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# TWO NEW CALANOID COPEPODS FROM THE GALAPAGOS ISLANDS: *PSEUDOCYCLOPS JUANIBALI* N. SP. AND *PSEUDOCYCLOPS SAENZI* N. SP.

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ABSTRACT

Two new calanoid copepods, *Pseudocyclops juanibali* n. sp. and *Pseudocyclops saenzi* n. sp. are described from anchialine pools in the Galapagos Islands. *Pseudocyclops juanibali* n. sp. is similar to *P. australis*, *P. mathewsoni*, *P. simplex, P. pacificus*, and *P. latisetosus*. A deep cleft on the distal margin of the endopod of the left leg five of males separates these six species from all other *Pseudocyclops* likely forming a species group. *Pseudocyclops juanibali* differs from other members of this group in the shape and number of elements on the distal exopodal segment of the left leg five of males. *Pseudocyclops saenzi* n. sp. is most similar to *P. rubrocinctus* and *P. steinitzi* but differs in the presence of a posterior seta on the basis of the leg five of females, a sclerotized seta on the exopod of the maxillule, and several differences in the shape and ornamentation of the leg five of males. A 569-base-pair region of the internal transcribed spacer 1 ribosomal DNA region (ITS-1) was amplified from specimens of *P. juanibali* and *P. saenzi*, and from specimens of *Pseudocyclops* that were morphologically identical to *P. juanibali* but from two different anchialine pools. The phylogenetic analysis of the ITS-1 region shows that *P. juanibali* and *P. saenzi* are genetically different from each other and, furthermore, that the specimens from the two other anchialine pools are genetically isolated from the former species, a finding that suggests cryptic speciation. The morphological and genetic evidence presented here, including confirmation of a close sibling species of *P. juanibali* from the Ryukyu Islands of Japan, demonstrate that vicariance and active migration are responsible for the observed distribution of species, with faunal exchange occurring between the Galapagos and the Caribbean and Western Pacific Oceans. However, although these copepods are able to cross the entire Pacific, such long-range migration is not the norm. They tend to have restricted distributions with minimal migration and gene exchange, even between habitats that are very close to each other such as the anchialine pools in the Galapagos.

KEY WORDS: anchialine pools, Copepoda, cryptic species, Galapagos Islands, *Pseudocyclops*

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## INTRODUCTION

Anchialine pools are dispersed throughout the Galapagos Archipelago. These are inland bodies of salt water with subterranean connections to the sea (Holthuis, 1973). They take the form of open pools along tectonic fault lines, pools in caves, and submerged cave passages; they exhibit tidal influence, and often the seawater is mixed with freshwater from the groundwater system or from rain resulting in a mixohaline environment (Sket, 1996; Iliffe, 2000; Santos, 2006). Studies of anchialine habitats in the Caribbean, Mediterranean, and Western Pacific have yielded many new species of invertebrates, including copepods. The only reported location of anchialine habitats in the eastern Pacific is the Galapagos Islands (Iliffe, 1991). As in other regions, these pools contain numerous undescribed species of specialized cave dwellers, including copepods.

Anchialine organisms exhibit disjunct but widespread distributions. Four models have been proposed to explain this pattern: 1) vicariance, 2) regression, 3) deep-sea origin, and 4) active migration (Iliffe, 2000; Kano and Kase, 2004). The vicariance model suggests that plate movements separated and spread the various anchialine faunal elements, emphasizing those that exhibit a Tethyan track. The assumption is that while the Tethys Sea was open and circumglobal, ancestors of this anchialine fauna were found throughout this warm sea. As the modern continents formed, the climate changed and the Tethyan Sea closed. Some of the Tethyan fauna found refuge in anchialine environments where they diversified and underwent speciation. This explanation for the biogeography of these animals is favored by many workers: Iliffe et al. (1984); Stock (1993, 1994); Jaume and Boxshall (1996); Boxshall and Jaume (2000); Danielopol et al. (2000); Humphreys (2000).

The regression model stipulates that anchialine fauna descended from shallow-water, epibenthic species that became stranded due to tectonic uplift and/or successive marine regressions and transgressions, leading to dispersal, isolation, and speciation (Stock, 1980; Holsinger, 1988; Suarez-Morales and Iliffe, 2007).

The deep-sea model suggests that the deep-sea and anchialine systems provide similar environments (dark and climatically stable with limited food resources) that are linked by crevices and fissures; the latter facilitate colonization from the depths up into newly formed anchialine settings (Hart et al., 1985; Manning et al., 1986; Boxshall, 1989; Iliffe, 1990).

Finally, the active migration model attempts to explain current anchialine faunal distributions by the colonization

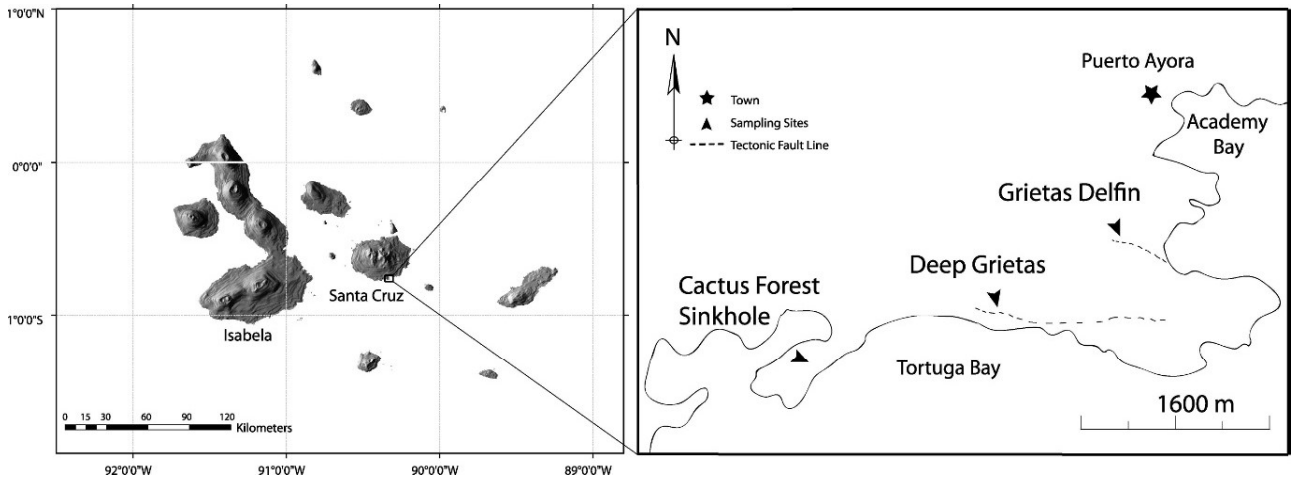


Fig. 1. The Galapagos Islands with expanded view of the location of the 3 anchialine pools sampled in the Island of Santa Cruz.

of new habitats through dispersal by ocean currents (Rouch and Danielopol, 1987; Iliffe, 2000; Kano and Kase, 2004).

Given the various studies supporting each of these models as cited above, it is obvious that the biogeography of the anchialine fauna cannot be fully explained by just one of them. The models are not mutually exclusive, and it is likely that each mechanism has contributed to the formation of the modern anchialine fauna.

Herein two new species of calanoid copepods belonging to Pseudocyclopidae are described from anchialine pools in the Galapagos. This family consists of demersal calanoid copepods found worldwide in temperate, subtropical, and tropical waters (Boxshall and Halsey, 2004). The ribosomal DNA region known as ITS1 is used in a phylogenetic analysis of specimens of these two species of *Pseudocyclops* from different anchialine pools. This region has been used successfully as a marker for phylogenetic and population analyses in crustaceans (Chu et al., 2001), including copepods (Schizas et al., 1999; Rocha-Olivares et al., 2001; Elvers et al., 2006; Ki et al., 2009; Figueroa, 2011). This is a non-coding region acting as a spacer between ribosomal genes. Because it is not transcribed, it lacks selective constraints, evolving freely in a neutral model (Marinucci et al., 1999). The morphological and genetic analysis presented gives insight into the colonization and speciation of *Pseudocyclops* in the Galapagos anchialine system.

## Materials and Methods

### Field Sampling

Copepods were collected from 20 anchialine pools on three different islands in the Galapagos Archipelago, Ecuador. Three of these pools contained undescribed species of *Pseudocylops* (Fig. 1). The first site, locally known as Grietas Delfin (00°45.426′S 90°18.932′W) is on the island of Santa Cruz, near the town of Puerto Ayora. It consists of two pools, open to the surface, about 300 m inland from the shore, located on a tectonic fault line running along an east-west transect. The site is described in detail by Iliffe (1991) and by Figueroa and Hoefel (2008). Among the copepods inhabiting this pool are several harpacticoid and cyclopoid species; the calanoids include a ridgewayiid, *Ridgewayia delfine* Figueroa and Hoefel, 2008, an epacteriscid, *Enantiosis galapagensis* Fosshagen, Boxshall, and Iliffe, 2001, the near-shore Galapagos endemic acartiid, *Acartia levequei* Grice, 1964, and the two new species of *Pseudocyclops* that are described in this paper. Samples from Grietas Delfin were taken on 30 January, 6 February, and 16 February 2005, by using two simple nets, one with 333 µm mesh and a mouth opening of 30 cm and one with 102 µm mesh and a mouth opening of 60 cm. These nets were towed at various depths from the surface to near the bottom (about 20 m depth) by swimming with snorkeling equipment and by pulling a line from shore.

Two other anchialine pools, one a sinkhole in the midst of a giant cactus forest (Cactus Forest Sinkhole, 00°45.884′S 90°20.430′W), and the other a pool in a deep crevice along a tectonic fault line (Deep Grietas, 00°45.735′S 90°19.742′W), contained specimens of *Pseudocyclops*, morphologically similar to the larger of the two species of *Pseudocyclops* found in Grietas Delfin. These pools were sampled using the same methods as in Grietas Delfin, on 25 March, 14 April, 19 April, 4 May, 10 May, 8 July, and 29 July 2005. The samples from all three sites were immediately split and preserved after collection, one half being placed in 10% buffered formalin solution and the other half in 97% ethanol.

### Morphological Analysis

Several males and females of each of the two new species were dissected and all limbs were mounted flat on a slide with glycerol. The morphology of each limb was then observed and photographed with the aid of a compound microscope at magnifications ranging from ×100 to ×1000. Drawings of each limb for species descriptions were prepared with the aid of a Wacom Intuos™ 3 graphic pen and tablet and Adobe™ CS3 software.

### Molecular Analysis

Adult male and female copepods preserved in ethanol were re-hydrated in Milli-Q water and DNA extraction was accomplished by standard proteinase-K digestion, using Qiagen's® DNAeasy kit. Polymerase chain reaction (PCR) primers F1665-18S (5′CCGTCGCTACTACCGATT-GAACG 3′) and R73-5.8S (5′GTGTCGATGTTCATGTGTCCTGC 3′) (Ryuji J. Machida personal communication) were used to obtain the ITS1 region of ribosomal DNA. PCR was carried out in a 50 µl reaction with the following reagents: 3 µl of Accuprime® *Taq* polymerase, 10 µl of purified DNA, 2.5 µl of each primer, 5 µl of Accuprime® Buffer II (includes dNTPs and $MgCl_2$), and 28 µl of water. Thermocyler conditions were: 3 minutes at 94°; followed by 30 cycles of 1 minute at 94°, 1 minute at 55°, and 1.5 minutes at 68°; followed by 5 minutes at 68° and then cooling to 4°. PCR product was loaded on a gel for electrophoresis; the gel was then checked under UV light for contamination and consistency of product. The PCR product was cleaned using the standard Montage® PCR product cleaning kit. Both the forward and reverse strands were sequenced at the Center for Genome Research and Biocomputing at Oregon State University.

A 569-base-pair region of ITS-1 was amplified from 6 specimens of *P. juanibali* and 4 specimens of *P. saenzi* from their type localities, 2 specimens each of *Pseudocyclops* from Cactus Forest sinkhole and Deep Grietas, and 1 specimen of *E. galapagensis* from the closely related

Epacteriscidae; which was used as an out-group (Genbank JF933915, JF933916, JF933917, JF933918, JF933919, JF933920, JF933921, JF933922, JF933923, JF933924, JF933925, JF933926, JF933927 and JF933928).

The chromatogram for each sequence was visually inspected and the forward and reverse sequences for each specimen were used to generate a consensus sequence. Sequences were aligned using ClustalW 1.4 and visually inspected. Phylogenetic analyses were performed with PAUP*4.0 (ver. 4.0b10; Swofford, 2009) using neighbor-joining (NJ), maximum-parsimony (MP), and maximum-likelihood (ML) methods. The model of molecular evolution was selected by the Akaike Information Criterion as implemented in ModelTest 3.7 (Posada and Crandall, 1998). Bootstrap re-sampling with random sequence additions and tree bisection-reconnection (TBR) branch swapping were used to assess nodal support of phylogenetic tree branches. A Bayesian analysis was also performed with MrBayes 3.1 (Huelsenbeck and Ronquist, 2001). MrModeltest 2.2 (Nylander, 2004) was used to select the best-fit model of evolution. Details of the implemented analysis are given in the Genetic Analysis section.

A transitional model with gamma distribution (TIM + G) was selected by Modeltest 3.7 (Posada and Crandall, 1998) as the best-fit model of molecular evolution based on the Akaike Information Criterion (AIC). This model was applied in PAUP*4.0 (ver. 4.0b10; Swofford, 2009) to reconstruct phylogenies with the following settings: MP- an heuristic search with 10,000 replicates, branch swapping with tree-bisection-reconnection, and bootstrap values from 10,000 replicates; ML- an heuristic search with 100 replicates, branch swapping with tree-bisection-reconnection, and bootstrap values from 100 replicates; NJ(HKY85 distance measure)- an heuristic search with 10,000 replicates, branch swapping with tree-bisection-reconnection, and bootstrap values from 10,000 replicates. A Bayesian analysis was also performed with MrBayes 3.1 (Huelsenbeck and Ronquist, 2001). Mrmodeltest 2.2 (Nylander, 2004) was used to select the best-fit model of evolution based on AIC scores, resulting in the Hasegawa-Kishino-Yano-85 model with invariable sites (HKY + I). The analysis was carried out with 1,000,000 generations, sampling every 100th generation. As suggested by Nylander (2004), the initial 25% (2500) of the sampled generations were omitted from the analysis.

## Systematics

Subclass Copepoda H. Milne Edwards, 1830
Order Calanoida G. O. Sars, 1901
Pseudocyclopidae Giesbrecht, 1893
*Pseudocyclops* Brady, 1872
***Pseudocyclops juanibali***, n. sp.
(Figs. 2-4)

Material Collected.—Some 25 specimens collected on 30 January 2005; 32 specimens collected on 6 February 2005, and 28 specimens collected on 16 February 2005, all from Grietas Delfin near the town of Puerto Ayora, on the Island of Santa Cruz, Galapagos, Ecuador (00°45.426′S 90°18.932′W). 20 ♀ and 20 ♂ were used for analysis, including dissection and measurements. Specimens other than those designated as types were kept in the author's personal collection.

Body Length.—Female. Total length: range = 0.69-0.74 mm (mean ± standard deviation = 0.72 ± 0.02 mm, $n = 20$); prosome length = 0.53-0.55 mm (0.54 ± 0.02 mm, $n = 20$); urosome length = 0.17-0.19 mm (0.18 ± 0.01 mm, $n = 20$). Male. Total length = 0.64-0.68 mm (0.66 ± 0.02 mm, $n = 20$); prosome length = 0.48-0.50 mm (0.49 ± 0.02 mm, $n = 20$); urosome length = 0.16-0.18 mm (0.17 ± 0.02 mm, $n = 20$).

Types.—Deposited in the Smithsonian Institution's National Museum of Natural History, Washington. Holotype: adult female (all limbs dissected), USNM 1144208; allotype: adult male (antennule and fifth leg dissected), USNM 1144209; paratypes 5 adult females, USNM 1144210, and 5 adult males, USNM 1144211. All collected on 16 February 2005 (00°45.426′S 90°18.932′W).

Description.—Female (holotype). Body (Figs. 2A, B) stout, transparent with several purple striations on anterior cephalosome. Prosome 6-segmented, cephalosome clearly separate from first pedigerous somite. Posteriolateral angles of prosome rounded and extending one third of the way along genital double somite. Large eye present in anterior section of cephalosome, red/orange-pigmented. Rostrum a simple strong process, produced ventrally. Urosome (Fig. 2C) 4-segmented, first 2 segments each with row of spines along posterior margin. Annal segment telescoped into preceding somite with thick row of very fine setae on dorsal surface. Genital double somite (Fig. 2C) symmetrical, with paired gonopores. Caudal rami (Fig. 2C) symmetrical bearing 6 setae each: 1 small, cylindrical seta on outer distal corner, followed medially by 2 plumose setae, 1 large, barbed seta, 1 plumose seta, and 1 small, naked distal seta on dorsal inner margin.

Antennules (Fig. 2D) symmetrical and 18-segmented, barely reaching first pedigerous somite. Armature of segments as follows: 1-10 (setae) +3ae (aesthetascs), 2-4, 3-4, 4-2, 5-2, 6-2, 7-2, 8-2, 9-2, 10-2, 11-2, 12-2, 13-2, 14-2, 15-2, 16-4, 17-2, 18-6 + 1ae.

Antenna (Fig. 3A) with coxa and basis partly fused. Coxa unarmed; basis with single distal seta. Endopod 3-segmented, first segment with 1 inner seta and patch of denticles on distal dorsal surface; second segment with 5 setae along inner margin and 4 distal setae, outer margin with denticles; third segment with 6 terminal setae, outer margin with denticles. Exopod indistinctly 6-segmented, first 3 segments partly fused; segments 1-3 each with single inner seta; segment 4 with 1 + 2 setae; segment 5 and terminal segment partly fused with 2 + 3 setae.

Mandible (Fig. 3B) with gnathobase bearing several short, stout teeth and with a thickened subapical ridge giving the appearance of an articulation. Basis of palp with 2 setae on inner margin. Endopod 2-segmented, first segment with 4 distal setae; second segment with 11 terminal setae. Exopod 4-segmented with setal formula 1, 1, 1, 3.

Maxillule (Fig. 3C) with precoxal endite bearing 7 ventral setae, 1 anterior seta and 4 posterior setae; coxal exite with 4 setae, coxal endite with 3 setae; basal exite with 1 seta, proximal and distal endites with 3 setae each; exopod with 3 + 7 setae, endopod unsegmented with 4 + 4 mid-ventral setae and 6 terminal setae.

Maxilla (Fig. 3D) with precoxal endite bearing 5 setae, coxal endite with 3 setae; proximal and distal basal endites with 3 setae each. Endopod 3-segmented; first segment with 6 setae on ventral lobe; second segment with 2 inner and 1 outer setae; distal segment with 5 setae.

Maxilliped (Fig. 3E) with 3 syncoxal endites bearing 1, 2, and 3 setae respectively; coxal endite bearing 3 setae. Basis with proximal endite bearing 3 setae, distal endite bearing 2. Endopod indistinctly 5-segmented with seta formula: 2, 2, 2, 3 + 1, 4.

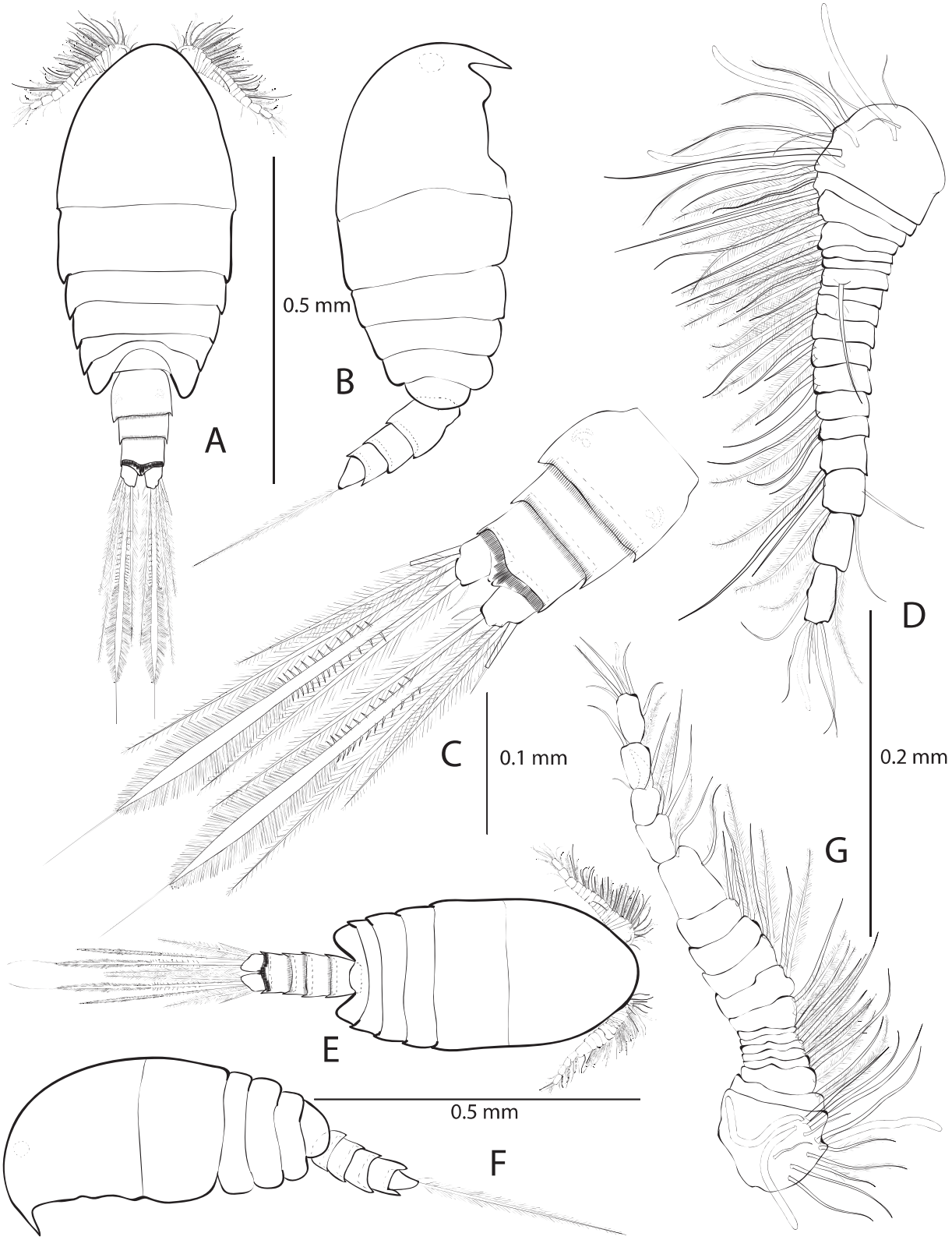


Fig. 2. *Pseudocyclops juanibali* n. sp. A, female, habitus, lateral; B, female, habitus, dorsal; C, female, urosome, ventral; D, female, antennule; E, male, habitus, lateral; F, male, habitus, dorsal; G, male, right antennule.

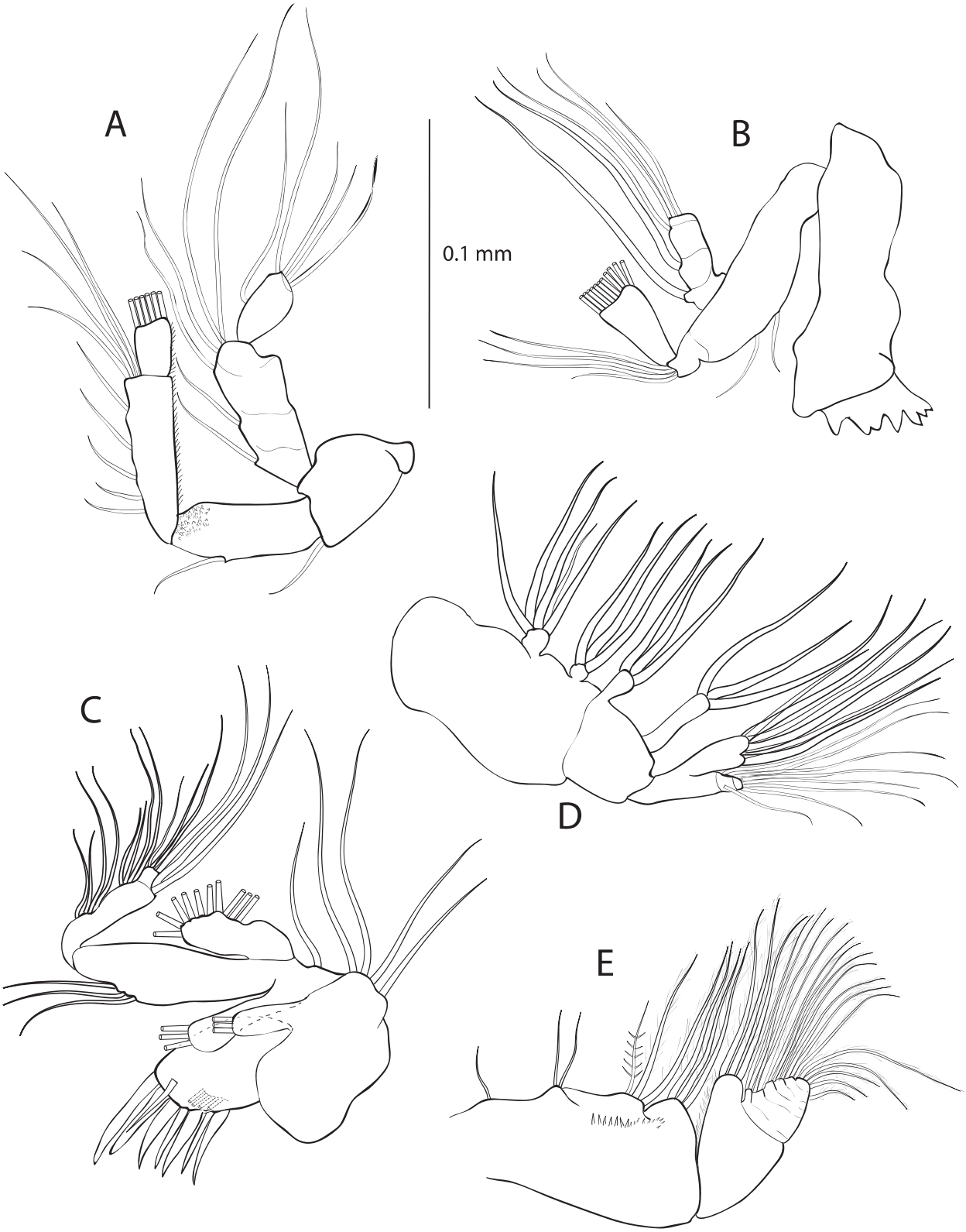


Fig. 3. *Pseudocyclops juanibali* n. sp., female mouthparts. A, antenna; B, mandible; C, maxillule; D, maxilla; E, maxilliped.

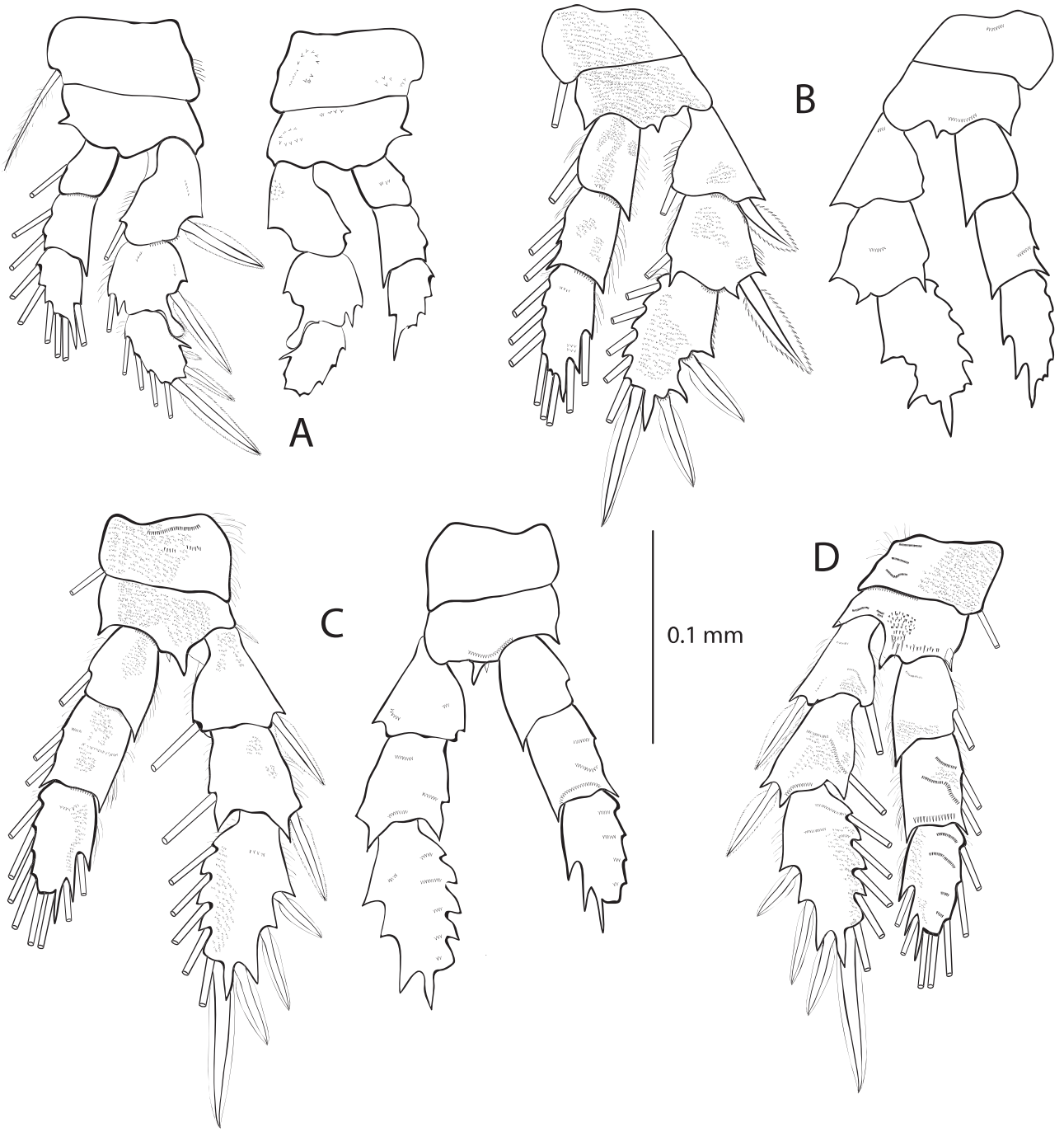


Fig. 4. *Pseudocyclops juanibali* n. sp., female legs 1-4 all posterior, with anterior surface spinulation shown to the right for legs 1-3. A, leg 1; B, leg 2; C, leg 3; D, leg 4.

Legs 1-5 (Figs. 4A-E and 5A) with 3-segmented rami. Seta and spine formulae given in Table 1. Inner coxal seta present on legs 1-4, absent on leg 5. Leg 1 with patches of denticles on inner margins of all 3 exopodal segments. Second exopodal segment with lamellate process on distal outer margin, between outer spine and insertion point of third segment. Legs 1-5 with strong spinulation on posterior surfaces. Legs 2-4 bearing row of spinules on anterior distal margins of exopodal and endopodal segments 1 and 2. Row of denticles on inner margin of each exopodal segment. All endopodal segments with rows of denticles on inner and outer margins. Basis of leg 3 bearing small outer spine. Basis of leg 4 with minute lateral seta. Leg 5 with inner distal margin of basis produced into strong spinular process. Coxa and basis lacking setae.

Male (allotype). Body (Figs. 2E, F) as in female, but slightly smaller. Urosome 5-segmented. Caudal rami symmetrical, bearing 6 setae each: 1 small naked seta on

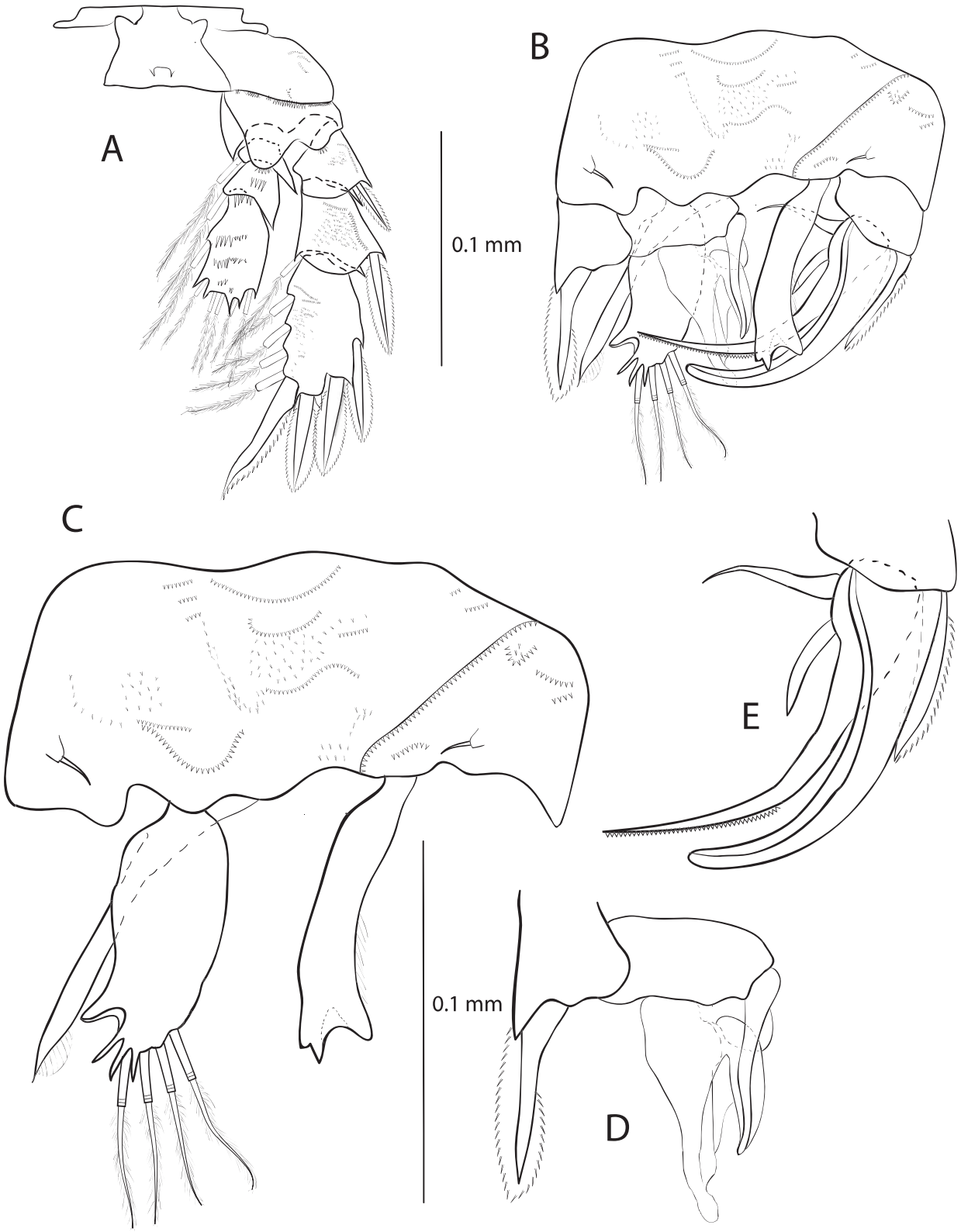


Fig. 5. *Pseudocyclops juanibali* n. sp., leg 5. A, female leg 5, posterior; B, male leg 5, posterior; C, male leg 5 coxa, basis, and endopods, posterior; D, male left leg 5 exopod, posterior; E, male right leg 5 exopod, posterior.

Table 1. Setae and spine formulae for legs 1-5 of female *Pseudocyclops juanibali*.

| | | | Exopod | | | Endopod | | |
|---|---|---|---|---|---|---|---|---|
| | Coxa | Basis | 1 | 2 | 3 | 1 | 2 | 3 |
| Leg 1 | 0-1 | 0-0 | I-1 | I-1 | II, I, 4 | 0-1 | 0-2 | 1, 2, 3 |
| Leg 2 | 0-1 | 0-0 | I-1 | I-1 | II, I, 5 | 0-1 | 0-2 | 2, 2, 4 |
| Leg 3 | 0-1 | I-0 | I-1 | I-1 | III, I, 5 | 0-1 | 0-2 | 2, 2, 4 |
| Leg 4 | 0-1 | 0-0 | I-1 | I-1 | III, I, 5 | 0-1 | 0-2 | 2, 2, 3 |
| Leg 5 | 0-0 | 0-0 | I-0 | I-1 | III, I, 4 | 0-1 | 0-1 | 2, 2, 2 |

outer distal corner, followed medially by 1 longer plumose seta, 2 barbed median setae (the second more robust but not as large as in female than the first), 1 long plumose seta, and 1 small naked seta on distal inner margin.

Left antennule as in female. Right antennule (Fig. 2G) 18-segmented, geniculate between segments 14-15. Armature of segments as follows: 1-11 + 3ae, 2-2, 3-2, 4-2, 5-2, 6-2, 7-2, 8-2, 9-2, 10-2, 11-2, 12-2, 13-2, 14-2, 15-4, 16-3, 17-2, 18-6 + 1ae. Distal margin of segment 16 projecting as a spinular process.

Mouthparts and legs 1-4 as in female. Leg 5 (Figs. 5B-E) biramous, asymmetrical, and strongly modified. Left Coxa fused with basis, right coxa separate from basis. Small setae present on posterior surface, one near outer margin on left basis, and one located medially of right basis. Right leg with 2-segmented exopod. First segment with lateral spine on outer margin and thick, elongate medial seta. Second segment elongate, with thick base and curved, spine-like distally serrated attenuation with 2 thick setae on proximal inner margin. Right endopod in form of elongate, lamellar, distally forked lobe with row of denticles along distal outer margin. Left basis with long, slender process originating on anterior surface near inner margin. Left leg with 2-segmented exopod. First segment bearing outer spine, with serrated margin. Second segment bearing 3 membranous processes, first 2 broad and leaf like, with marginal folds; third elongate and slender. Left endopod an unsegmented lobe, with 4 tooth-like attenuations on distal end bearing 4 setae and with pronounced cleft on distal inner margin.

Remarks.—*Pseudocyclops juanibali* is most similar to *Pseudocyclops australis* Nicholls, 1944, found in Australia and Japan. Nicholls (1944) described *P. australis* from a female and an immature copepodid from Southern Australia. Later, Tanaka (1967) assigned male and female specimens of *Pseudocyclops* from Kyushu, Japan, to *P. australis*. It is difficult to ascertain if indeed Nicholls' *P. australis* and Tanaka's specimens are conspecific due to the lack of detail in Nicholls' description of this species. Nevertheless, there are several key characters that are shared by *P. australis*, Tanaka's specimens and *P. juanibali*. They all have 18-segmented antennules, an outer spine on the basis of leg 3 (Nicholl's did not describe this limb but it is present on Tanaka's specimens), an inner seta on exopodal segments 1 and 2 of leg 5 in females, 4 setae and a distal cleft on the endopod and similar complex structure of the exopod of left leg 5 in males. *Pseudocyclops juanibali* differs from *P. australis* and Tanaka's specimens in having 6 setae on the terminal endopodal segment of leg 5 in females, 4 present in *P. australis* and Tanaka's specimens, in the fusion pattern of the coxal and basal segments of leg 5 in males, *P. juanibali* having all 3 segments fused with only the right basis separate, but *P. australis* having only the 2 left segments fused, leaving the right coxa and basis separate. The female genital double-somite is ornamented with spines in *P. juanibali*, but naked in *P. australis*. *Pseudocyclops juanibali* has a mandible with a 4-segmented exopod; that of *P. australis* has a 5-segmented exopod. Finally, although both species have a complex terminal exopodal segment on leg 5 in males, the details of this segment are different. Instead of having 1 long and slender and 2 broad, leaf-like processes, *P. australis* has 3 and 1 such processes, respectively.

Several male and female specimens of *Pseudocyclops* from the Cactus Forest sinkhole and Deep Grietas were dissected and examined. These specimens seemed to belong to *P. juanibali* inasmuch as they are morphologically identical to specimens from the type locality except for some minor differences in details of leg 5 in males observed in one specimen from Deep Grietas. This particular male lacked the characteristic distal cleft on the endopod of the left leg 5; a fifth seta is present instead. Further sampling is needed at these two sites to collect more specimens of these *Pseudocyclops*.

Etymology.—The species name is derived from a partial abbreviation in honor of Ingeniero Juan Aníbal Figueroa Bastidas, professor at the Universidad Central del Ecuador. He was a mentor to many generations, always leading by example.

### ***Pseudocyclops saenzi***, n. sp.
(Figs. 6-9)

Material Collected.—Some 18 specimens collected on 30 January 2005; 21 specimens collected on 6 February 2005, and 16 specimens collected on 16 February 2005, all from Grietas Delfin near the town of Puerto Ayora, on the Island of Santa Cruz, Galapagos, Ecuador (00°45.426′S 90°18.932′W). 20 ♀ and 20 ♂ were used for analysis, including dissection and measurements. Specimens other than those designated as types were kept in the author's personal collection.

Body Length.—Female. Total length: range = 0.59-0.63 mm (mean ± standard deviation = 0.61 ± 0.02 mm, $n = 20$); prosome length = 0.44-0.48 mm (0.46 ± 0.02 mm, $n = 20$); urosome length = 0.14-0.16 mm (0.15 ± 0.01 mm, $n = 20$). Male. Total length = 0.53-0.57 mm (0.55 ± 0.02 mm, $n = 20$); prosome length = 0.38-0.42 mm (0.50 ± 0.02 mm, $n = 20$); urosome length = 0.14-0.16 mm (0.15 ± 0.02 mm, $n = 20$).

Types.—Deposited in the Smithsonian Institution's National Museum of Natural History, Washington. Holotype: adult female (all limbs dissected), USNM 1144212; allotype: adult male (antennule and fifth legs dissected), USNM 1144213; paratypes 5 adult females, USNM #1144214, and 5 adult males, USNM 1144215. All collected on February 16, 2005 (00°45.426′S 90°18.932′W).

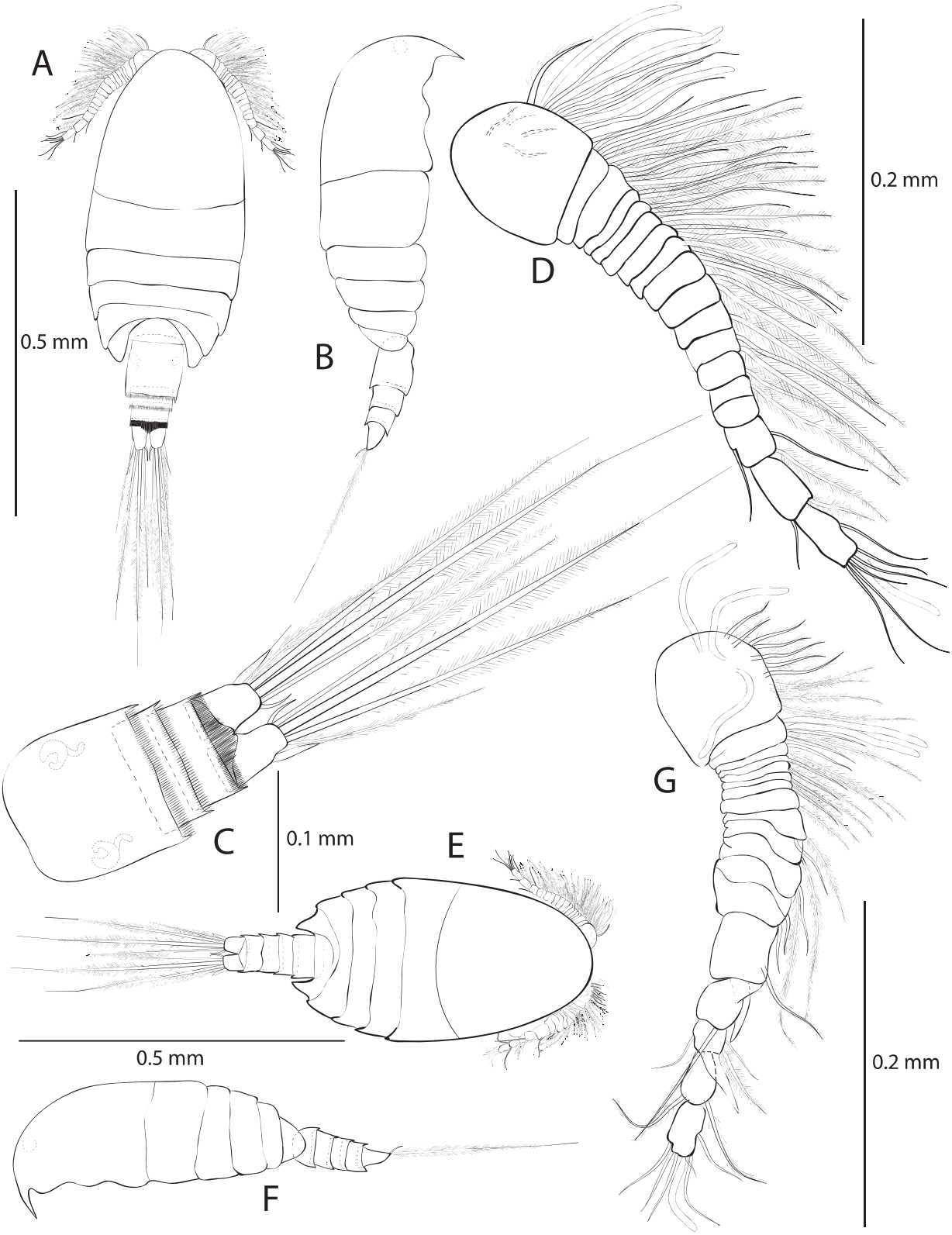


Fig. 6. *Pseudocyclops saenzi* n. sp. A, female, habitus, lateral; B, female, habitus, dorsal; C, female, urosome, ventral; D, female, antennule; E, male, habitus, lateral; F, male, habitus, dorsal; G, male, right antennule.

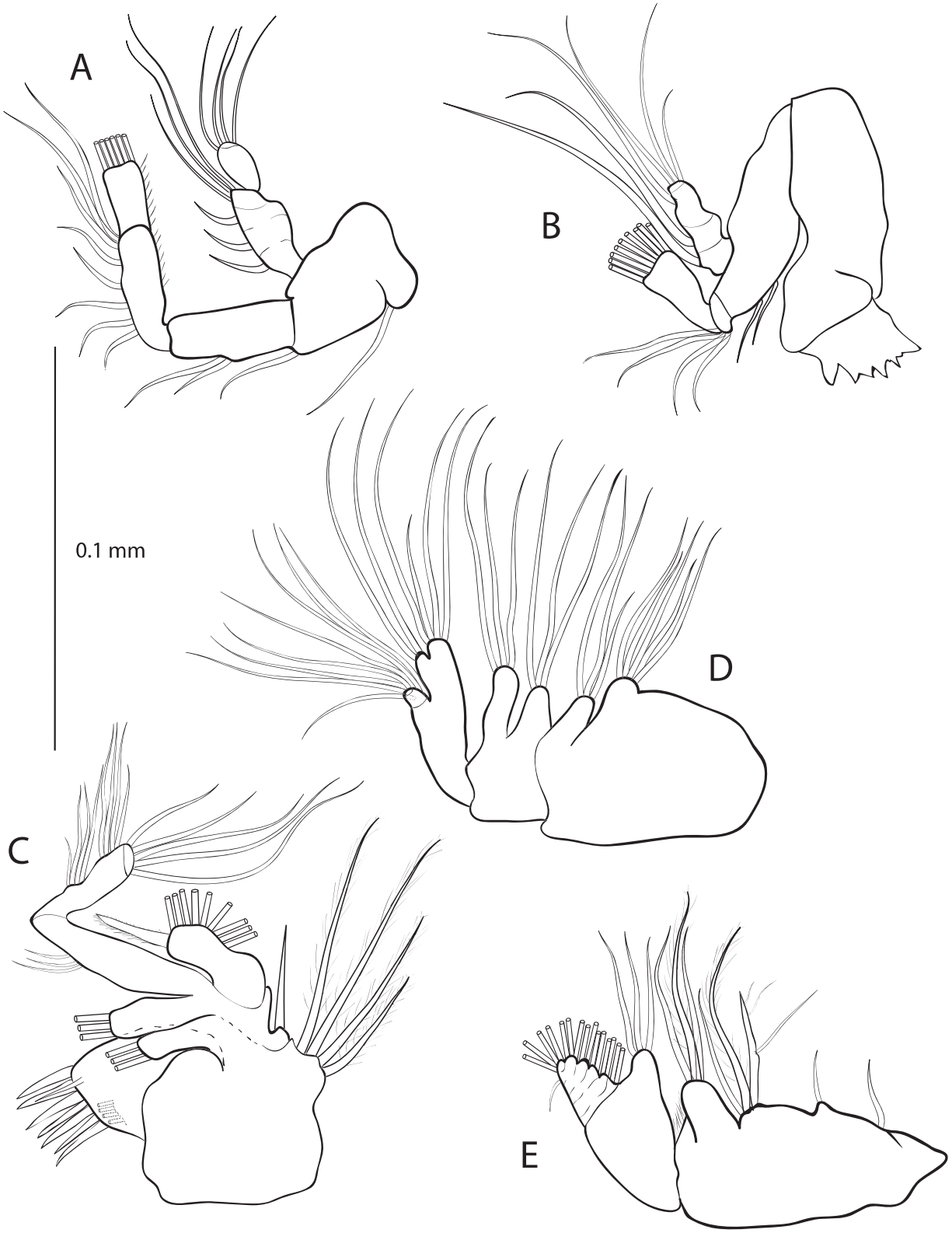


Fig. 7. *Pseudocyclops saenzi* n. sp., female mouthparts. A, antenna; B, mandible; C, maxillule; D, maxilla; E, maxilliped.

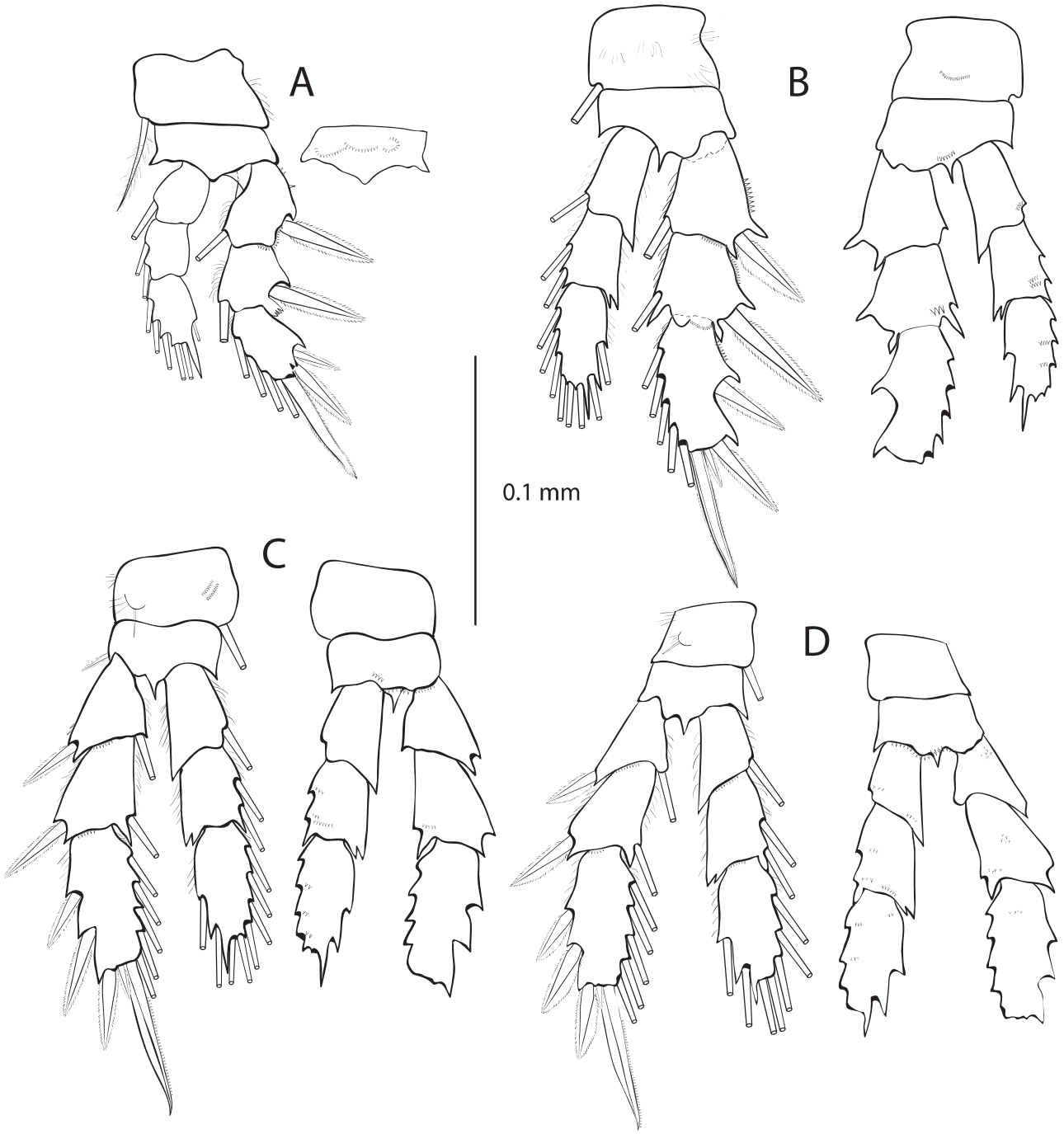


Fig. 8. *Pseudocyclops saenzi* n. sp., female legs 1-4 all posterior, with anterior surface spinulation shown to the right for legs 1-3. A, leg 1; B, leg 2; C, leg 3; D, leg 4.

Description.—Female (holotype). Body (Figs. 6A, B) stout, transparent with purple striations on cephalosome. Prosome 6-segmented, cephalosome clearly separate from first pedigerous somite. Posteriolateral angles of prosome rounded, extending one third of the way along genital double somite. Large eye present in anterior section of cephalosome, orange-pigmented. Rostrum a simple strong process, produced ventrally. Urosome (Fig. 6C) 4-segmented, first 2 segments each with row of spines along posterior margin. Annal segment telescoped into preceding somite with thick row of very fine setae on dorsal surface. Genital double somite symmetrical with paired gonopores. Caudal rami symmetrical, bearing 6 setae each: 1 small, blade-like seta on outer distal corner, followed medially by longer plumose seta, 2 long barbed median setae (the second more robust than the first), 1 long, plumose seta, and 1 small, naked distal seta on dorsal inner margin.

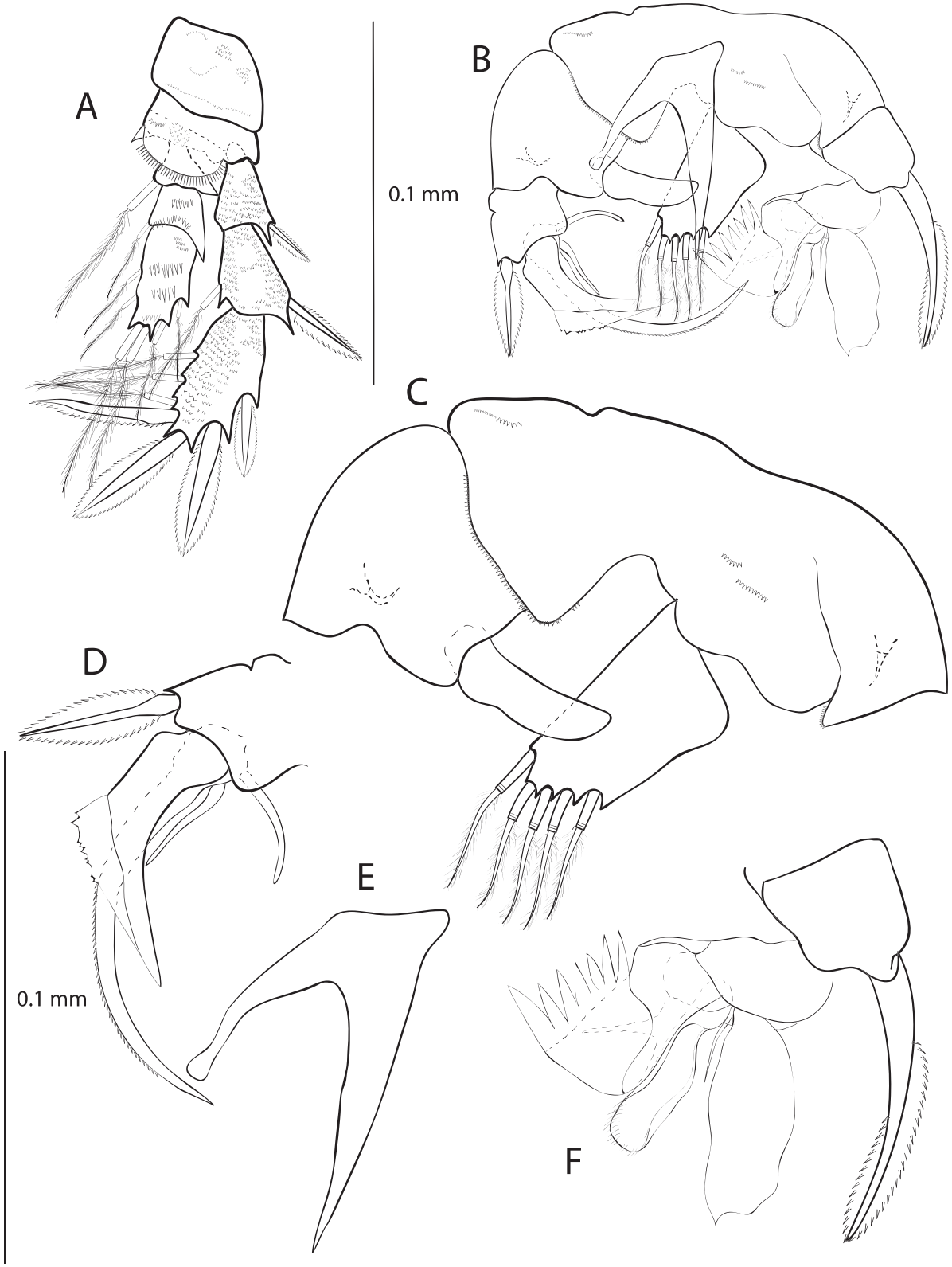


Fig. 9. *Pseudocyclops saenzi* n. sp. leg 5. A, female leg 5, posterior; B, male leg 5, anterior; C, male leg 5 coxa, basis, and endopods, anterior; D, male right leg 5 exopod, anterior; E, male leg 5, v-shaped coxal process, anterior; F, male left leg 5 exopod, anterior.

Antennules (Fig. 6D) symmetrical, 18-segmented, barely reaching first pedigerous somite. Armature of segments as follows: 1-11 + 3ae, 2-4, 3-5, 4-2, 5-2, 6-2, 7-2, 8-2, 9-2, 10-2, 11-1, 12-2, 13-1, 14-1, 15-0, 16-3, 17-2, 18-6 + ae.

Antenna (Fig. 7A) with coxa and basis partly fused. Coxa and basis each with single setae. Endopod 3-segmented, first segment with 2 inner seta; second segment with 5 setae along inner margin and 3 distal setae, outer margin with denticles; third segment with 6 terminal setae, outer margin with denticles. Exopod 6-segmented, first 3 segments partly fused; segments 1-3 each with single inner seta; segment 4 with 3 distal setae; segment 5 with 2 setae; terminal segment with 3 distal setae.

Mandible (Fig. 7B) with gnathobase bearing several short, stout teeth; with a thickened subapical ridge giving the appearance of an articulation. Basis of palp with 2 setae on inner margin. Endopod 2-segmented, first segment with 4 distal setae; second segment with 9 terminal setae. Exopod 4-segmented with setal formula 1, 1, 1, 3.

Maxillule (Fig. 7C) with precoxal endite bearing 7 ventral setae, 1 anterior seta and 4 posterior setae; coxal exite with 4 setae, coxal endite with 3 setae; basal exite with 1 seta, proximal and distal endites with 3 setae each; exopod with 3 + 7 setae (innermost distal setae sclerotized), endopod unsegmented with 4 + 4 mid-ventral setae and 5 terminal setae.

Maxilla (Fig. 7D) with precoxal endite bearing 5 setae, coxal endite with 3 setae; proximal and distal basal endites with 3 setae each. Endopod 3-segmented; first segment with 6 setae on ventral lobe; second segment with 2 inner and 1 outer setae; distal segment with 5 setae.

Maxilliped (Fig. 7E) with 3 syncoxal endites bearing 1, 1, and 3 setae, respectively; coxal endite bearing 3 setae. Basis with proximal endite bearing 3 setae, distal endite bearing 2. Endopod indistinctly 5-segmented with setae formula: 2, 2, 2, 3 + 1, 4.

Legs 1-5 (Figs. 8A-D and 9A) with 3-segmented rami. Seta and spine formulae are given in Table 2. Inner coxal seta present on legs 1-4, absent on leg 5. Leg 1 with patches of denticles on inner margins of all 3 exopodal segments. Second exopodal segment with lamellate process on distal outer margin, between outer setae and insertion point of third segment, toothed at tip. Legs 1-5 with spinulation on posterior surfaces. Legs 2-4 bearing row of spinules on anterior distal margins of exopodal and endopodal segments 1 and 2. Row of denticles on inner margin of each exopodal segment. All endopodal segments with rows of denticles on inner and outer margins. Basis of leg 3 bearing small outer spine. Distal exopodal segments on legs 2-4 bearing a minute terminal spine in addition to large terminal spine. Leg 5 with inner distal margin of basis produced into small spinular process. Coxa and basis lacking setae. Posterior surfaces of endopodal and exopodal segments with strong spinulation and denticles.

Male (allotype). Body (Figs. 6E, F) as in female, but slightly smaller. Urosome 5-segmented. Left antennule and caudal rami as in female. Right antennule (Fig. 6G) 18-segmented, geniculate between segments 14-15. Armature of segments as follows: 1-11 + 3ae, 2-3 + 2ae, 3-5, 4-2, 5-2, 6-2, 7-2, 8-1, 9-2, 10-1, 11-2, 12-1, 13-1, 14-1, 15-1, 16-3, 17-2, 18-6 + ae. Segments 14 and 16 each with distal margin expanded into spinular process.

Table 2. Setae and spine formulae for legs 1-5 of female *Pseudocyclops saenzi*.

| | | | Exopod | | | Endopod | | |
|---|---|---|---|---|---|---|---|---|
| | Coxa | Basis | 1 | 2 | 3 | 1 | 2 | 3 |
| Leg 1 | 0-1 | 0-0 | I-1 | I-1 | II, I, 4 | 0-1 | 0-2 | 1, 2, 3 |
| Leg 2 | 0-1 | 0-0 | I-1 | I-1 | II, II, 5 | 0-1 | 0-2 | 2, 2, 4 |
| Leg 3 | 0-1 | I-0 | I-1 | I-1 | III, II, 5 | 0-1 | 0-2 | 2, 2, 4 |
| Leg 4 | 0-1 | 0-0 | I-1 | I-1 | III, II, 5 | 0-1 | 0-2 | 2, 2, 3 |
| Leg 5 | 0-0 | 0-0 | I-0 | I-1 | III, I, 3 | 0-1 | 0-1 | 2, 2, 2 |

Mouthparts and legs 1-4 as in female. Leg 5 (Figs. 9B-F) biramous, asymmetrical, strongly modified. Right coxa separate from basis, left coxa partly fused with basis. Small setae present on posterior surface, one near outer margin on left basis and one located medially of right basis. Right leg with 2-segmented exopod. First segment with lateral spine on outer margin and broad, blade-like medial seta with proximal outer margin flanged and serrated. Second segment elongate, with thick base and slightly curved, spine-like attenuation with 2 thick setae on proximal inner margin. Right endopod a simple elongate lobe. Left basis with long, v-shaped process originating on anterior surface near inner margin. Left leg with 2-segmented exopod, first segment bearing a serrated outer spine, second segment bearing a slender seta and three membranous processes. First process broad and leaf-like; second more slender but also leaf-like, with hairs along marginal folds and long finger-like projection at base; third process much larger, medially folded, with 7 finger-like projections at its terminal end. Left endopod a simple lobe, its distal end bearing 5 setae.

Remarks.—*Pseudocyclops saenzi* is similar to *P. rubrocinctus* Bowman and Gonzalez, 1961, from the Caribbean side of Panama, and *P. steinitzi* Por, 1962 from the Red Sea (Bowman and Gonzalez, 1961; Por, 1962). All three species have a similar body shape and segmentation, and all have nearly identical mouthparts. The thoracic legs are also very similar. There is no inner basal seta on the leg 1, and the female leg 5 has 4 inner setae on the terminal exopodal segment, 6 setae on the terminal segment of the 3-segmented endopod, and an inner seta on the first exopodal segment. They also all have 5 setae on the endopod of left leg 5 in males. *Pseudocyclops saenzi* can be distinguished from these other two species by a sclerotized seta on the exopod of the maxillule, and differences in the details of leg 5 in males. The medial seta on the left exopodal segment of leg 5 in males of *P. saenzi* is short and stout, while it is elongate with a narrower distal attenuation in both, *P. rubrocinctus* and *P. steinitzi*. The distal left exopodal segment of leg 5 in males is simpler in P. *steinitzi* than in *P. saenzi* and *P. rubrocinctus*, with fewer elements present. Finally, *P. saenzi* has a v-shaped process on the anterior surface near the inner margin; in *P. steinitzi* and *P. rubrocinctus* this process is simple and elongate.

Etymology.—The species name is in honor of Ingeniero Carlos Metolio Sáenz Olmedo, who was a professor at the

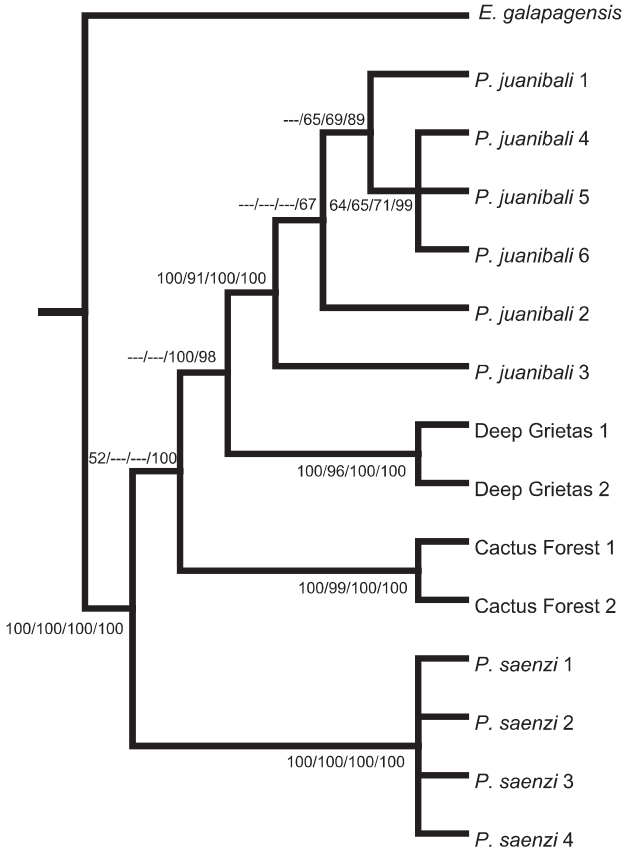


Fig. 10. Phylogenetic tree of *Pseudocyclops* based on a 569-base-pair region of ITS-1. Branch values correspond to bootstrap support for maximum parsimony, maximum likelihood, neighbor joining, and Bayesian posterior probabilities (--- means that the branch failed to get 50% bootstrap support).

high school and university level and a civil engineer whose work in rural communities across Ecuador improved the lives of many.

## Genetic Analysis

Analysis of the sequences of *Pseudocyclops* resulted in four trees for MP, 1 for ML, and three for NJ. Strict consensus trees were constructed for MP and NJ. The resulting tree topology for MP, ML and NJ methods was similar to that of the Bayesian analysis (Fig. 10). All four methods resulted in four well-supported clades for *P. juanibali*, specimens from Deep Grietas, specimens from Cactus Forest sinkhole, and *P. saenzi* respectively. The main differences among methods included for MP there was a collapse of two branches within the *P. juanibali* clade, and a collapse of the branch for the *P. juanibali* and Deep Grietas clades. For the ML method, a collapse of one branch in the *P. juanibali* clade, a collapse of the branch that generates the *P. juanibali* and Deep Grietas clade and a collapse of the branch of the *P. juanibali*, Deep Grietas, and Cactus Forest sinkhole clades. Finally, for NJ there was a collapse of a branch within the *P. juanibali* clade and a collapse of the branch for the *P. juanibali*, Deep Grietas and Cactus Forest sinkhole clades.

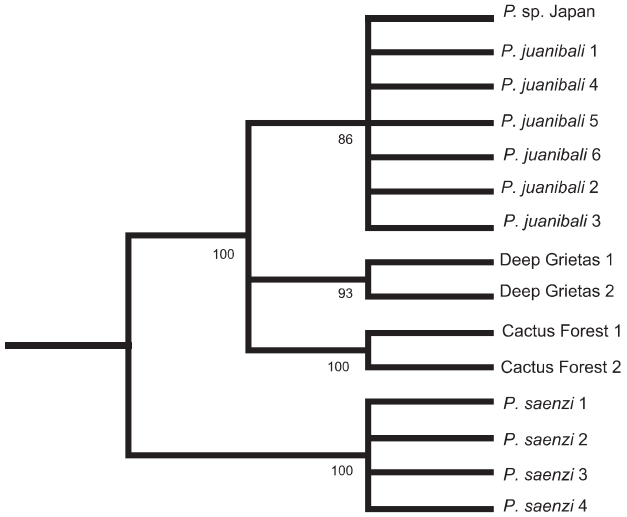


Fig. 11. Phylogenetic tree of *Pseudocyclops* including a specimen of *Pseudocyclops* from Japan, based on a 114-base-pair region of ITS-1. Branch values correspond to bootstrap support for Bayesian posterior probabilities.

Huys et al. (2006) sequenced a 1785-base-pair region of the 18S small subunit ribosomal gene of an un-identified *Pseudocyclops* that was collected in May 2000 with a sledge net off Nagannu Island, Ryukyu Islands, Japan; this sequence is available in GenBank (Benson et al., 2005), accession number AY626994. The first 114-base-pairs of this sequence correspond to the last 114-base-pairs of the ITS-1 region used in my analysis. A new alignment was prepared only using this 114-base-pair region for all the sequences of *Pseudocyclops*, and this showed an exact match between the specimen of *Pseudocyclops* from Japan and all six specimens of *P. juanibali*. This sequence is different however from the sequences for the other examined *Pseudocyclops*, by 1.8% to 18%. A Bayesian analysis was then performed with MrBayes 3.1 (Huelsenbeck and Ronquist, 2001) using the same settings as before. These shorter sequences generated a similar phylogenetic tree to that based on the entire 569-base-pair sequences. Well-supported branches first separated *P. saenzi* from all other *Pseudocyclops* and also supported separate clades for the population of *Pseudocyclops* from the Cactus Forest sinkhole, that from Deep Grietas, and *P. juanibali* including the specimen of *Pseudocyclops* from Japan (Fig. 11). A female voucher specimen of this *Pseudocyclops* from Nagannu Island was deposited in the Natural History Museum in London (NHM reg. no. 2005.42). This specimen was requested and permission was obtained to perform a dissection. It proved to be morphologically indistinguishable from females of *P. juanibali* from the Galapagos.

## Discussion

The ITS-1 region clearly shows that the two new species, *Pseudocyclops juanibali* and *P. saenzi*, are genetically different. It also shows that, although the two populations of *Pseudocyclops* from Cactus Forest sinkhole and Deep Grietas are both morphologically identical to *P. juanibali*,

they are genetically isolated from it and each other, forming separate clades. These populations form a *Pseudocyclops* species complex, which demonstrates that cryptic speciation is occurring in the anchialine environments of the Galapagos.

Pseudocyclopidae, established by Giesbrecht (1893), is monogeneric. *Pseudocyclops* contains 39 named species, including the two described in this paper. Incomplete descriptions have made it difficult to assign the species of *Pseudocyclops* to species groups. Nevertheless, four groups were characterized by Ohtsuka et al. (1999): the *lepidotus*-group (*P. lepidotus* Barr and Ohtsuka, 1989 and *P. ornaticauda* Ohtsuka, Fosshagen, and Putchakarn, 1999), the *kulai*-group (*P. kulai* Othman and Greenwood, 1989, and *P. ensiger* Ohtsuka, Fosshagen, and Putchakarn, 1999), the *crassiremis*-group (*P. crassiremis* Brady, 1872, *P. bahamensis* Fosshagen, 1968, *P. oliveri* Fosshagen, 1968, and *P. minutus* Ohtsuka, Fosshagen, and Putchakarn,1999), and the *magnus*-group (*P. magnus* Esterly, 1911, *P. latens* Gurney, 1927, *P. xiphophorus* Wells, 1967, *P. bilobatus* Dawson, 1977, and *P. schminkei* Chullasorn, Ferrari, and Dahms, 2010). These groupings only include 13 species at present, leaving 26 species yet to be classified.

The four groups determined by Ohtsuka et al. (1999) were not defined on the basis of apomorphies, and many taxonomically interesting traits of the males and females were not considered (Frank D. Ferrari, personal communication). Cladistically valid species-groups must be defined on the basis of apomorphies; and to date, only Ferrari et al. (2010, 2011) have attempted to diagnose apomorphies for Pseudocyclopidae. Unfortunately many of the descriptions of *Pseudocyclops* are incomplete and lack the necessary detail for a morphological analysis of apomorphies in this monogeneric family. Therefore a complete cladistic reconstruction of this family will not be possible until someone works with the various type materials of *Pseudocyclops* and revises the species descriptions to clearly characterize apomorphic states. In the absence of such an analysis, species similarities based on combinations of mostly plesiomorphic characters, such as those presented by Ohtsuka et al. (1999), do provide an initial classification framework of likely phylogenetic relationships that can be validated, amended, or discarded by future studies.

Based on the available morphological descriptions, it is likely that an *australis* species-group exhists that includes: *P. australis*, *P. mathewsoni* Fosshagen, 1968, *P. simplex* Sewell, 1932, and *P. juanibali* (presumably also *P. pacificus* Vervoort, 1964 and *P. latisetosus* Sewell, 1932, but the descriptions of these two species are incomplete and based only on males). Members of this potential group have several plesiomorphic (ancestral) characters such as a leg 5 in females with a 3-segmented endopod, 4 inner setae on the terminal exopodal segment of the same leg, thoracic somites bearing legs 4 and 5 separate, and 2 spines on the exopod of the right leg in males. However, there are also several common apomorphic (derived) characters, such as barbed caudal setae instead of plumose ones and the absence of an inner basal seta on leg 1. Finally, there is a clear synapomorphy (shared derived trait) for this group, the presence of a deep cleft on the distal margin of the endopod of left leg 5 in males. This last character, and the combination of the aforementioned ancestral and derived characters, suggests that these animals are closely related.

It is likely that *P. rubrocinctus*, *P. steinitzi*, and *P. saenzi* also form a species-group, but the incomplete description of *P. steinitzi* limits the morphological analysis of this potential group to just *P. rubrocinctus* and *P. saenzi*. They share a few ancestral characters including the 3-segmented endopod of leg 5 in females, with 4 inner setae and 6 terminal setae on the third exopodal segment of the same leg. They also share several apomorphic traits: absence of an inner seta on the first exopodal segment of leg 5 in females, 5 setae on the left endopod of leg 5 in males, barbed caudal setae, absence of an inner basal seta on leg 1, and presence of only 1 seta on the second syncoxal endite of the maxilliped (2 is ancestral). These latter characters set them apart from other *Pseudocyclops*.

There seem to have been two colonization events for the *Pseudocyclops* spp. found in the Galapagos, leading to the *P. juanibali* species-complex on the one hand and *P. saenzi* on the other. An ancestral form of the *australis*-group probably arrived at the Islands from the Western Pacific and gave rise to *P. juanibali*. The several plesiomorphic characters that define this group suggest a Western Pacific origin. Proponents of a Tethyan origin for this fauna argue that such a transfer would have had to occur during the existence of the warm, shallow and circumtropical Tethys Sea. The large genetic distances among the three members of the *P. juanibali* species-complex in the Galapagos imply that these animals were likely one of the first colonizers of the Archipelago.

Although designating the specimen from Japan as conspecific with *P. juani*bali seems like a foregone conclusion, it must be emphasized that females of *Pseudocyclops* alone do not have enough distinguishing characters to differentiate the various species in this genus; males are needed for specific determination. Therefore, assigning a specimen to a new species based only on the morphology of a female is not recommended. Furthermore, the genetic identity between the specimen from Japan and *P. juanibali* is only based on a very short DNA sequence that only demonstrates that the specimen of *Pseudocyclops* from Japan is more closely related to *P. juanibali* than to the specimens of *Pseudocyclops* from Deep Grietas, Cactus forest sinkhole, or *P. saenzi*. Hence, although the genetic match and morphological identity of the hitherto unidentified specimen of *Pseudocyclops* from Japan with *P. juanibali* does not imply conspecificity, it does show that they are closely related and it suggests that a secondary exchange between the Western and Eastern Pacific is occurring in the present-day ocean. These epibenthic animals have evidently been able to cross the Eastern Pacific Barrier, a vast expanse of deep ocean (Darwin, 1859; Lessios et al., 1998; Lessios and Robertson, 2006).

The origins of *P. saenzi* are not as clear. Its closest relatives are found in the Caribbean (*P. rubrocinctus*) and the Red Sea (*P. steinitzi*). It seems likely that the ancestral form of *P. saenzi* crossed the seaway at Panama before the isthmus closed, arriving from the Caribbean and speciating in the Galapagos. The reverse scenario, however, is also

possible: an ancestral form arriving in the Galapagos from the west, just like the ancestor of *P. juanibali*, and subsequently crossing into the Caribbean

Among the above-mentioned four evolutionary models suggested for anchialine fauna, it seems that in the Galapagos, for *Pseudocyclops*, as for *Ridgewayia* (Figueroa, 2011), a combination of the vicariance model and the active migration model is responsible for the observed species distributions. There was clearly faunal exchange between the Galapagos Islands and the Caribbean before the closure of the Panamanian seaway, followed by speciation through vicariance. In addition, though, active migration and colonization with subsequent speciation is also occurring in the present-day ocean. This is demonstrated by the colonization of Isabela, a geologically young island, by *Ridgewayia* (Figueroa, 2011), and also by the extraordinary genetic and morphological similarities between *P. juanibali* and the specimens of *Pseudocyclops* from Japan. Although the morphological and genetic evidence shows that these copepods are able with time to cross the entire Pacific, it also shows that such long-range migration is not the norm. These copepods tend to have restricted distributions with minimal migration and gene exchange, even between habitats that are very close to each other such as different anchialine pools on the Island of Santa Cruz in the Galapagos.

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