hickey DA.** 2007. DNA barcoding: how it
618 complements taxonomy, molecular phylogenetics and population genetics. *TRENDS in*
619 *Genetics* 23(4):167–172 https://doi.org/10.1016/j.tig.2007.02.001
620 **Hajibabaei M, De Waard JR, Ivanova NV, Ratnasingham S, Dooh RT, Kirk SL, Mackie**
621 **PM, Hebert PDN.** 2005. Critical factors for assembling a high volume of DNA barcodes.
622 *Philosophical Transactions of The Royal Society of London B Biological Science*
623 360:1959–1967 https://doi.org/10.1098/rstb.2005.1727
624 **Hall TA.** 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis
625 program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95–98.
626 **Hamel JF, Ng PKL, Mercier A.** 1999. Life cycle of the pea crab *Pinnotheres halingi* sp. nov.,
627 and obligate symbiont of the sea cucumber *Holothuria scabra* Jaeger. *Ophelia* 50(3):149–
628 175 https://doi.org/10.1080/00785326.1999.10409393
629 **Hebert PND, Cywinska A, Ball SL, De Waard JR.** 2003. Biological identification through
630 DNA Barcodes. *Proceedings of the Royal Society of London. Series B, Biological Science*
631 270 (1512):313–321 https://doi.org/10.1098/rspb.2002.2218
632 **Honey-Escandón M, Solís-Marín FA.** 2018. A revision of *Holoturia* (*Halodeima*) *kefersteinii*
633 (Selenka, 1867) and the revival of *Holothuria inornata* Semper, 1868 from sea cucumbers

collected in Mexico and Central America. *Zootaxa* 4377(2):151–77 https://doi.org/10.11646/zootaxa.4377.2.1

Hurtado LA, Mateos M, Santamaria CA. 2010. Phylogeography of supralitoral rocky intertidal *Ligia* isopods in the Pacific region from Central California to Central Mexico. *PLoS ONE* 5(7):e11633 https://doi.org/10.1371/journal.pone.0011633

Hurtado LA, Frey M, Guebe P, Pfeiler E, Markow TA. 2007. Geographical subdivision, demographic history and gene flow in two sympatric species of intertidal snails, *Nerita scabricosta* and *Nerita funiculate*, from the tropical eastern Pacific. *Marine Biology* 151:1863–1873 https://doi.org/10.1007/s00227-007-0620-5

Ivanova NV, De Waard JR, Hebert, PDN. 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes* 6:998-1002 https://doi.org/10.1111/j.1471-8286.2006.01428.x

Laurenzano C, Mantelatto FLM, Schubart CD. 2013. South American homogeneity versus Caribbean heterogeneity: populations genetic structure of the western Atlantic fiddler crab *Uca rapax* (Brachyura, Ocypodidae). *Journal of Experimental Marine Biology and Ecology* 449:22–27 https://doi.org/10.1016/j.jembe.2013.08.007

Lefébure T, Douady CJ, Gouy M, Gibert J. 2006. Relationship between morphological and molecular divergence within Crustacea: Proposal of a molecular threshold to help species delimitation. *Molecular Phylogenetics and Evolution* 40(2006):435–447 https://doi.org/10.1016/j.ympev.2006.03.014

Manning RB. 1993. Three genera remove from the synonymy of *Pinnotheres* Bosc, 1802 (Brachyura: Pinnotheridae). *Proceedings of the Biological Society of Washington* 106(3): 523–531.

Matzen da Silva J, Creer S, dos Santos A, Costa AC, Cunha MR, Costa FO, Carvalho GR. 2011. Systematic and evolutionary insights derived from mtDNA COI barcode Diversity in the Decapoda (Crustacea: Malacostraca). *PloS ONE* 6(5):1–15, e19449 https://doi.org/10.1371/journal.pone.0019449

Nauck E. 1880. Das Kaugerüst der Brachyuren. *Zeitschrift für wissenschaftliche Zoologie* (*Leipzig*) 34:1–69.

Nei M, Kumar S. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York

Ng PKL, Manning RB. 2003. On two new genera of pea crabs parasitic in holothurians (Crustacea: Decapoda: Brachyura: Pinnotheridae) from the Indo-West Pacific, with notes on allied genera. *Proceedings of the Biological Society of Washington* 116:901–919.

Nobili G. 1901. Decapodi raccolri dal Dr. Filippo Silvestri nell' America meridionale. *Bollettino del Musei di Zoologia ed Anatomia comparata della R. Univerith di Torino* 16(402):1–16.

Ocampo EH, Nuñez JD, Lizarralde MS, Cledón M. 2011. Larval development of *Calyptraeotheres garthi* (Fenucci, 1975) (Brachyura, Pinnotheridae) described from laboratory-reared material, with notes of larval character use on Pinnotheridae systematics. *Helgoland Marine Research* 65:347–359 https://doi.org/10.1007/s10152-010-0228-x

Ocampo EH, Nuñez JD, Cledón M, Baeza JA. 2012. Host-specific reproductive benefits, host selection behaviour and host use pattern of the pinnotherid crab *Calyptraeotheres granthi*. *Journal of Experimental Marine Biology and Ecology* 429: 36–46 https://doi.org/10.1016/j.jembe.2012.06.009

Ocampo EH, Robles R, Terossi M, Nuñez JD, Cledón M, Mantelato FL. 2013. Phylogeny, phylogeography, and systematics of the American pea crab genus *Calyptraeotheres*,

680 Campos, 1990, inferred from molecular markers. *Zoological Journal of the Linnean*
681 *Society* 169:27–42 https://doi.org/10.1111/zoj.12045
682 **Palacios-Theil E, Cuesta JA, Felder DL.** 2016. Molecular evidence for non-monophyly of the
683 pinnotheroid crabs (Crustacea: Brachyura: Pinnotheroidea), warranting taxonomic
684 reappraisal. *Invertebrate Systematics* 30:1–27 https://doi.org/10.1071/IS15023
685 **Paz-García DA, Chávez-Romo HE, Correa-Sandoval F, Reyes-Bonilla H, López-Pérez A,**
686 **Medina-Rosas P, Hernández-Cortés MP.** 2012. Genetic connectivity patterns of corals
687 *Pocillopora damicornis* and *Porites panamensis* (Anthozoa: Scleractinia) along the West
688 Coast of Mexico. *Pacific Science* 66(1): 43–61 https://doi.org/10.2984/66.1.3
689 **Perez-Miguel M, Drake P, García-Raso EJ, Mamán-Menéndez J, Navas IJ, Cuesta AJ.**
690 2019. European Pinnotheridae (Crustacea, Decapoda, Brachyura): species, distribution,
691 host use and DNA barcode. *Marine Biodiversity* 49:57–68 https://doi.org/10.1007/s12526-
692 017-0754-8
693 **Poeppig E.** 1836. Crustacea Chilensia nova aut minus nota descripsit. *Archiv Für*
694 *Naturgeschichte* 2(1):133–145.
695 **Posada D, Buckley TR.** 2004. Model selection and model averaging in phylogenetics:
696 advantages of Akaike information criterion and Bayesian approaches over Likelihood
697 Ratio Test. *Systematic Biology* 53(5):739–808
698 https://doi.org/10.1080/10635150490522304
699 **Prieto-Rios, E, Solís-Marín FA, Borrero-Pérez GH, Díaz-Jaimes P.** 2014. Filogeografía de
700 Holothuria (*Halodeima*) *inornata* Semper, 1868 (Echinodermata: Holothuroidea). *Revista*
701 *Peruana de Biología* 21(2):155–162 https://doi.org/10.15381/rpb.v21i2.9818

Prosser S, Martínez-Arce A, Elías-Gutiérrez M. 2013. A new set of primers for COI amplification from freshwater microcrustaceans. *Molecular Ecology Resources* 13:1151–1155 https://doi.org/10.1111/1755-0998.12132

Rathbun MJ. 1904. Description of three new species of American crabs. *Proceedings of the Biological Society of Washington* 4(17):161–162.

Rathbun MJ. 1918. The grapsoid crabs of America. *Bulletin of the United States National Museum* 97:1–461 https://doi.org/10.5479/si.03629236.97.i

Ratnasingham S, Hebert PDN. 2013. A DNA-based registry for all animal species: The Barcode Index Number (BIN) system. *PLoS ONE* 8(7):e66213 https://doi.org/10.1371/journal.pone.0066213

Rossi N, Mantelatto FL. 2013. Molecular analysis of the freshwater prawn *Macrobrachium olfersii* (Decapoda, Palaemonidae) supports the existence of a single species throughout its distribution. *PLoS ONE* 8(1):e54698 https://doi.org/10.1371/journal.pone.0054698

Salgado-Barragán J. 2015. A new species of *Pinnixa* (Crustacea: Brachyura: Pinnotheridae) from Mazatlan, Sinaloa, Mexico. *Revista Mexicana de Biodiversidad* 86(3):629–636 http://dx.doi.org/10.1016/j.rmb.2015.03.001

Santos-Beltrán C, Salazar-Silva P. 2011. Holothuroideos (Echinodermata: holoturoidea) de playas rocosas, zona norte de Bahía Banderas, Nayarit, México. *Ciencia y Mar* 15(45):3–11.

Schmitt WL, McCain JC, Davidson ES. 1973. Fam. Pinnotheridae, Brachyura I: Decapoda I. In: Gruner HE, Holthuis LB, eds. *Crustaceorum Catalogus* 3:1–160.

Solís-Marín FA, Arriaga-Ochoa JA, Laguarda-Figueras A, Frontana-Uribe SC, Durán-González A. 2009. *Holoturoideos (Echinodermata: Holothuroidea) del Golfo de California.* CONABIO-UNAM-ICMyL, México, 177 pp.

Spielmann G, Diedrich J, Haszprunar G, Busch U, Huber I. 2019. Comparison of three DNA marker regions for identification of food relevant crustaceans of the order Decapoda. *European Food Research and Technology*, 245:987-995 https://doi.org/10.1007/s00217-018-3199-9

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30:2725–2729 https://doi.org/10.1093/molbev/mst197

Wares JP, Gaines SD, Cunningham, CW. 2001. A comparative study of asymmetric migration events across a marine biogeographic boundary. *Evolution* 55:295–306 https://doi.org/10.1111/j.0014-3820.2001.tb01294.x

Will WK, Rubinoff D. 2004. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics* 20:47–55 https://doi.org/10.1111/j.1096-0031.2003.00008.x

Zhang C, Li Q, Wu X, Liu Q, Cheng Y. 2017. Genetic diversity and genetic structure of farmed and wild Chinese mitten crab (*Eriocheir sinensis*) populations from three major basins by mitochondrial DNA COI and Cyt b gene sequences. *Mitochondrial DNA PART A* 2–9 https://doi.org/10.1080/24701394.2017.1404048

Zhou H, Xu J, Yang M, Wu B, Yan B, Xiong Y. 2015. Population genetic diversity of sesarmid crab (*Perisesarma bidens*) in China based on mitochondrial DNA. *Mitochondrial DNA, Early Online* 1–8 https://doi.org/10.3109/19401736.2015.1015002

Figure 1

Sampling sites

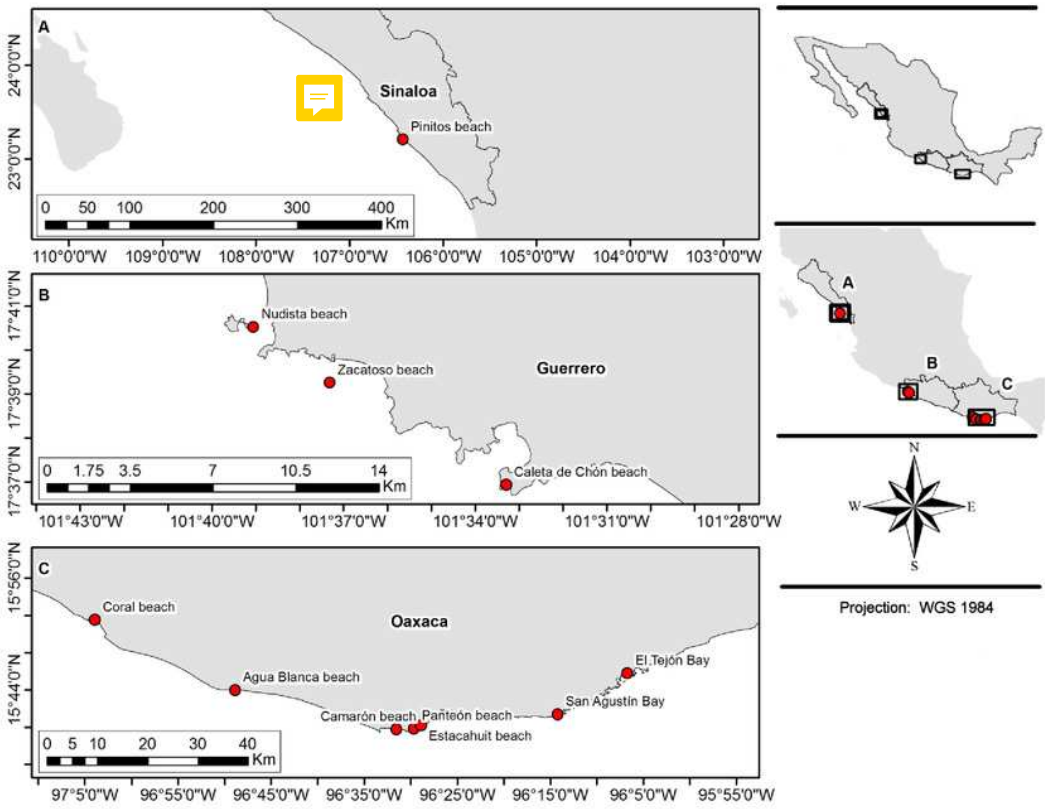

A
Sinaloa
Pinitos beach
0 50 100 200 300 400 Km
24°0'0"N
23°0'0"N
110°0'0"W 109°0'0"W 108°0'0"W 107°0'0"W 106°0'0"W 105°0'0"W 104°0'0"W 103°0'0"W
B
Nudista beach
Zacatoso beach
Guerrero
Caleta de Chón beach
0 1.75 3.5 7 10.5 14 Km
17°41'0"N
17°39'0"N
17°37'0"N
101°43'0"W 101°40'0"W 101°37'0"W 101°34'0"W 101°31'0"W 101°28'0"W
C
Coral beach
Oaxaca
El Tejón Bay
Agua Blanca beach
San Agustín Bay
Camarón beach
Panteón beach
Estacahuit beach
0 5 10 20 30 40 Km
15°56'0"N
15°44'0"N
97°5'0"W 96°55'0"W 96°45'0"W 96°35'0"W 96°25'0"W 96°15'0"W 96°5'0"W 95°55'0"W
A
B
C
N
W
E
S
Projection: WGS 1984

Figure 2

Holothuriophilus trapeziformis Nauck, 1880

A–C, male from Panteon 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.

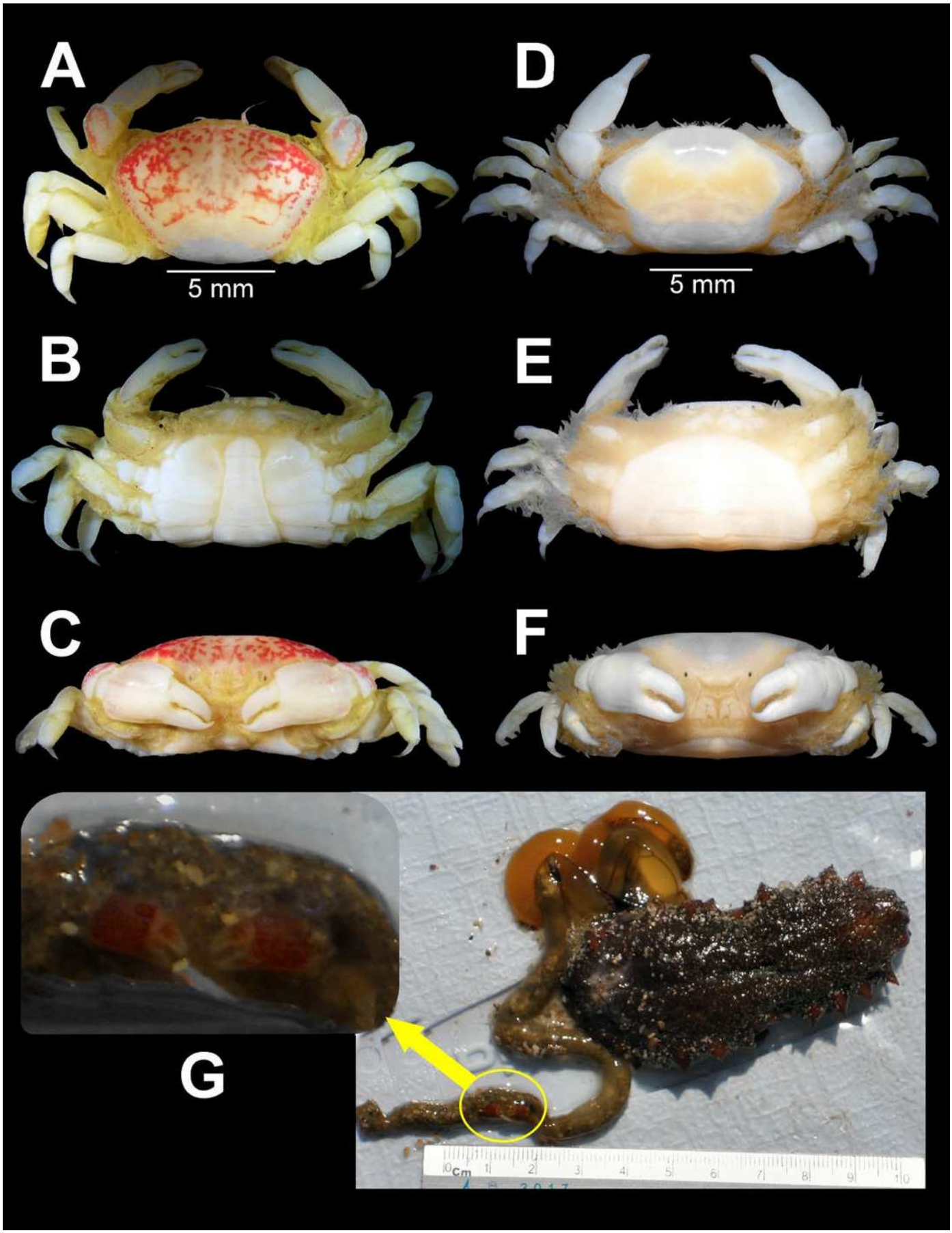

A
5 mm
D
5 mm
B
E
C
F
G

Figure 3

Holothuriophilus trapeziformis Nauck, 1880

A–D, male from Panteon beach, Oaxaca, Mexico (UMAR-DECA-308): A, dorsal view; B, frontal view; C, third-fourth sternal plate; D, abdomen; E, abdominal view of the left first gonopod; F, abdominal view of the left second gonopod; A, C, hollow circles indicating pits. Fine dots indicating pilosity. A–D, half of the illustration without ornamentation.

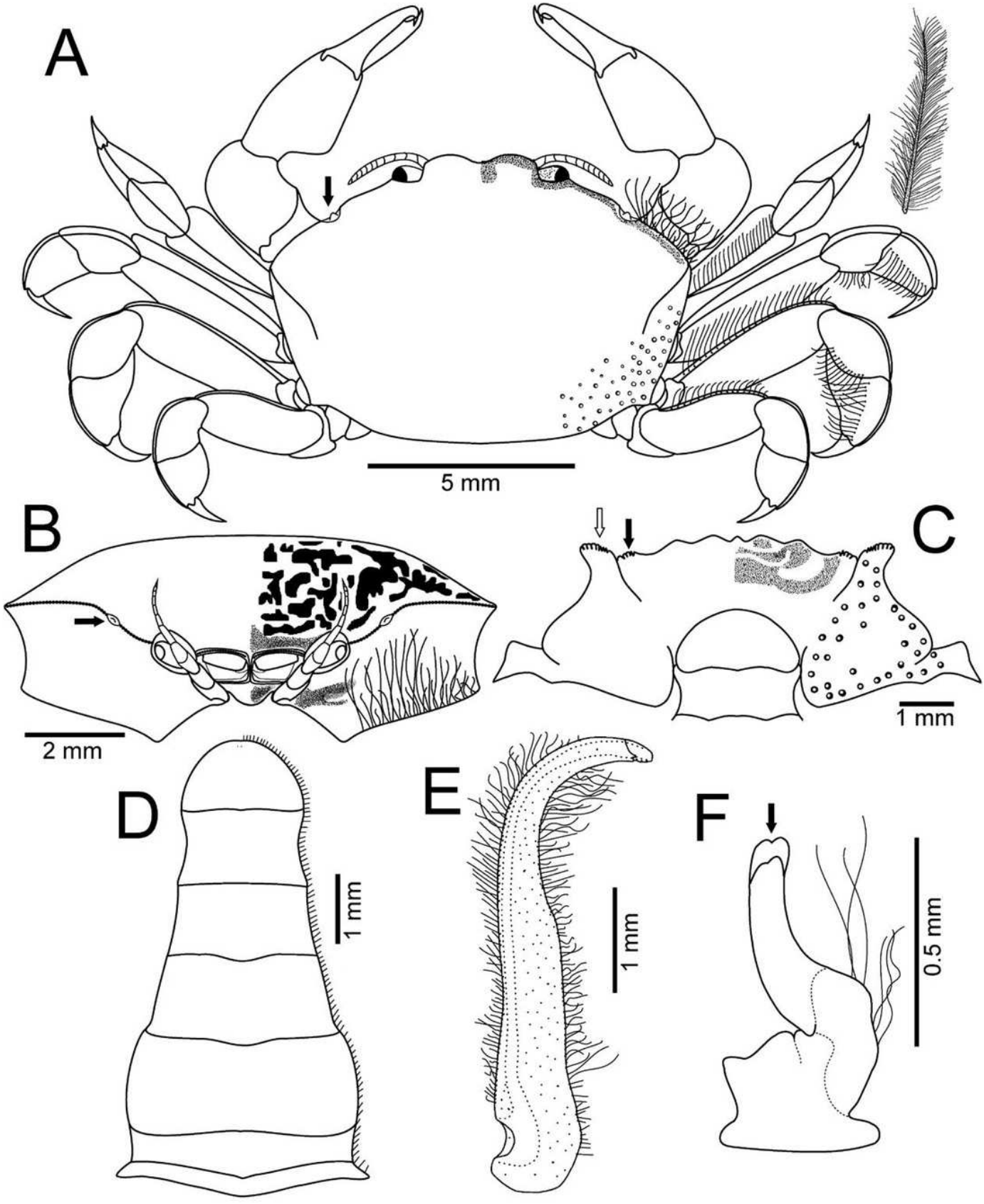

A
5 mm
B
2 mm
C
1 mm
D
1 mm
E
1 mm
F
0.5 mm

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; D, third maxilliped: a, ischiomerus; b, carpus; c, propodus; d, dactylus; bold arrow indicating a projection. E, exopod of the third maxilliped. F, chela; bold arrow indicating mid-posterior teeth; dotted arrow, indicating the lamella; clear arrow, medio-distal projection; bold arrow in the inferior part, indicating granules.

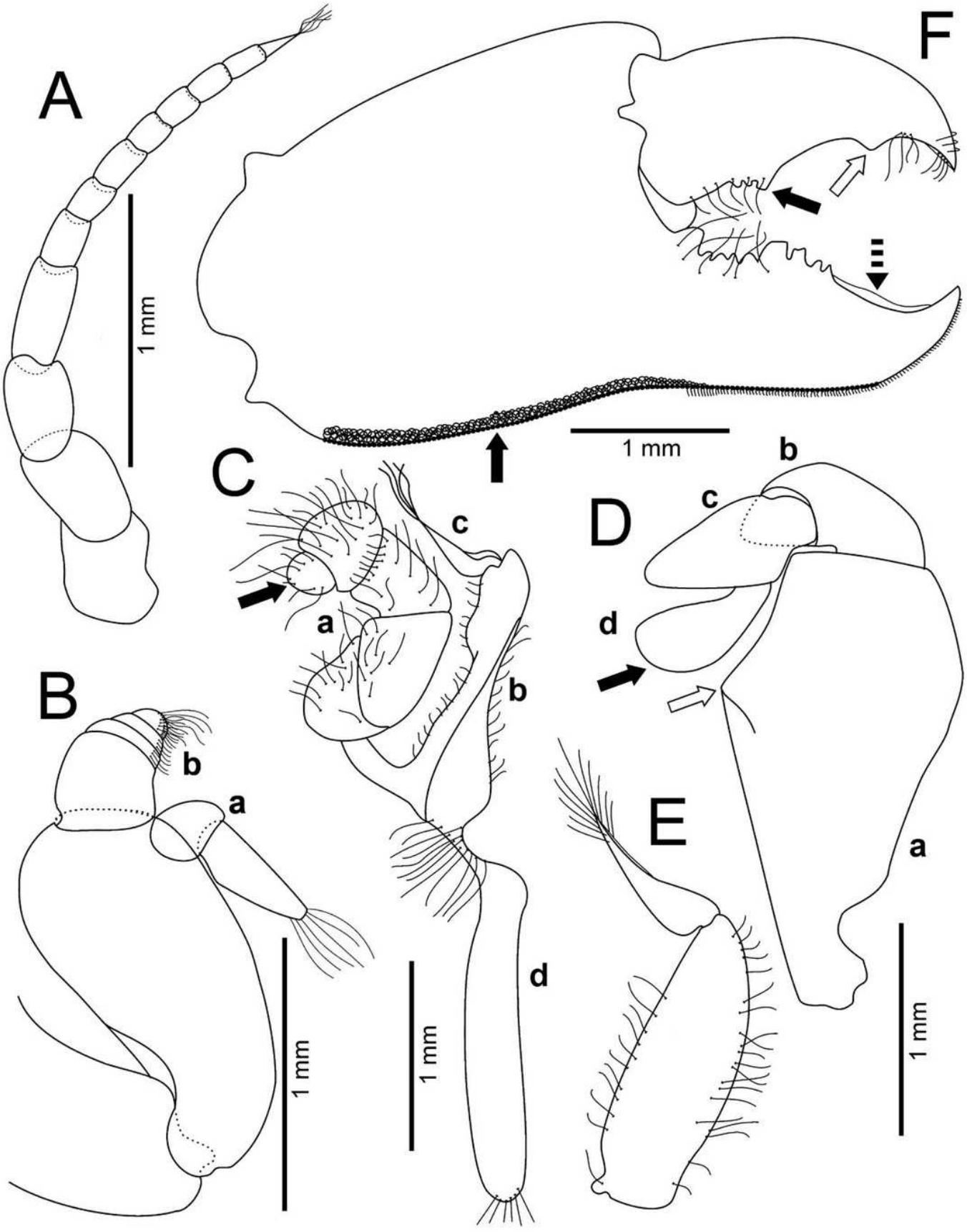

A
1 mm
F
1 mm
C
c
a
b
d
1 mm
D
b
c
d
a
1 mm
B
b
a
1 mm
E

Figure 5

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

A 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; B, F, J, right chela, external view; C, G, K, left Mxp3 endopod, external view; D, H, L, first gonopod, abdominal view; e, gonopod tip, abdominal view; f, gonopod tip, sternal view. Descriptions are in the main text.

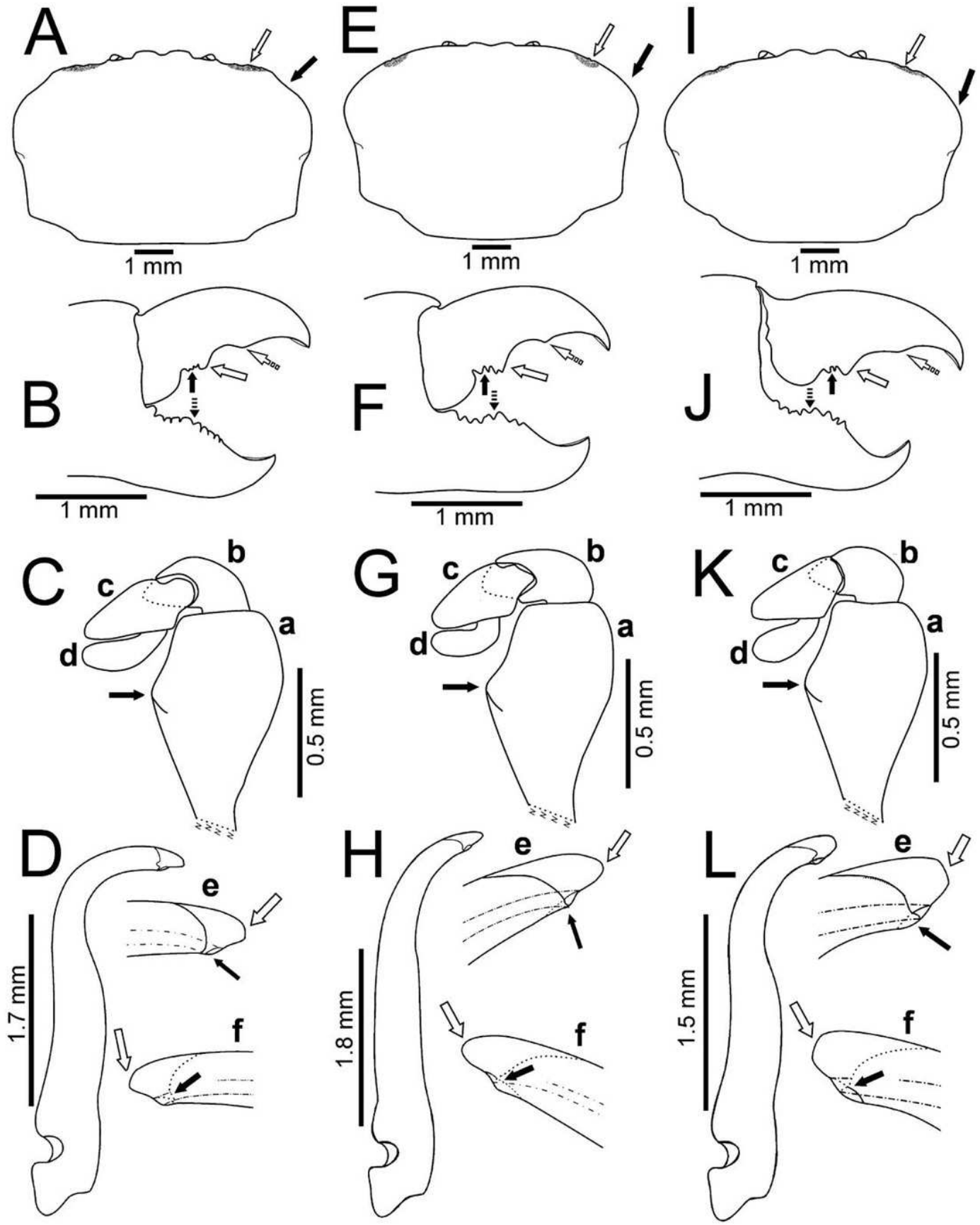

A
E
I
1 mm
1 mm
1 mm
B
F
J
1 mm
1 mm
1 mm
C
G
K
b
c
a
d
0.5 mm
0.5 mm
0.5 mm
D
H
L
e
f
1.7 mm
1.8 mm
1.5 mm

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; B, E, H, right chela, external view; C, F, I, left Mxp3 endopod, external view. Descriptions are in the main text.

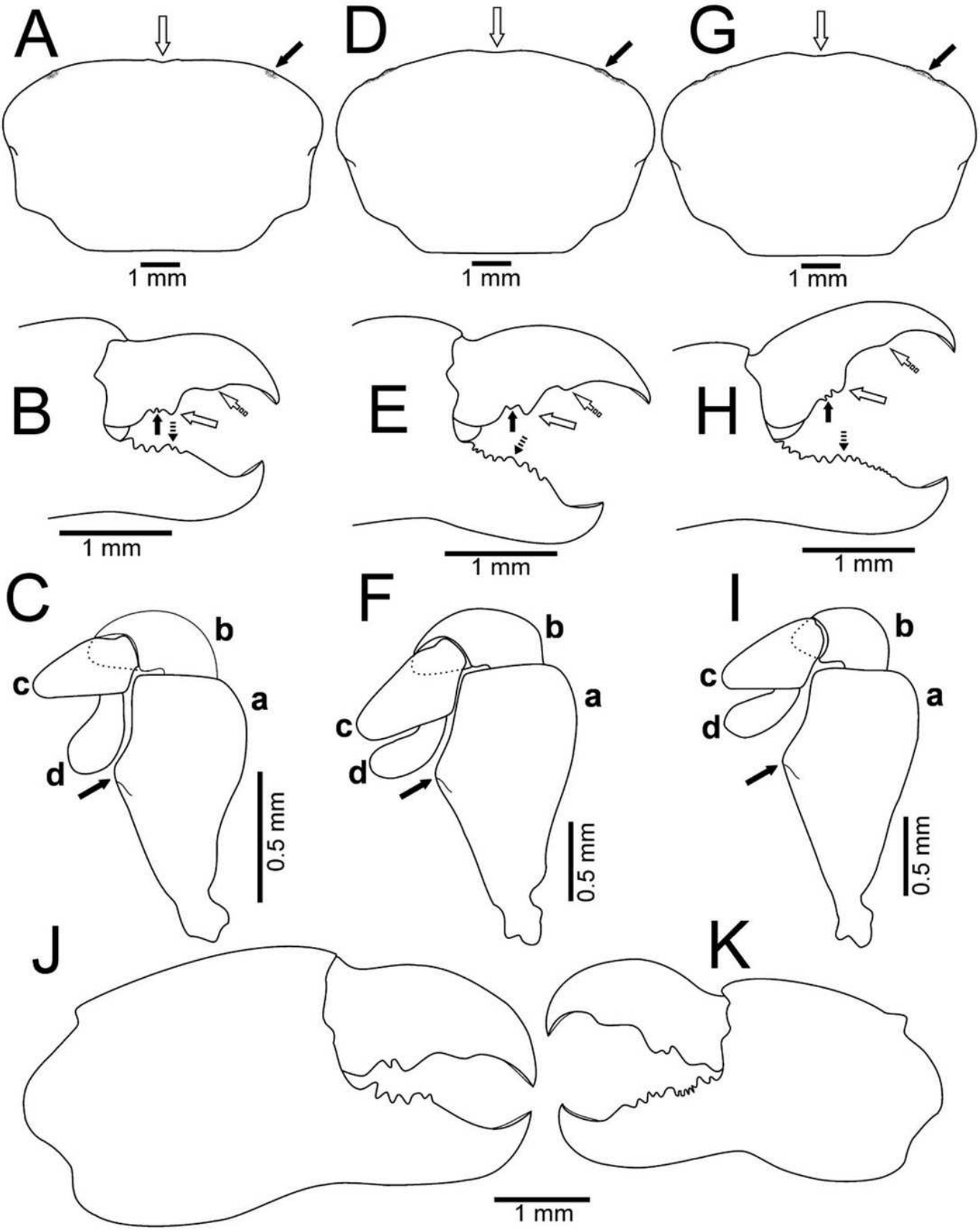

A
D
G
1 mm
1 mm
1 mm
B
E
H
1 mm
1 mm
1 mm
C
F
I
b
c
a
d
0.5 mm
J
K
1 mm

Figure 7

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

A–D, *H. trapeziformis* from Camaron beach, Oaxaca, Mexico (UMAR-DECA-1163): A, carapace; B, third maxilliped; C, chela; D, ovigerous abdomen. E–H, *H. pacificus* holotype of from San Vicente, Chile (Taken from Garth 1957): E, carapace; F, third maxilliped; G, chela; H, abdomen. I-J, lectotype of *H. trapeziformis* from Mazatlan, Mexico (Taken from Ahyong & Ng 2007): I, dorsal view of carapace; J, third maxilliped. K, *H. trapeziformis* from Guerrero, third maxilliped of the adult female (Taken from Campos, Peláez-Zárate, Solís-Marín 2012). Scale of E= x3.5, F= x18.6, G= x4.6, H= x2.9 (*fide* Garth 1957).

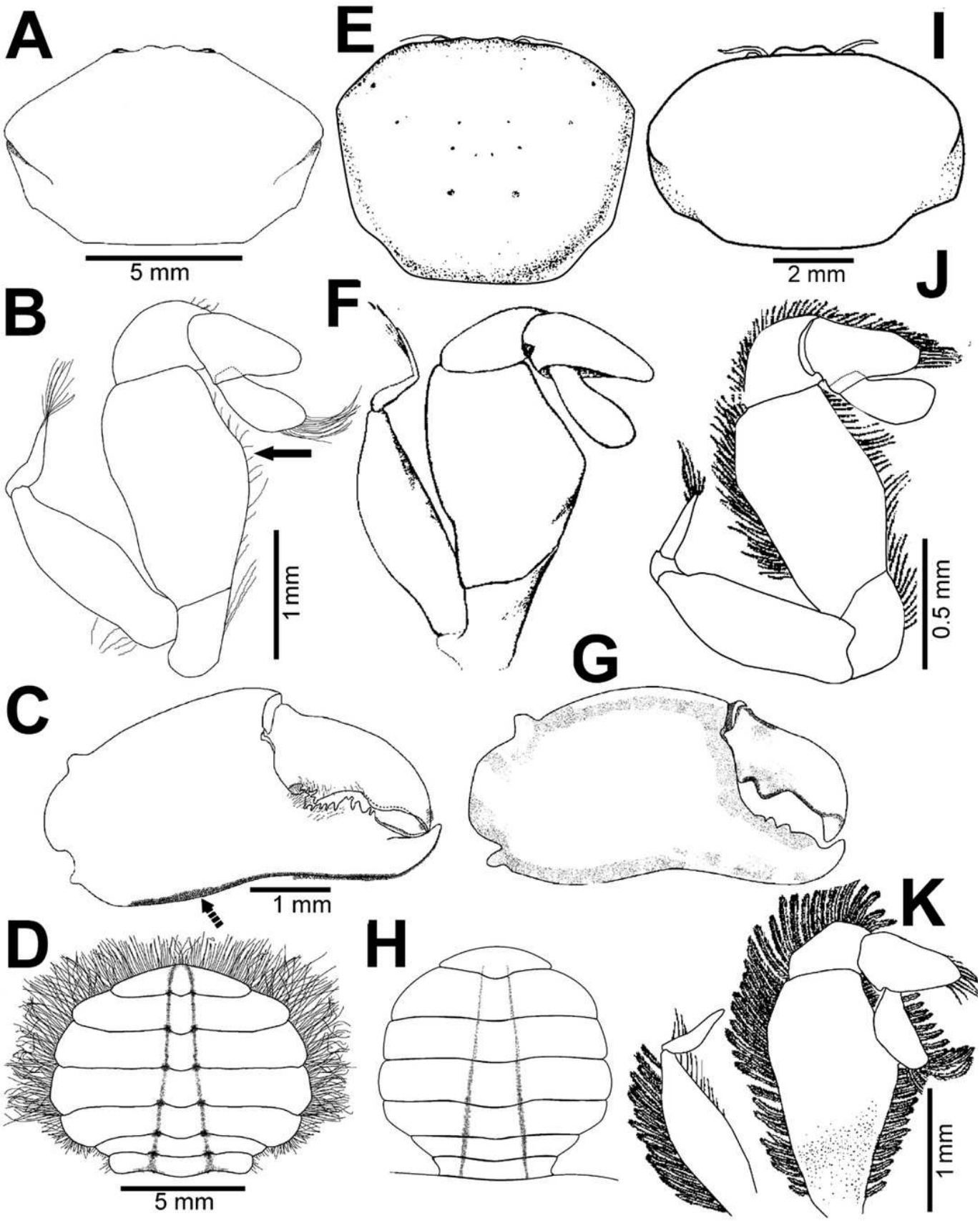

A
5 mm
E
I
2 mm
B
1mm
F
J
0.5 mm
C
1 mm
G
D
5 mm
H
K
1mm

Figure 8

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

A, D, third xilliped; a, dactylus; b, propodus; c, exopod flagellum. B, F, abdomen. C, F, first gonopod. A–C, from Panteon beach, Oaxaca, Mexico; C, abdominal view of the gonopod, Mexico (UMAR-DECA-308). D–F, from Talcahuano, Chile (Taken from Garth 1957).

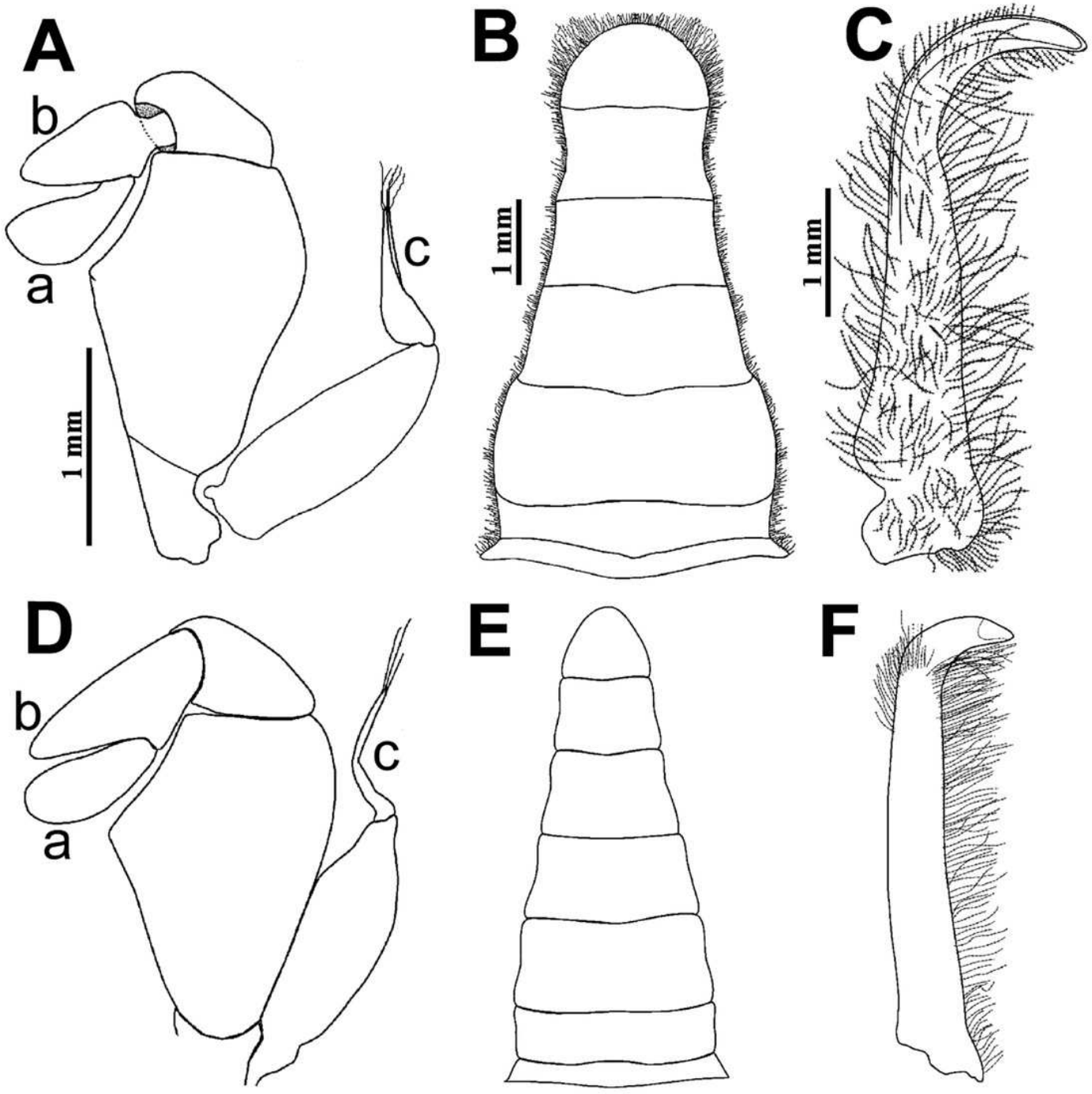
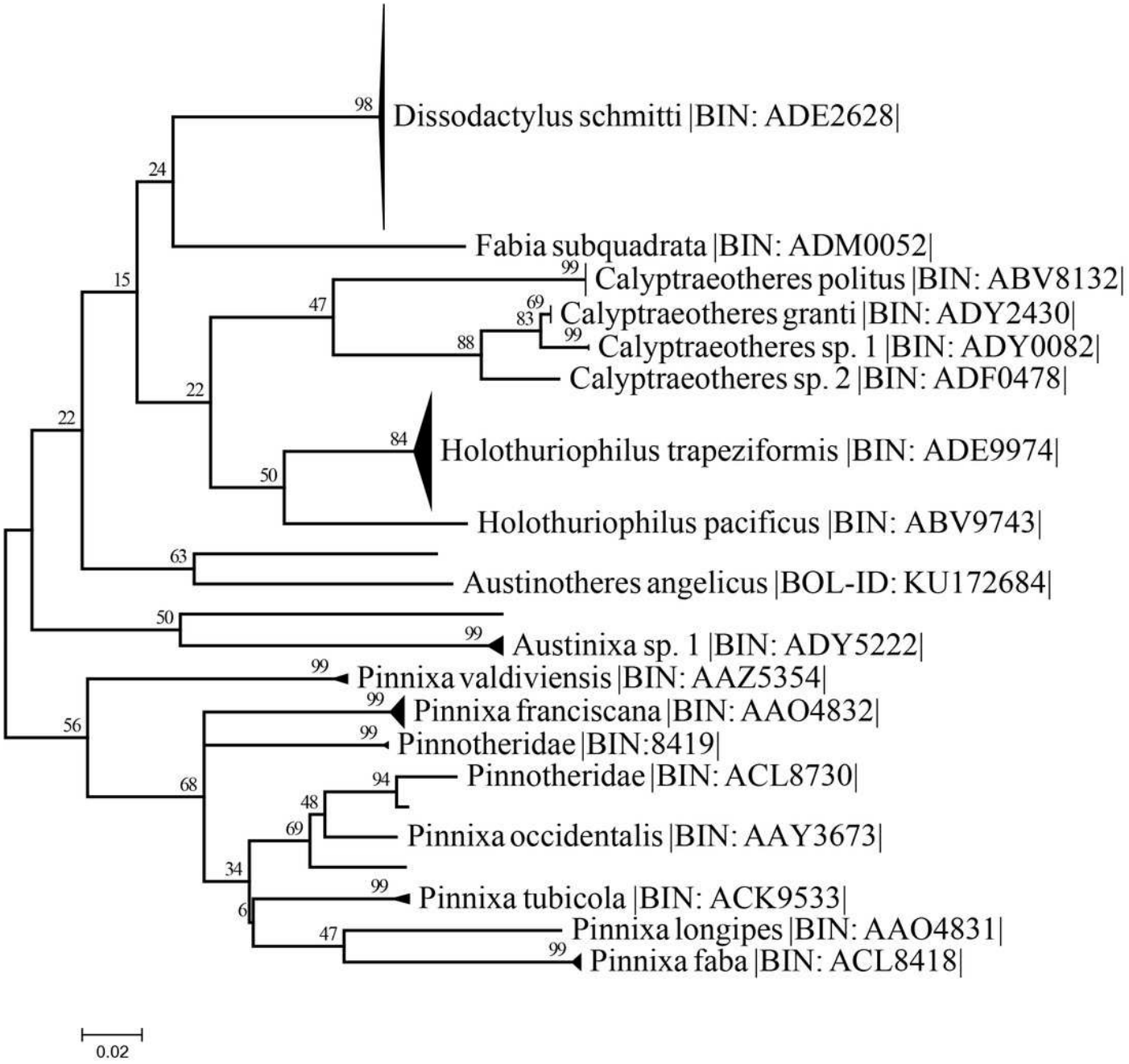

A
b
a
c
1 mm
B
1 mm
C
1 mm
D
b
a
c
E
F

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).


98
Dissodactylus schmitti |BIN: ADE2628|
24
Fabia subquadrata |BIN: ADM0052|
99
Calyptraeotheres politus |BIN: ABV8132|
15
47
69
83
Calyptraeotheres granti |BIN: ADY2430|
99
88
Calyptraeotheres sp. 1 |BIN: ADY0082|
Calyptraeotheres sp. 2 |BIN: ADF0478|
22
22
84
Holothuriophilus trapeziformis |BIN: ADE9974|
50
Holothuriophilus pacificus |BIN: ABV9743|
63
Austinotheres angelicus |BOL-ID: KU172684|
50
99
Austinixa sp. 1 |BIN: ADY5222|
99
Pinnixa valdiviensis |BIN: AAZ5354|
99
Pinnixa franciscana |BIN: AAO4832|
56
99
Pinnotheridae |BIN:8419|
68
94
Pinnotheridae |BIN: ACL8730|
48
69
Pinnixa occidentalis |BIN: AAY3673|
34
99
Pinnixa tubicola |BIN: ACK9533|
6
47
Pinnixa longipes |BIN: AAO4831|
99
Pinnixa faba |BIN: ACL8418|
0.02