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2013-12-13

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## On Tetragnonipitidae (Crustacea, Copepoda) from the Channel of São Sebastião, Brazil, with description of their nauplii and two new species of *Phyllopodopsyllus*

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### Abstract

*Phyllopodopsyllus iuanamai* sp. nov. and *Phyllopodopsyllus pseudokunzi* sp. nov. are described from specimens collected in the Channel of São Sebastião (State of São Paulo, Brazil). The nauplii of *P. iuanamai*, *P. pseudokunzi*, *P. aegypticus* Nicholls, 1944 are described, additional information is given on nauplii and morphology of the adult *P. setouchiensis* Kitazima, 1981, and on the nauplius of *Laophontella horrida* (Por, 1964). These represent first records of *P. aegypticus* and *L. horrida* in Brazilian waters.

**Key words:** Harpacticoida, ontogeny, South America, taxonomy

### Introduction

The family Tetragnonicepsidae, corrected by Wells (1967) to Tetragnonipitidae, was erected by Lang (1944) to receive the genera *Tetragnoniceps* Brady, 1880, *Pteropsyllus* T. Scott, 1906, *Phyllopodopsyllus* T. Scott, 1906, *Diagnoniceps* Willey, 1930 and *Paraphyllopodopsyllus* Lang, 1944 (see Lang 1948). To these, several other genera were added later such as *Laophontella* Thompson & Scott, 1903.

Several of these genera were synonymized (Lang, 1965) and some removed from the Laophontidae T. Scott, 1904 to the Tetragnonipitidae. *Laophontella* and *Willeyella* Por, 1964 were synonymized (Por 1964) as well as *Phyllopodopsyllus* and *Paraphyllopodopsyllus* (see Lang 1965). *Fearia* Coull, 1971 is a junior synonym of *Tetragnoniceps* (see Kunz 1984).

Boxshall & Halsey (2004) considered Tetragnonipitidae as a very speciose family in the tropics, where they can be found in various marine sediments at different depths. Though predominantly marine, Karanovic *et al.* (2001) reported the occurrence of representatives of the family in subterranean continental waters.

The Tetragnonipitidae is now composed of 12 genera (Wells 2007), the most representative being *Phyllopodopsyllus* and *Tetragnoniceps*, with 48 and 10 species respectively. The genera *Oniscopsis* Chapuis, 1955, *Phyllopodopsyllus*, *Protognoniceps* Por, 1964 and *Tetragnoniceps* had already been collected off the São Paulo State by Rocha *et al.* (2011).

This study amplifies the family distribution with first record of the *Phyllopodopsyllus aegypticus* Nicholls, 1944 and *Laophontella horrida* (Por, 1964). in Brazilian waters and adds two new species to those cited by Wells (2007).

### Material and methods

Sediment (sand and gravel) were taken with bottom samplers or manually in São Sebastião Channel at 5–7 m depth in the following localities: off Itaçuca (Guaecá point), in Sítio de Calhetas, in Cigarras, in Pitangueiras, in São Francisco beaches (off São Sebastião city), and in Parcel da Praia Grande (off São Sebastião island).

The bottom samples were brought to the laboratory, and after having been washed in sea water using 0.25–0.50 mm meshed sieves, were placed in plastic containers (30 cm long, 10 cm wide, 10 cm deep). The animals were kept alive in these containers in the laboratory of the Centro de Biologia Marinha (CEBIMar) near to a window but away from the direct sun at about 25°C. The 34‰ salinity from the site of collection was kept constant by occasional addition of freshwater.

Specimens of *Phyllopodopsyllus* were easily kept alive and reproducing several weeks in this micro ecosystem. Subsamples were taken weekly out of the containers and examined under magnification lens.

Whole specimens were examined in temporary lactic acid mounts. The material was preserved in 70% ethanol. Dissections were made in glycerin and the dissected parts were placed on slides and sealed with Glyceel®.

A Nikon Labophot®, Zeiss Axioskop compound microscope equipped with differential interference contrast, digital camera Nikon Coolpix 995® and camera lucida were used to examine and prepare the illustrations of the specimens.

For confocal laser scanning microscopy (CLSM), before dissection the specimens were stained with Congo Red and mounted on slides following the procedure described by Michels & Büntzow (2010), using a Leica TCS SP5 (Leica, Wetzlar, Germany) equipped with a Leica DM5000 B upright microscope (Leica, Wetzlar, Germany) and 3 visible-light lasers (DPSS 10 mW 561 nm; HeNe 10 mW 633 nm; Ar 100 mW 458 nm, 476 nm, 488 nm and 514 nm), combined with the software LAS AF 2. 2. 1.—Leica Application Suite Advanced Fluorescence (Leica, Wetzlar, Germany).

Series of stacks were obtained, collecting overlapping optical sections throughout the whole preparation; the optimal number of scans and the imaging settings according to the software, are given in Table 1. Final images were obtained by maximum projection, and CLSM illustrations were composed and adjusted for contrast and brightness using the software Adobe Photoshop CS4 (Adobe Systems, San José, U.S.A.).

The terminology applied to the segmentation and setation of body appendages was adopted from Huys *et al.* (1991). The terminology used for description of naupliar stages followed Dahms (1990). Abbreviations used: NI–NVI = nauplius 1 to nauplius 6; P1–P6 = leg 1 to leg 6; ae = aesthetask; enp = endopod; exp = exopod; A1 = antennule; A2 = antenna; Mx = maxilla.

The type material was deposited in the collection of the Museu de Zoologia, Universidade de São Paulo (MZUSP).

**TABLE 1.** Microscope lens and confocal laser scanning microscopy (CLSM) settings used for the observation of the specimens; Ch1 and Ch2 = detection channels 1 and 2.

Lens	HC PL APO CS (High-grade colour-corrected Plan Apochromat lens for confocal)
Objective	10X
Numerical aperture	0.4
Immersion	No immersion - air
Excitation wavelength	561 nm
Laser intensity	80%
Excitation beam splitter	Double dichroic filter 488/561
Detected emission wavelength	Ch1: 575–646 nm Ch2: 646–775 nm
Detector gain	584.3–596.1 V
Amplitude offset	-3.5–5.6 %
Electronic zoom	2X
Pinhole aperture	30 µm
Image format	2048 x 2048 dpi

## Systematics

Family Tetragonicipitidae Lang, 1944

Genus *Phyllopodopsyllus* T. Scott, 1906

***Phyllopodopsyllus iuanamai* sp. nov.**

(Figs. 1–10)

**Type material.** One female, holotype (MZUSP 28031) and 15 paratype females; 9 paratype males; 6 copepodites and 7 nauplii paratypes (MZUSP 28030). Collection sites: São Sebastião, Guaecá Beach, Itaçucê (23°50'00"S 15°26'62"W), Pepato, A. coll. São Sebastião Is., Parcel da Praia Grande (23°51'30"S 45°25'00"W), Oliveira, J. M. coll.

**Type locality.** Itaçucê, Guaecá Beach, São Sebastião (23°50'00"S 45°26'62"W).

**Diagnosis.** A *Phyllopodopsyllus* with paired dorsal spines on the urosomites; with hook-like processes on the second segment of the antennule; anal operculum margin fringed with a row of very fine setules, leg 4 endopod 2-segmented; female caudal ramus 1.4 times as long as anal somite and strongly dilated proximally; male caudal ramus 3.5 times as long as anal somite.

**Description of adults.** Female (Figs 1A–C): Total body length from 770–950 µm (n = 6, X = 860 µm). Color when alive light brown. Dorsal red spot (“eye”) anteriorly. Body with 10 somites. Dorsal surface of cephalosome and first free somite as in Figure 2A. Pedigerous somites with 2 long sensilla dorsally, one on each lateral distal site. Numerous minute dots either following the striae or dispersed irregularly dorsally and laterally (Fig. 2A). Posterior margin of somites ornamented with close packed transparent setules forming a hyaline fringe all around the segment. Epimeral lappets short, rounded. Somite bearing P5 and double genital somite with weak (somite bearing P5) or strong (double genital somite) pairs of dorsal spiniform processes (Fig. 2B). Ventral margin below brood pouch (P5) with patch of setules. Genital double somite with suture visible dorsally, laterally and ventrally (Figs 1A–B, 3C). Anal operculum (Figs 2B, 3A) finely setulose and with a lateral small sensillum on each side.

Caudal rami (Figs 3A–C) pear shaped and with a dorsal ridge, inner margins developed into very thin lateral transparent expansions, with setulose ridge on dorsal and full of setulose striae on the inner side. Pores scattered along outer margin of the rami. Each ramus with 7 setae (Figs 3A–C), seta V long and proximally globular.

Rostrum (Figs 2A, 4A–B): flat, defined at base, with two frontal sensilla and with setules along the ventral distal margin.

Antennule 9-segmented (Fig. 4C). Segment-1 about as long as all following segments summed together. Segment-2 with prominent pointed outer hook about twice as long as supporting segment. Setal formula: 1, 9, 8, 4+1 ae, 2, 4, 2, 2, 7+1 ae.

Antenna (Fig. 4D) biramous. Basis unarmed. Exopod 1-segmented with 3 elements: a bipinnate short, a bipinnate long seta and a unipinnate middle seta. Endopod 2-segmented, segment-1 unarmed, segment-2 with 7 setae (4 geniculated), 2 small setae laterally, pre-terminal spinules and 2 spinulose frills, one median and one pre-terminal.

Mandible (Figs 4E–F) with well-developed coxal gnathobase with 3 main tooth-like processes and a row of spines. Basis prominent, bearing 3 terminal setae and a tuft of setules on medial face. Exopod 1-segmented with 4 setae, 3 lateral and 1 apical. Endopod with 2 medial and 8 distal setae.

Maxillule (Fig. 5A) with well-developed praecoxal arthrite bearing 10 distal elements, and 2 surface setae. Coxa with 6 setae on endite and with 1 long seta on exite. Basis with 7 setae. Endopod and exopod 1-segmented, with 4 and 3 setae respectively.

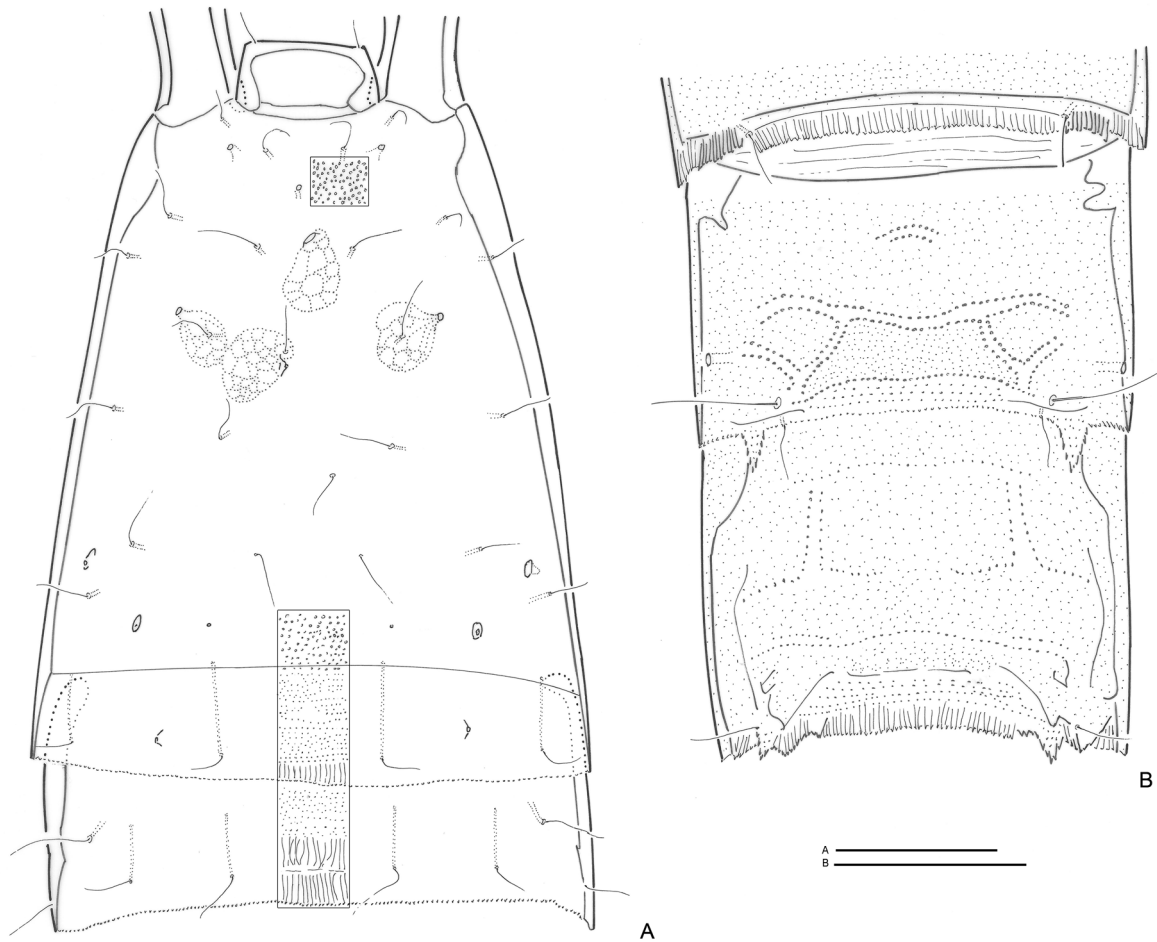
Maxilla (Fig. 5B) with 4 syncoxal endites bearing 2, 1, 3, 3 setae on proximal to distal segment, respectively. Basis armed with 2 spines and 2 setae. Endopod 2-segmented, proximal segment with 2, distal segment with 3 setae.

Maxilliped (Fig. 5C) prehensile. Syncoxa with 3 bipinnate setae. Basis with 1 seta, and a row of spinules along inner margin. Endopod 1-segmented with long geniculated claw and 2 slender setae.

Leg 1 (Fig. 5D) biramous. Coxa ornamented with rows of spinules. Basis with tufts of spinules, bearing 1 small seta on distal outer corner and 1 bipinnate spine on proximal inner margin. Exopodal segments with small spinules along outer margin and exp-2 with setules along inner margin. Enp-1 6.3 times longer than enp-2, with small spinules along outer margin and long setules along inner margin.



**FIGURE 1.** *Phyllopodopsyllus iuanamai* sp. nov. (female). Confocal laser scanning microscopy images. (A) habitus, dorsal; (B) habitus, lateral; (C) habitus, ventral. Scale bar: 50  $\mu$ m.



**FIGURE 2.** *Phyllopodopsyllus iuanamai* sp. nov. (female). (A) cephalosome and first free somite with detail of ornamentation, dorsal; (B) P5-bearing somite and double genital somite, dorsal view. Scale bars: 50  $\mu$ m.

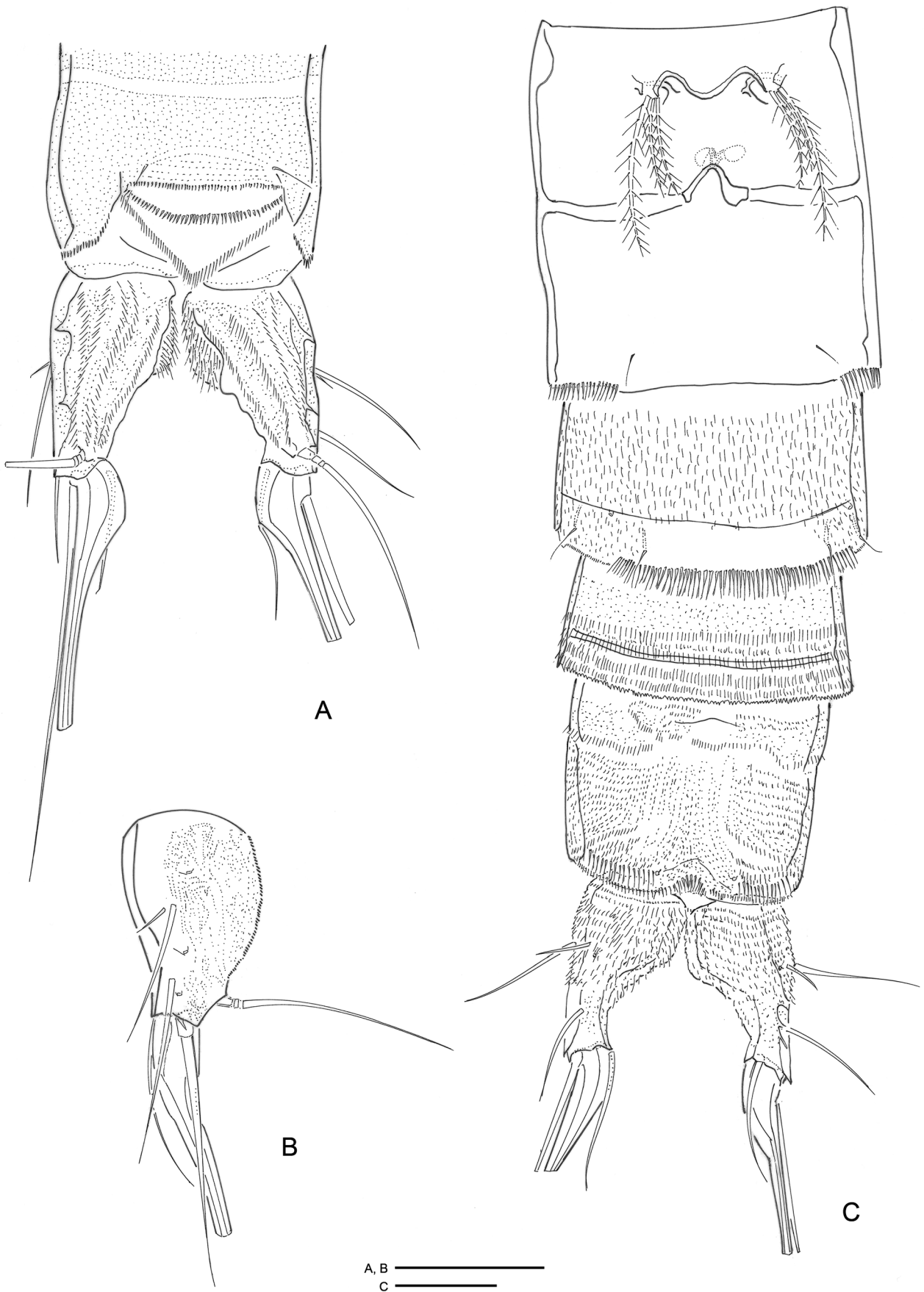
Legs 2–4 biramous (Figs 6A–C). Coxa ornamented with rows of spinules. Basis with rows of spinules, bearing one seta on outer corner, and spiniform projection on distal rim between exopod and endopod and on distal inner corner. Exopod 3-segmented. Endopod 2-segmented. Inner and outer margins of exopods and endopods of P2–4 ornamented with setules and spinules, spiniform projections on distal outer corner of segments of P2–3 and on first segments of P4 exopod and endopod. Armature formula as follows:

	Exopod	Endopod
Leg 1	0. 0. 022	1. 020
Leg 2	1. 0. 122	1. 021
Leg 3	1. 0. 122	1. 021
Leg 4	1. 1. 123	1. 021

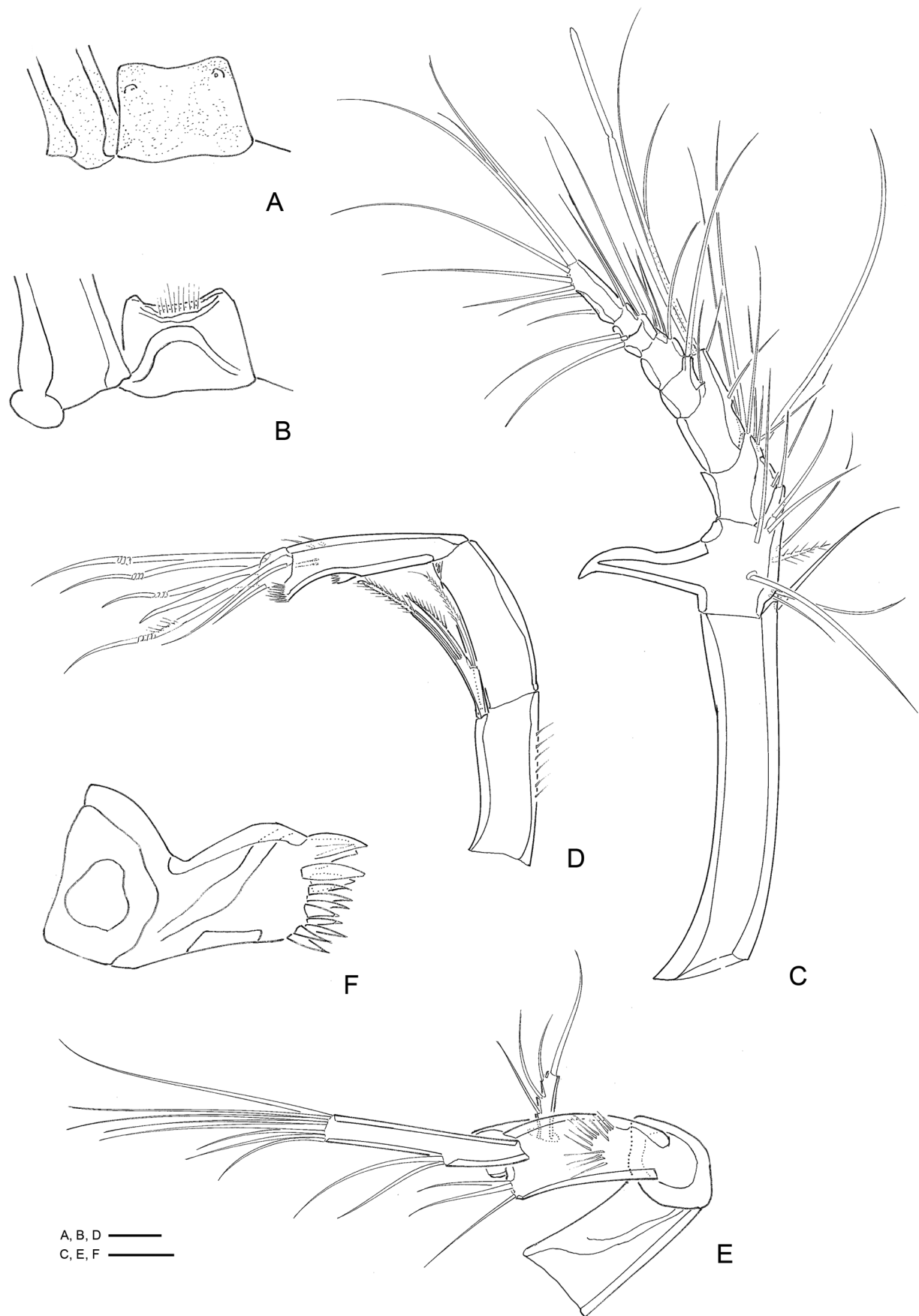
Leg 5 (Fig. 6D) large and foliaceous. Outer margin from proximal to distal with row of setules, 1 proximal slender seta (remnant of outer setophore), 1 seta inserted medially directed to inwards, 1 pinnate spine, 1 seta, 1 pointed process, 2 setae, 1 pointed projection and 1 bipinnate seta. Distal area with fine setules. Outer margin with 4 bipinnate setae.

Genital field as in Fig. 3C.

Leg 6 (Fig. 3C) represented by 3 setae on each side of genital pore.



**FIGURE 3.** *Phyllopodopsyllus iuanamai* sp. nov. (female). (A) anal somite and caudal rami, dorsal; (B) caudal ramus, lateral; (C) urosome, ventral (P5-bearing somite omitted). Scale bars: 40  $\mu$ m (C), 50  $\mu$ m (A, B).

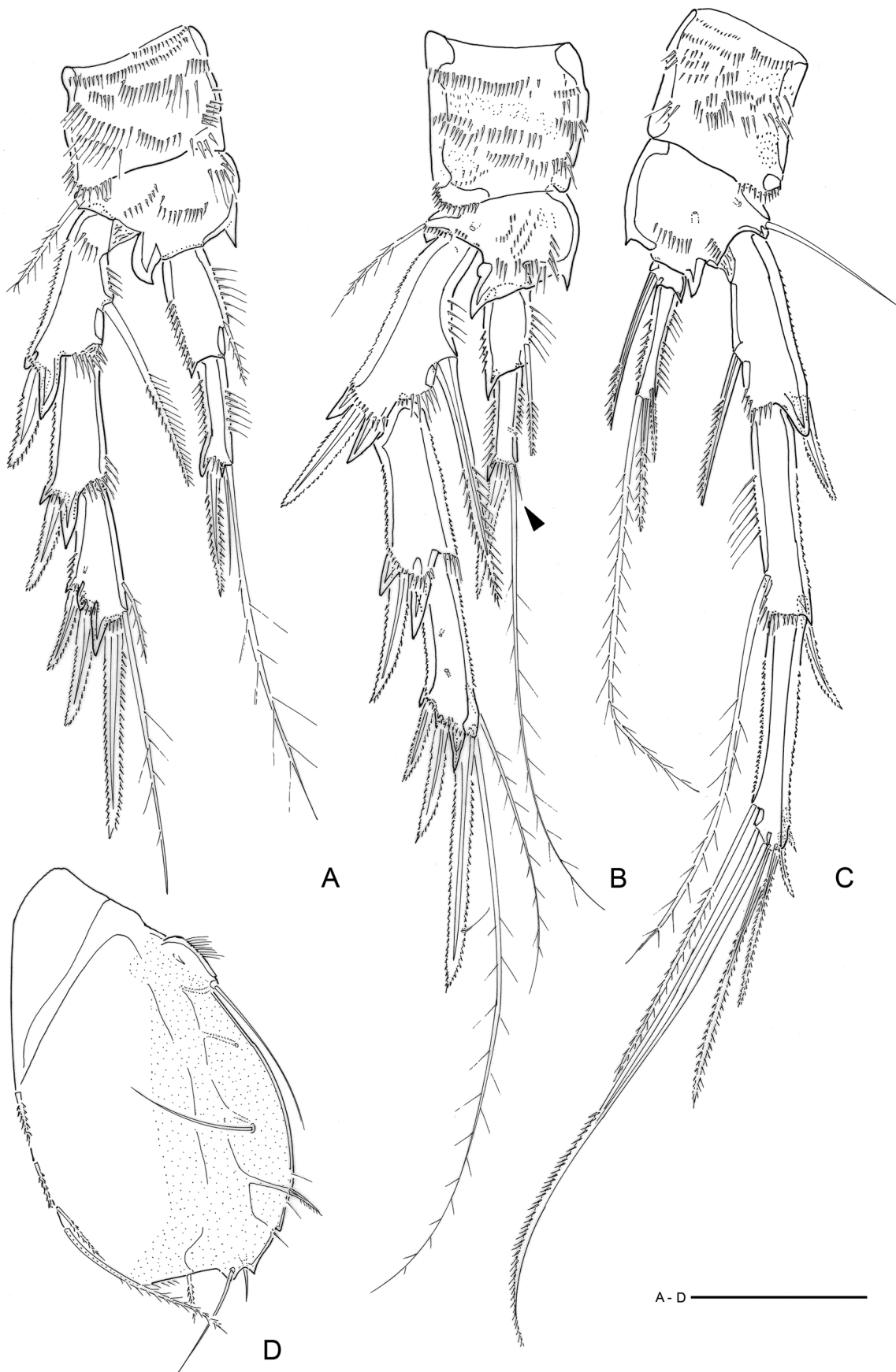


**FIGURE 4.** *Phyllopodopsyllus iuanamai* sp. nov. (female). (A) rostrum, dorsal; (B) rostrum, ventral; (C) antennule; (D) antenna; (E) mandible; (F) mandibular gnathobase. Scale bars 20  $\mu$ m.





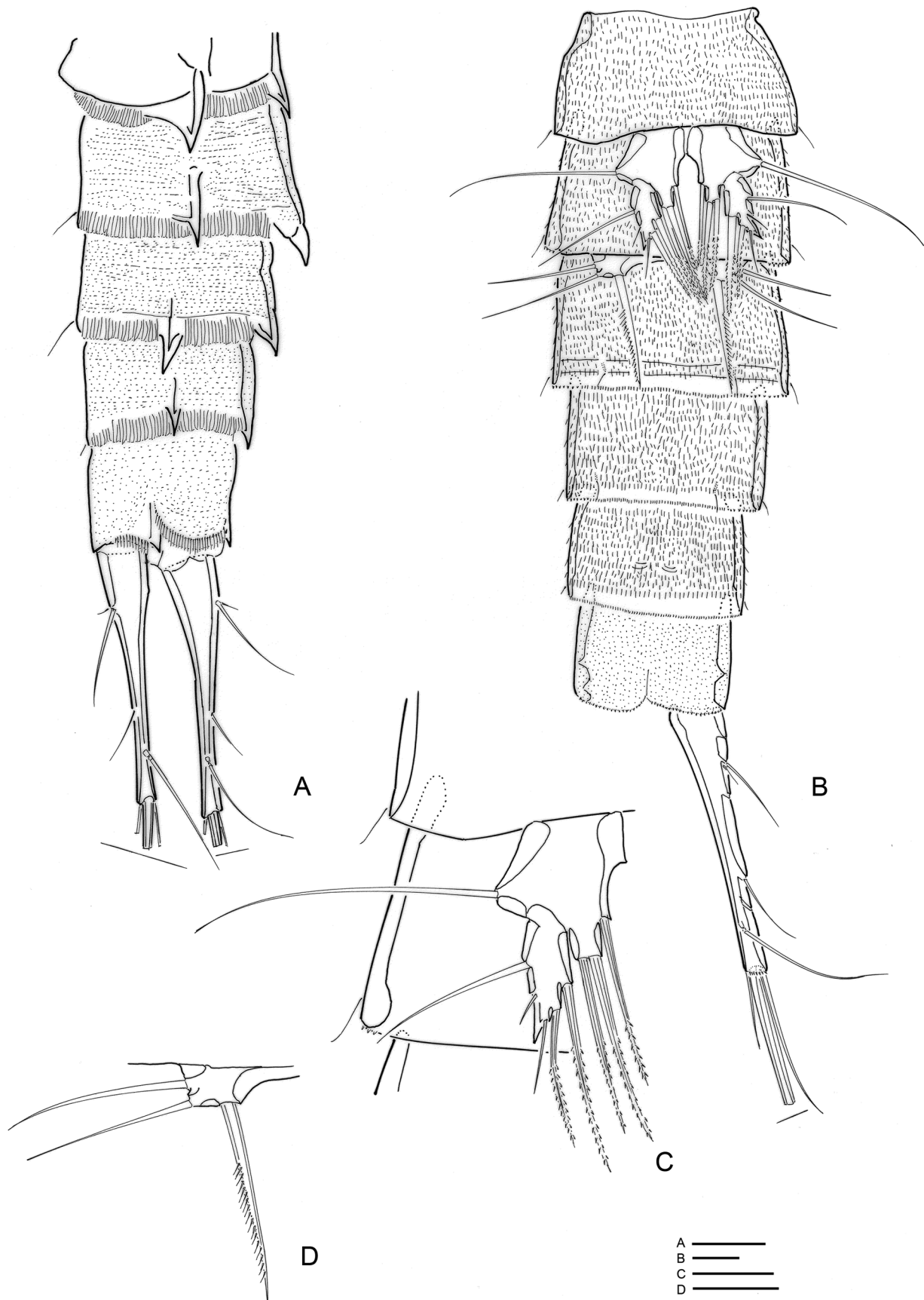
**FIGURE 5.** *Phyllopodopsyllus iuanamai* sp. nov. (female). (A) maxillule; (B) maxilla; (C) maxilliped; (D) P1, anterior. Scale bars: 20  $\mu$ m.



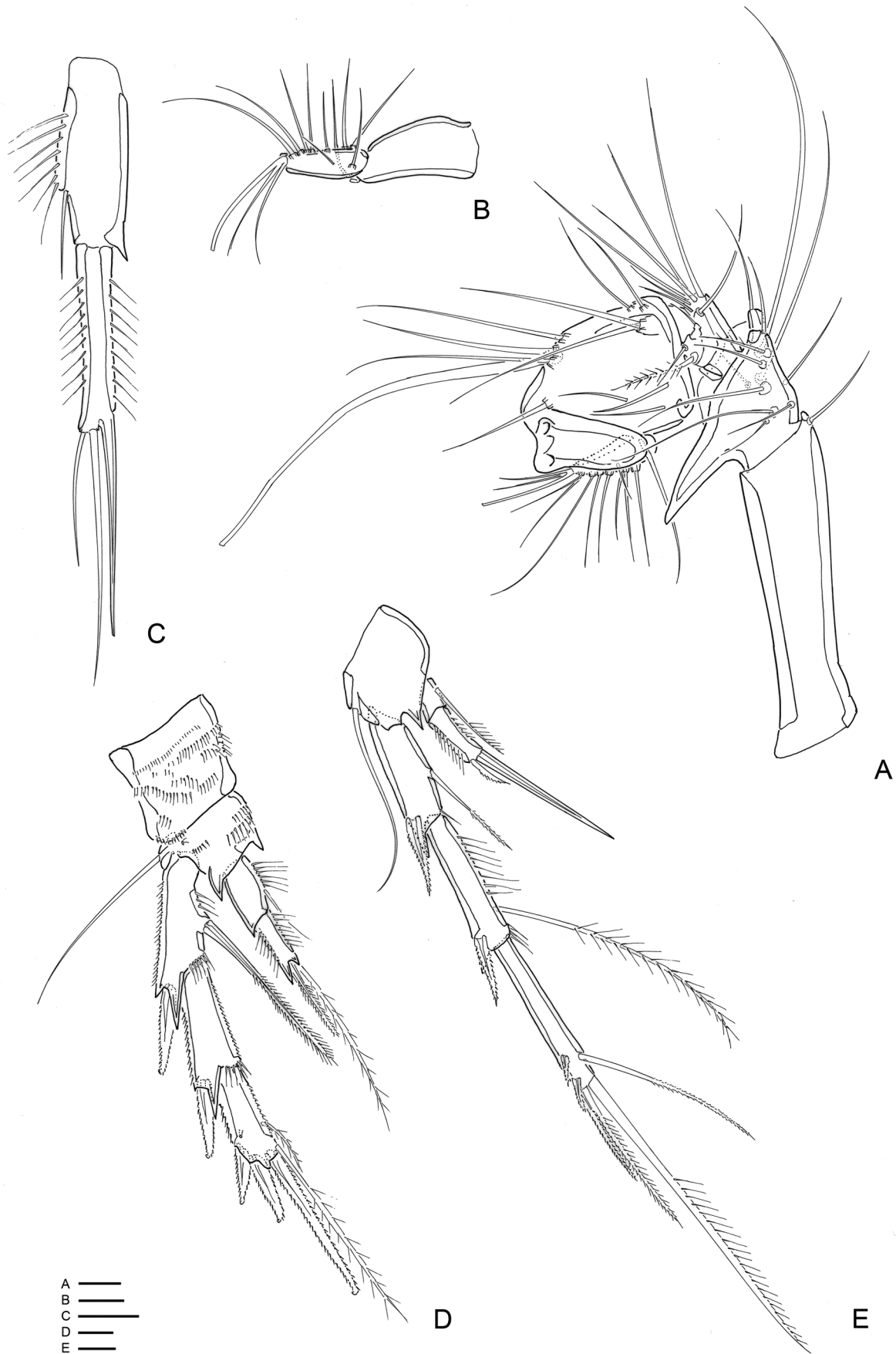
**FIGURE 6.** *Phyllopodopsyllus iuanamai* sp. nov. (female). (A) P2, anterior; (B) P3, anterior, P3 enp-2 inner seta arrowed; (C) P4, anterior; (D) P5. Scale bar: 50  $\mu$ m (A–C), 90  $\mu$ m (D).



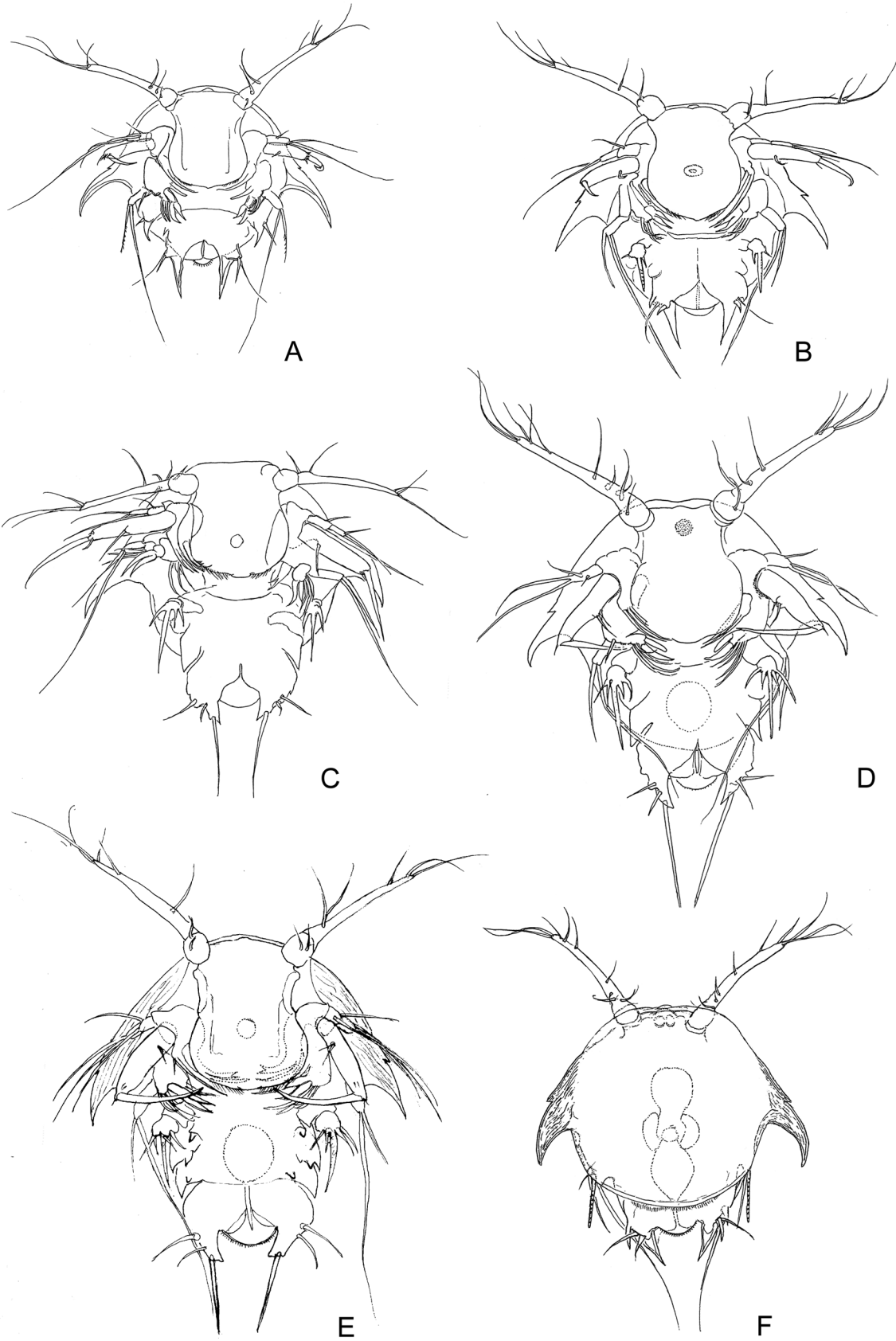
**FIGURE 7.** *Phyllopodopsyllus iuanamai* sp. nov. (male). confocal laser scanning microscopy images. (A) habitus, dorsal; (B) habitus, lateral; (C) habitus, ventral. Scale bar: 50  $\mu$ m.



**FIGURE 8.** *Phyllopodopsyllus iuanamai* sp. nov. (male). (A) urosome, dorsolateral (P5-bearing somite omitted); (B) urosome, ventral (C) P5, (D) P6. Scale bars: 50  $\mu$ m.



**FIGURE 9.** *Phyllopodopsyllus iuanamai* sp. nov. (male). (A) antennule; (B) antenna; segment 5 and 6; (C) P2 endopod; (D) P3, anterior; (E) P4, posterior. Scale bar: 10  $\mu$ m.



A - F ———

**FIGURE 10.** *Phyllopodopsyllus iuanamai* sp. nov. (nauplii). (A) NII, ventral; (B) NIII, ventral; (C) NIV, ventral; (D) NV, ventral; (E) NVI, ventral; (F) NVI, dorsal. Scale bars 50  $\mu$ m.

Male: Total body length from 600–722  $\mu\text{m}$  long ( $n = 6$ ,  $X = 678 \mu\text{m}$ ). Body cylindrical, 10 somites (Figs 7A–C). Prosome tergites with rounded epimeral lappets and hyaline margin. Urosomites with very prominent paired pointed processes (Figs 7A–B, 8A) dorsally, except on anal somite. Sensilla covering the whole body laterally and dorsally, except on wide prosome hyaline margin (Figs 8A–C).

Caudal rami fusiform, about 8 times as long as wide, 2.5 times as long as anal somite, with proximal third 3 times wider than distal third and armed with 7 setae. Seta V well developed and remaining setae as in figures 8A–B.

Rostrum, antenna, mandible, maxillule, maxilla and maxilliped similar to those of female.

Antennule sub-chirocer. 6-segmented (Fig. 9A). Segment 1: 3 times as long as segment 2; segment 2 with spiniform process. Setal formula: 1, 12, 10, 11+1 ae, 1, 11+1 ae.

Leg 2 exopod as in female. Endopod (Fig. 9C) first segment with naked seta and setules along inner margin; second segment slender, with setules along inner and outer margin and 3 apical elements: 2 naked setae and 1 outermost setae strong and basally fused to the segment.

Leg 3 (Fig. 9D) exopod-1–2 as in female, exopod-3 with reduced setae. Endopod first segment with naked seta and setules along inner margin; second segment with setules along inner and outer margin and 2 apical elements.

Leg 4 (Fig. 9E) exopod as in female. Endopod first segment with delicate unipinnate seta; second segment with 2 apical elements.

Leg 5 (Fig. 8C), with distinct basoendopod carrying outer seta and 3 elements; exopod tapering distally and with 5 elements: 2 inner subdistal, 1 apical and 2 outer ones.

Leg 6 (Fig. 8D) a process bearing 3 setae (innermost strong and unipinnate).

**Description of naupliar stages.** The naupliar stages obtained were NII–VI.

NII (Fig. 10A): body 132  $\mu\text{m}$  long, roundish, dorsal shield or scutum with two lateral, pointed acute processes and two posterior pointed acute processes limiting a deep round indentation area surrounding the anal region ventrally. The body is divided into cephalic or front area and hind body by a transversal suture. A small round area with setulose edge protrudes beyond the cephalic shield of the nauplius between the two acute posterior processes. A small lateral process next to each of the acute posterior processes carries two setae. Labrum (posteriorly rounded) is wide and reaches the middle of the body. Two minute pointed processes are present in the ventral body wall laterally below the transversal suture. Antennules uniramous, 3-segmented with first segment very short unarmed, second segment bears one ventral seta, third segment very long and thin, with 7 setae. Antenna: coxa (probably with masticatory process not seen), basis with two long spines turned medially, 1-segmented endopodite and 1-segmented exopodite. Endopodite carries a stout claw terminally extending medially and a little seta proximally. The insertion point of the claw is surrounded by a row of setules. The exopodite has a lateral outer seta and two long terminal setae. Mandible with coxa, basis, 1-segmented endopod and exopod. Basis carries a finger-like process turned medially. Exopodite with 2 long setae terminally and one short lateral seta proximally. Endopodite 2-segmented with first segment carrying two small setae and second segment with a scissor-like structure composed of two foliaceous or spatulate pointed setae. Maxillule: Anlage present in the form of a pointed lateral process bearing a little lateral outer spine.

NIII (Fig. 10B): Differs from NII as follows: 144  $\mu\text{m}$  long. A1 with 4–5 instead of 7 setae (their number varies in the same specimen and from nauplius to nauplius), two additional small round lateral lobes on ventral body wall in place of the two minute posterior pointed processes below the transversal suture. Antennal exopod with two segments (one seta on first and two setae on second segment). Mandible endopod with additional seta on first segment. Maxillule Anlage with changes, three setae appear instead of one. The middle seta is longer and stouter than the other two. Masticatory process not clearly seen.

NIV (Fig. 10C): Differs from NIII as follows: 180  $\mu\text{m}$  long from anterior cephalic region to end of posterior spines; cephalic shield much shorter; posterior pointed processes shorter and wider, bearing a longer inner caudal seta and two lateral small setae. Posterior edges or margins of hind body with deeper lateral indentations in ventral area forming a lateral lobe with pointed edge on each side; antennal exopod with one seta added terminally. Coxal maxillular lobe extends transversally posterior to transversal suture. Maxillule divided into two segments, with addition of one more seta to distal segment.

NV (Fig. 10D): Differs from NIV as follows: 250  $\mu\text{m}$  long. A1 distal segment with 7–9 setae. A2 with one inner seta added medially on exopod, one seta added to endopod in A2. Terminal seta on posterior region longer

NVI (Fig. 10E): Differs from NV as follows: 290  $\mu\text{m}$  long. Terminal caudal seta shortened. Second segment of

A1 with 2 setae. A2 with masticatory process clearly visible; number of setae on endopod diminishes to 3 or 4. Posterior edge or margin of the dorsal shield finely setulose. Marginal indentations on the ventral body wall separate the anlagen of the future maxilla, maxilliped, and legs.

**Etymology.** The species name is derived from the Tupi-Guarani (a South-American native language) *iu* (meaning spine, projection) and *anama* (meaning strong, stout).

**Biological note.** One of the most common and abundant species along São Sebastião coastal substrates. Reproduction most intense during spring time.

***Phyllopodopsyllus pseudokunzi* sp. nov.**

(Figs. 11–16)

**Type material.** Collected in Parcel da Praia Grande, São Sebastião Island (23°49'02"S 45°24'19"W). Four females (2 ovigerous), 14 Feb. 2007, Oliveira, J. M. coll. Holotype and paratypes deposited under same registration number - MZUSP 28029). The probable nauplius of the species collected in 23°50'00"S 45°26'82"W. The nauplii separated from the sample were very damaged and covered with debris, after a period of very rough sea.

**Type locality.** Parcel da Praia Grande, São Sebastião Is. (23°49'02"S 45°24'19"W).

**Diagnosis.** A *Phyllopodopsyllus* with foliaceous caudal rami inserted vertically (like the caudal fin of a fish) in the last urosomite, antennules with first segment very elongated; hook-like processes turned posteriorly on the second segment; leg 4 endopod 2-segmented; female caudal rami 2.5 times longer than anal segment, almost oval in shape, dilated distally and straight at insertion point in anal segment.

**Description of adults.** Female (Figs 11A–C): Total body length from 680–830 µm long (n = 4, X = 751 µm). Color when alive light yellow-brown. Body with 10 somites, with hyaline frill on 2<sup>nd</sup> to 4<sup>th</sup> posterior urosomite margins. Very fine setules cover the whole body, rami included. Genital double somite with subdivision line visible dorsally and laterally. Genital area as in Fig. 12B. Anal operculum with 2 minute sensilla laterally, one on each side, but inserted at a distance anteriorly to the operculum which has a setulose margin (Fig. 12A).

Caudal rami (Figs 12A–C) 2.5 times longer than anal segment, almost oval in shape, dilated distally and straightened at insertion point in anal segment. Seta IV and V not observed in any of the 4 specimens examined, excepting for a small triangular process which seems to show 2 terminal insertion points located where the insertion points of the setae should be (Fig. 12C). Two setae are present marginally (seta I or II and seta III); dorsal articulated seta present.

Rostrum flat. Defined at base, with two frontal sensilla.

Antennule (Fig. 13A) 8-segmented. Segment-1 very long, 4 times longer than wide, about as long as 5 following segments summed together. Segment-2 with prominent pointed hook turned posteriorly. Setal formula: 1, 9, 7, 4+1 ae, 2, 3, 4, 7+1 ae.

Antenna (Fig. 13B) biramous. Basis unarmed with row of short spinules along inner margin. Exopod 1-segmented with 3 bipinnate setae (one of which confluent with exopod). Endopod 2-segmented, segment-1 unarmed with row of short spinules along inner margin, segment-2 with 7 terminal setae (one unipinnate), 3 pre-terminal setae (two lateral) and rows of spinules (1 median and 2 marginal).

Mandible (Fig. 13C) coxal gnathobase with 2 tooth-like processes, row of spines and 1 pinnate seta. Basis with tuft of spinules and two bipinnate setae. Exopod 1-segmented with 3 setae (1 lateral and 2 apical). Endopod 1-segmented with 2 medial and 6 terminal setae.

Maxillule (Figs 13D–F) damaged, arthrite with 6 spines and 3 bipinnate setae apically and 2 marginal setae. Coxa with 6 setae on endite and 1 seta on exite (displaced during animal manipulation). Basis with 7 setae. Endopod with 4 bipinnate setae. Exopod missing.

Maxilla (Fig. 14A) with 4 syncoxal endites bearing 2, 1, 3, 3 spines proximal to distal segment, respectively. Basis armed with 1 spine and 2 setae. Endopod 2-segmented, proximal segment with 3, distal segment with 4 setae.

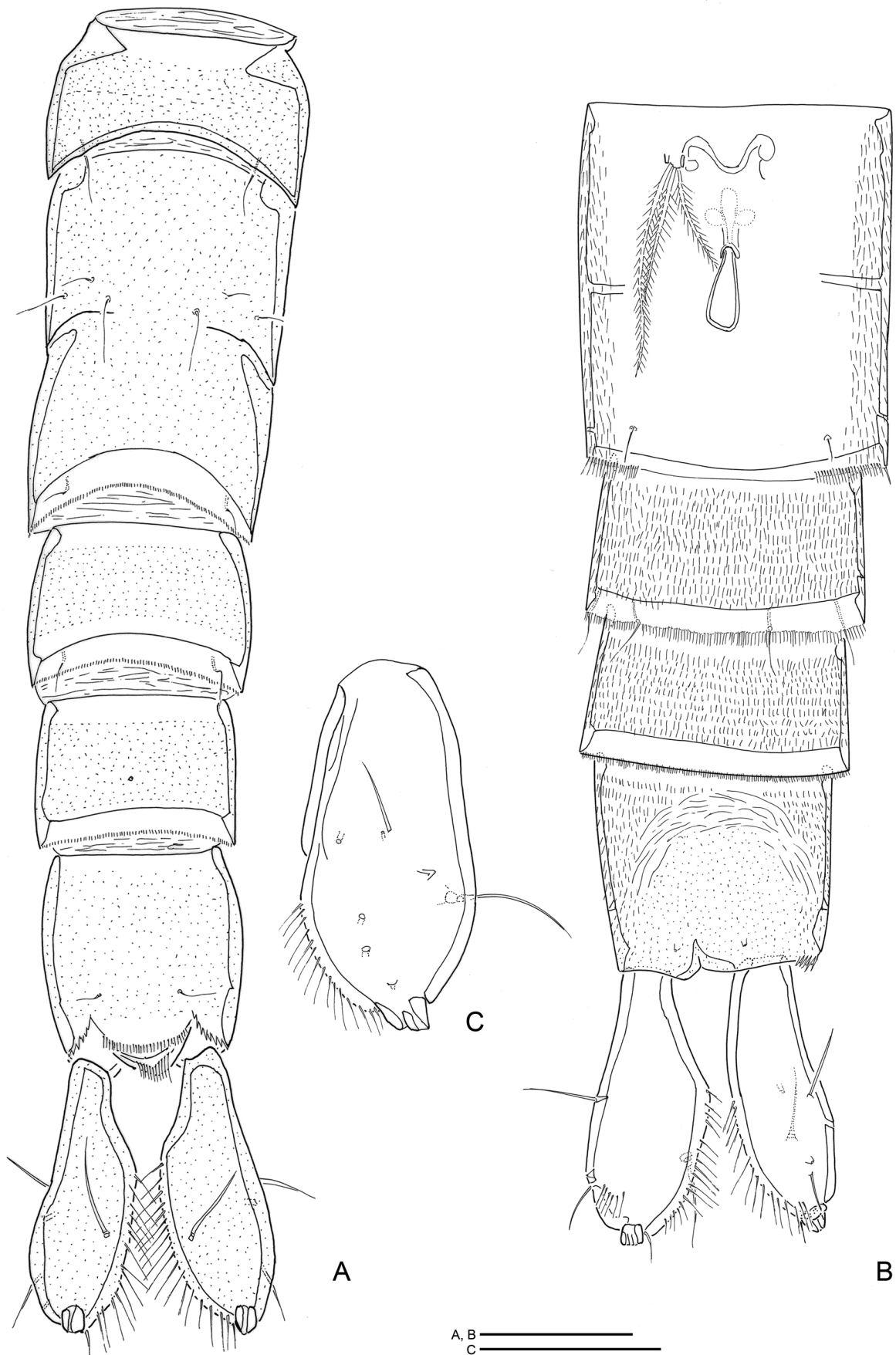
Maxilliped (Fig. 14B) prehensile. Syncoxa with 3 bipinnate setae. Basis with 1 seta. Endopod 1-segmented, with terminal claw and 2 accompanying setae.

Leg 1 (Fig. 14C) biramous. Coxa ornamented with rows of small spinules. Basis with rows of small spinules, bearing 1 bipinnate seta near proximal inner corner and 1 missing element on outer margin (arrowed). Exopodal segments with small spinules along outer margin and exp-2 with setules along inner margin. Enp-1 4.5 times longer than enp-2, with spinules marginally; enp-2 with small spinules along outer margin.

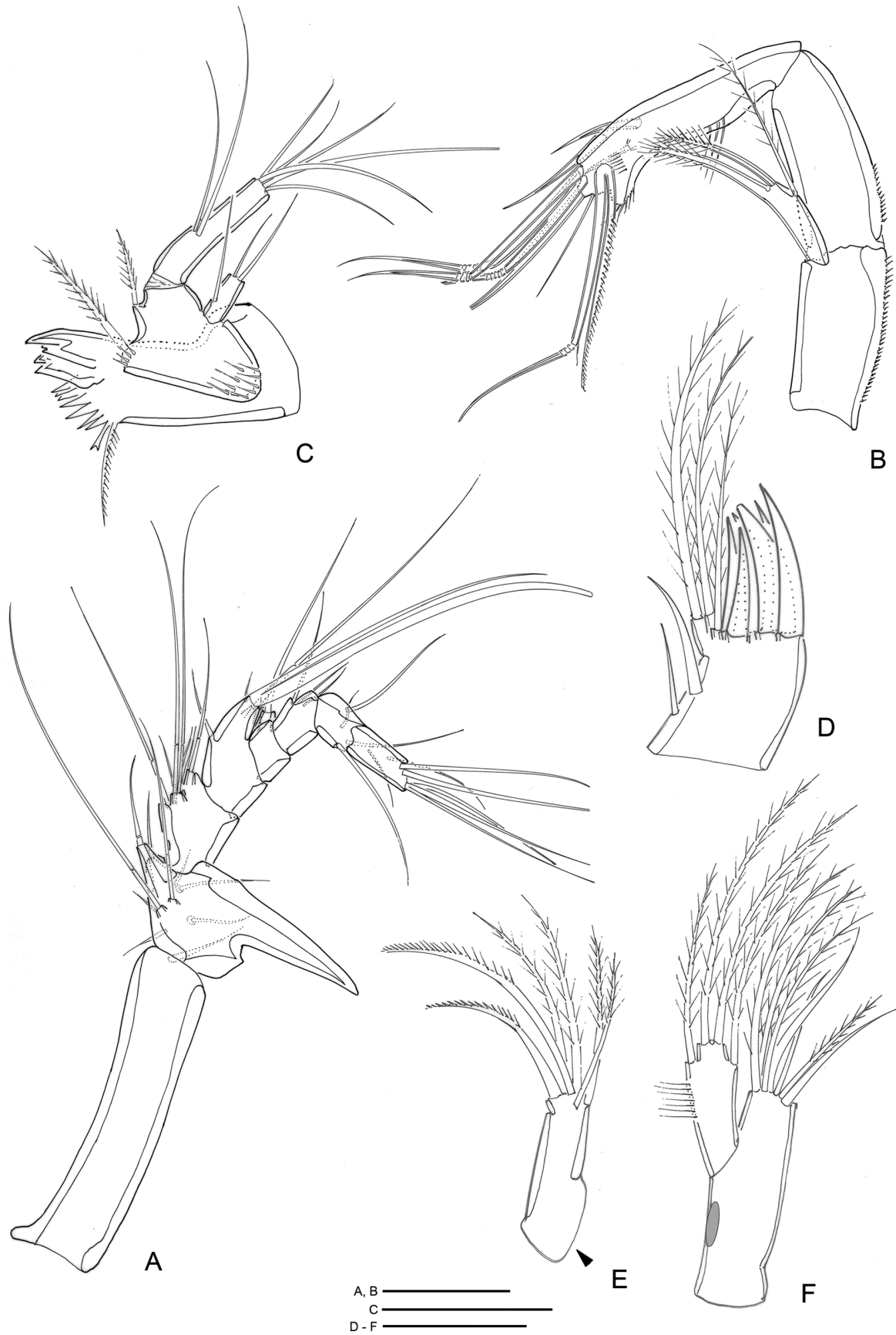




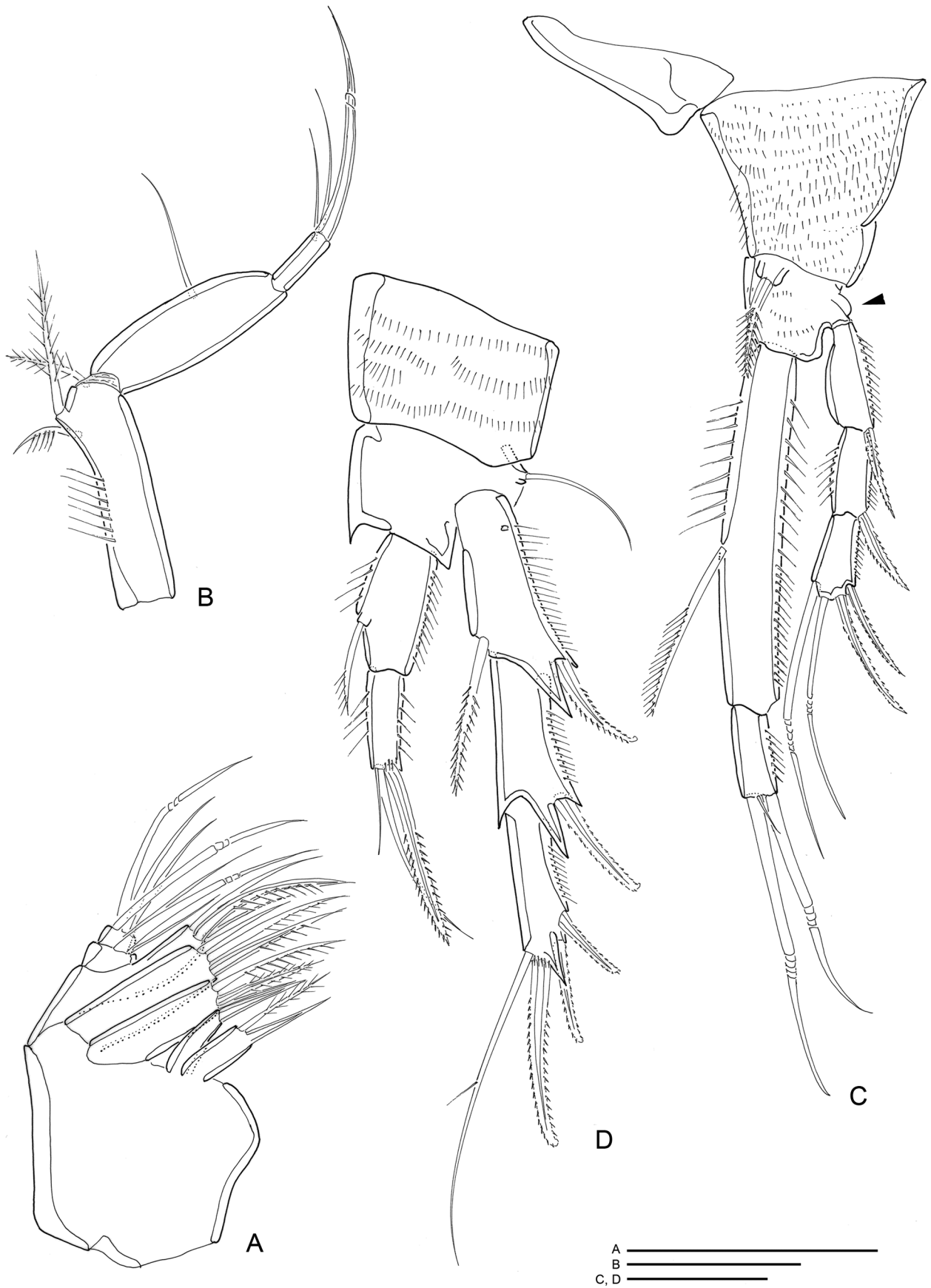
**FIGURE 11.** *Phyllopodopsyllus pseudokunzi* sp. nov. (female). Confocal laser scanning microscopy images. (A) habitus, dorsal; (B) habitus, lateral; (C) habitus, ventral. Scale bar: 50  $\mu$ m.



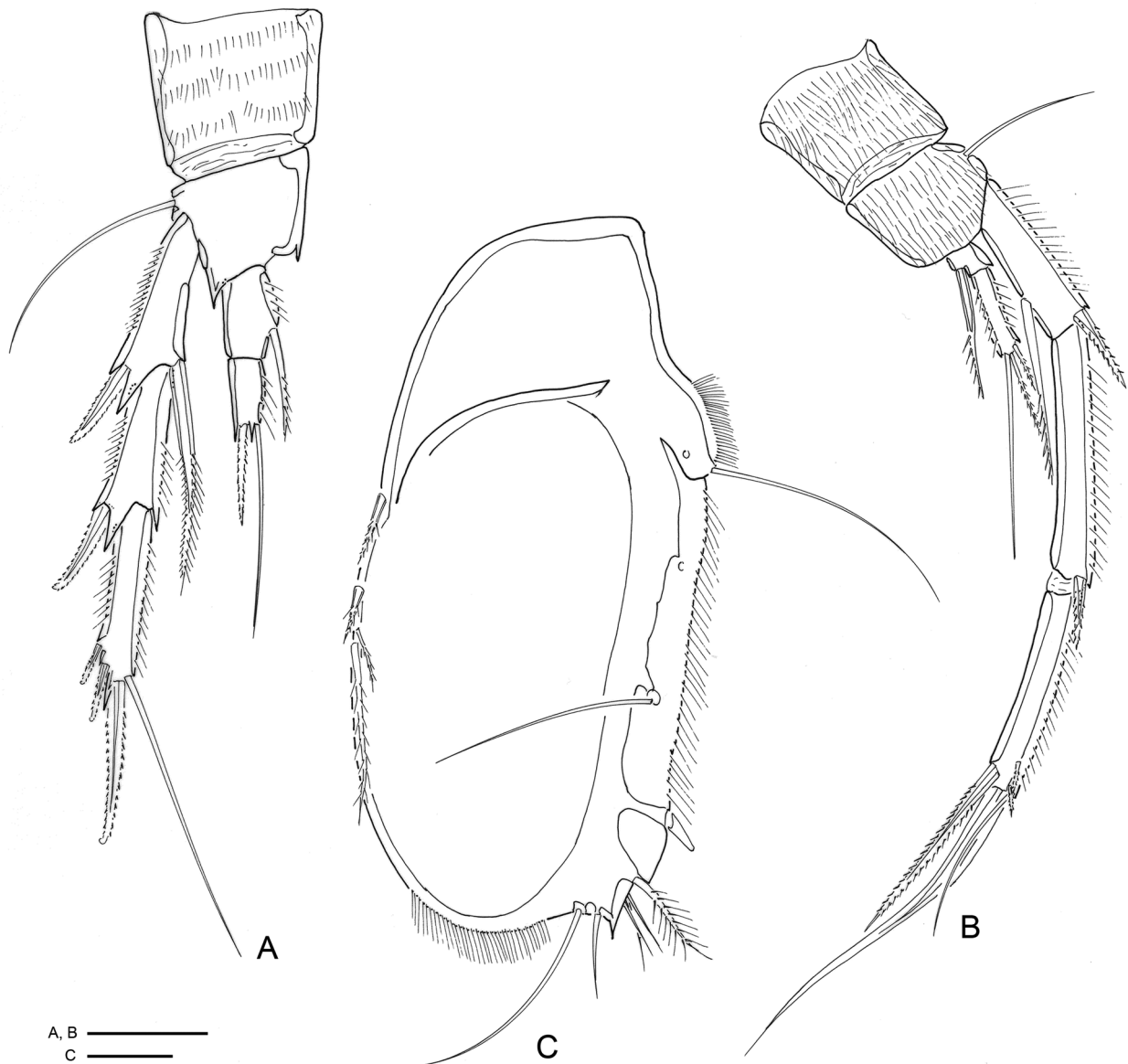
**FIGURE 12.** *Phyllopodopsyllus pseudokunzi* sp. nov. (female). (A) urosome, ventral (P5-bearing somite omitted); (C) caudal ramus, lateral. Scale bar: 50  $\mu$ m.



**FIGURE 13.** *Phyllopodopsyllus pseudokunzi* sp. nov. (female). (A) antennules; (B) antenna; (C) mandible; (D) arthrite of maxillule; (E) coxa of maxillule, missing element arrowed; (F) basis and endopod of maxillule, exopod missing. Scale bars: 25  $\mu$ m.



**FIGURE 14.** *Phyllopodopsyllus pseudokunzi* sp. nov. (female). (A) maxilla; (B) maxilliped; (C) P1, anterior, missing element arrowed; (D) P2, anterior. Scale bars 25  $\mu$ m.



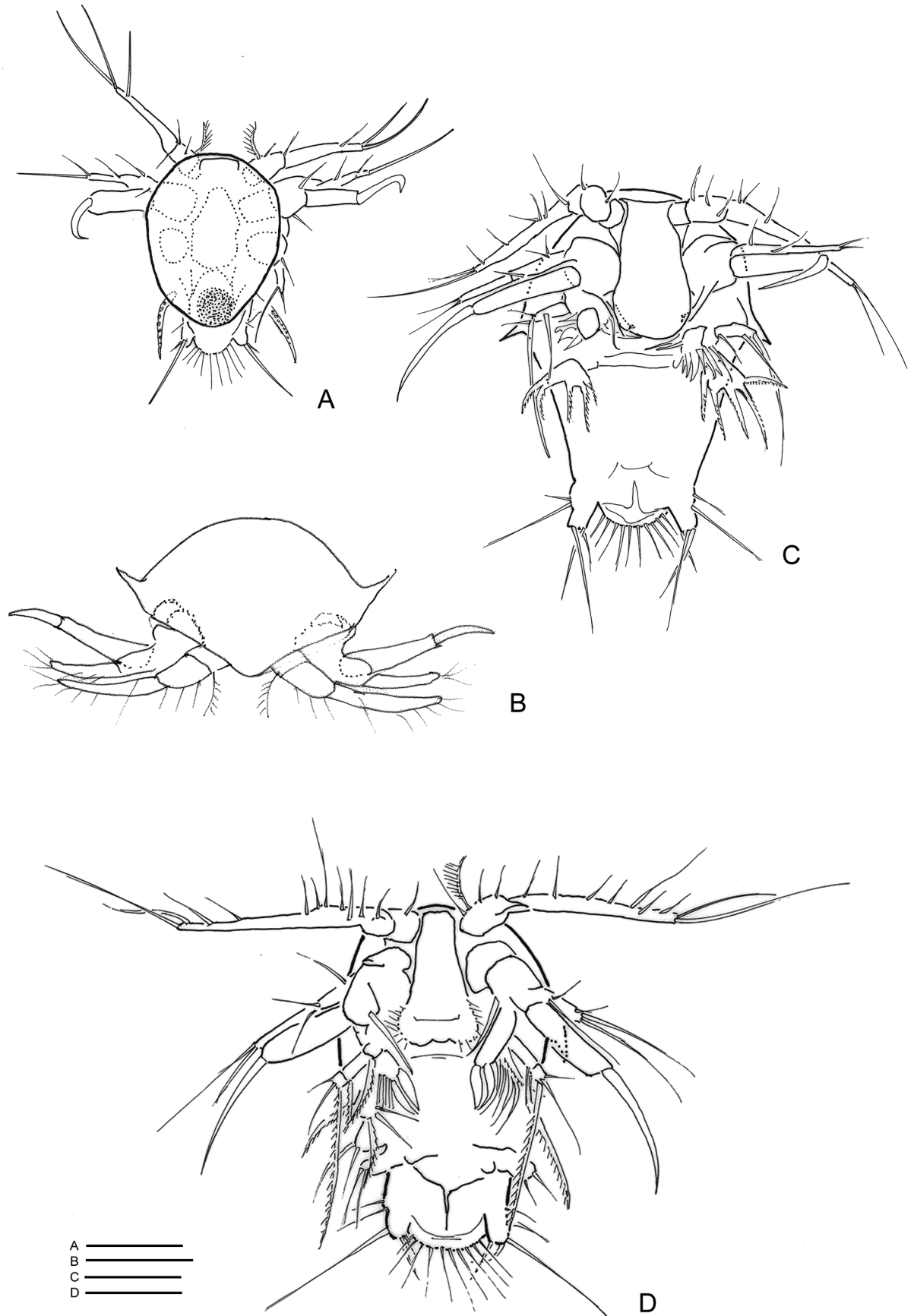
**FIGURE 15.** *Phyllopodopsyllus pseudokunzi* **sp. nov.** (female). (A) P3, anterior; (B) P4, anterior; (C) P5. Scale bars: 25  $\mu$ m.

Legs 2–3 biramous (Figs 14D, 15A). Coxa ornamented with rows of spinules. Basis bearing 1 seta on outer corner, and spiniform projection on distal rim between exopod and endopod and on distal inner corner. Exopod 3-segmented. Endopod 2-segmented. Inner and outer margins of exopods and endopods ornamented with setules and spinules, spiniform projections on distal outer corner of exopods.

Leg 4 biramous (Fig. 15B). Coxa ornamented with rows of spinules. Basis with rows of spinules, bearing 1 seta on outer corner. Exopod 3-segmented; elongated segments with setules along outer margin. Endopod 2-segmented; enp-2 with setules marginally.

Legs 1–4 (Figs 14C–D, 15A–B) armature formula as follows:

	Exopod	Endopod
Leg 1	0. 0. 022	1. 030
Leg 2	1. 0. 022	1. 030
Leg 3	1. 0. 022	1. 020
Leg 4	1. 0. 122	1. 030



**FIGURE 16.** *Phyllopodopsyllus pseudokunzi* sp. nov. (nauplii). (A) NII, dorsal; (B) NIV, frontal; (C) NIV, ventral; (D) NVI, ventral. Scale bars: 45  $\mu$ m.

Leg 5 (Fig. 6D) large and foliaceous. Outer margin from proximal to distal with row of setules, 1 proximal slender seta (remnant of outer setophore), setules along margin, 1 seta inserted medially directed to inwards, 1 spine, 1 bippinate seta, 3 long spinules, 1 pointed process and 2 setae. Distal area with fine setules. Outer margin with 4 bipinnate setae.

Genital field as in Fig. 12B.

Leg 6 (Fig. 12B) with 3 bipinnate setae on genital segment.

**Description of naupliar stages.** This tetragonicipitid nauplius (Figs 16A–D), collected in the region, is the probable larva of this species because the females, sampled at the time, were ovigerous and the eggs in the brood-pouches were ripe (transparent), so we expected to find nauplii. Those figured here were the only tetragonicipitid nauplii not yet identified which occurred in the region. The naupliar stages collected were NII, IV and VI.

NII (Fig. 16A) 70  $\mu\text{m}$  long. Dorsal shield oval, longer than wide, posterior region of body not covered by shield. Labrum straight. Antennule 3-segmented, second segment bearing a pinnate seta and a simple seta, third segment bearing 4 setae. Antenna biramous: exopod with 2 lateral and one terminal seta, near to endopod insertion point an anterior seta; endopod one segmented with long hook-like terminal spine, below insertion point of endopod a posterior seta. Mandible with one-segmented exopod bearing 2 terminal setae and at insertion point another outer seta. Anlage of maxillule present in form of a thick spiny seta. Posterior region of body rounded, ornamented with a row of long setules. Two lateral ventral processes, one on each side of posterior region, bear a longer inner and a shorter outer seta. Another outer lateral seta inserted pre-terminally.

NIV (Figs 16B–C) differs from NII as follows: 155  $\mu\text{m}$  long, one lateral pointed process on each side of the first third of dorsal shield. Body longer, posteriorly wider and rounded, with strong indentation ventrally towards anal opening. On each side of the anal indentation a rectangular process bearing two lateral outer setae and three terminal setae of different lengths. Labrum is long, anteriorly straighter and posteriorly wider. Antennal exopod two-segmented: three setae on first segment and two terminal setae on second, long basal process directed medially partly covered by labrum is visible. Mandible: coxa with two seta-like processes directed medially towards labrum; exopod one-segmented bearing two terminal setae and a proximal seta; endopod bearing two thick pointed setae and two or more simple setae. Maxillule anlage hand-like, a more or less triangular structure bearing five thick pointed setae.

NVI (Fig. 16D) differs from NIV as follows: Body 155  $\mu\text{m}$  long; processes lateral to anal region shorter, anal region wider, outermost terminal seta on lateral process longer. Two lateral processes appear on each side of ventral body wall, bearing two setae each representing the anlage or primordium of future appendages. Antennule with increased number of setae (12–13) Labrum longer and straighter finely setulose along posterior margin. Antenna: coxa bears a masticatory process, and two pinnate setae turned medially towards labrum. Basis bears two pinnate setae turned posteriorly. Exopod: long, 4 or 5 segmented. Each segment bears one seta, except the last segment with two terminal setae. Mandible: endopod with 2 foliaceous or spatulate setae and four or five setae; exopod two or three segmented with a seta proximally, a lateral and distal seta on second segment and two terminally. Maxillule with 5 strongly setulose spines in last stages. Anlagen of maxilla and of maxillipeds: lateral processes of ventral body bearing a pair of setae each. Posterior region with 2 lateral processes bearing 3 lateral setules and, terminally, 1 longer and 1 shorter seta.

**Remarks.** The nauplii separated from the sample were very damaged and covered with debris, collected after a period of very ruff sea.

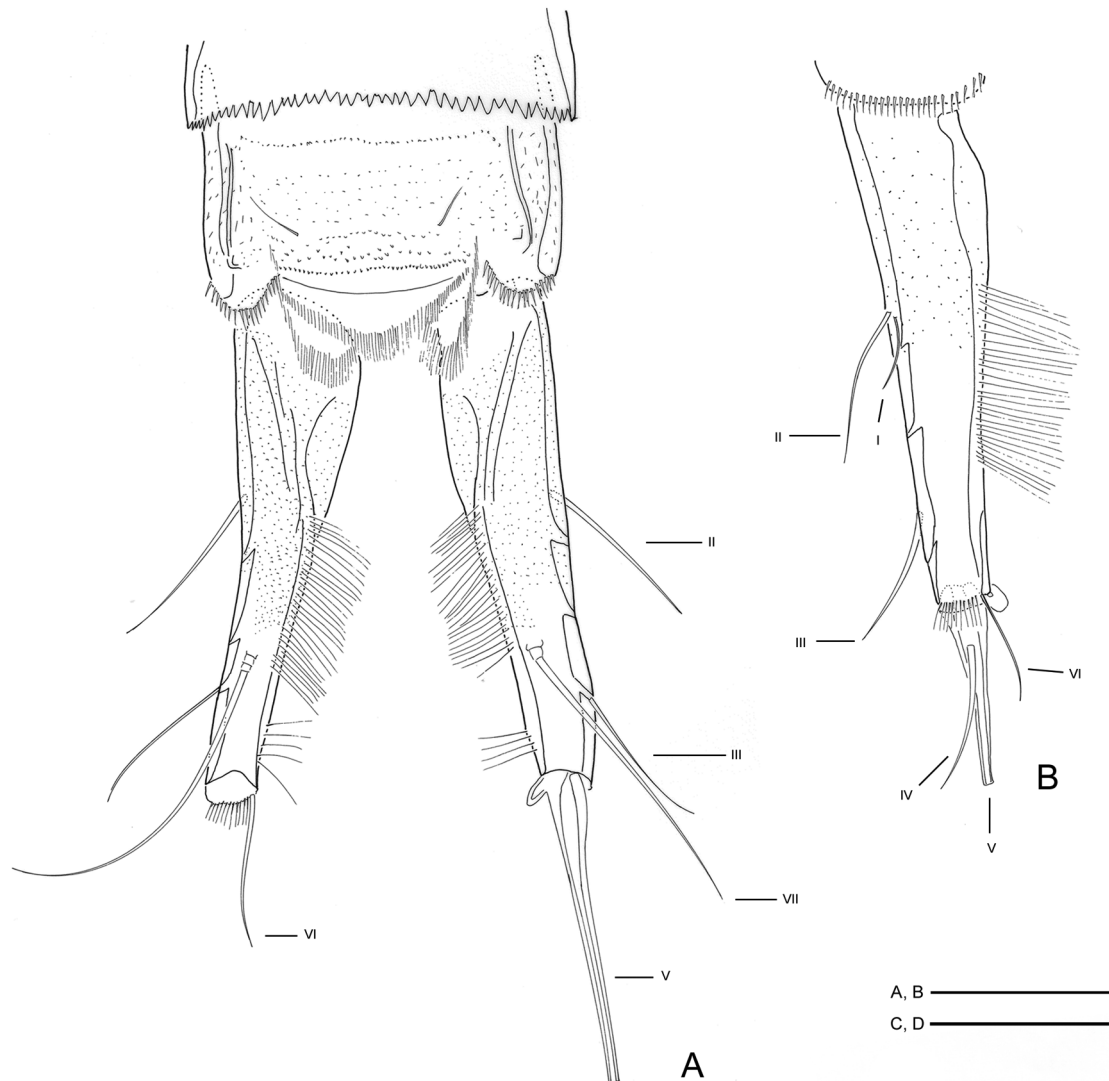
**Etymology.** The name species is derived from Greek *pseudo* (meaning false—used in science to denote a false resemblance to something). “Pseudo” and “kunzi” meaning “apparently like kunzi”.

### ***Phyllopodopsyllus setouchiensis* Kitazima, 1981**

(Figs. 17 & 18)

**Material examined.** São Sebastião, Guaecá Beach, Itaçucê (23°50'00"S 15°26'62"W): One female, 03 Mar. 2005; 3 females, 2 males, 1 copepodite and 5 nauplii, 18 May 2005; 2 females and 2 males, 08 Sep. 2005, in the intertidal zone; A. Pepato coll.

**Description of naupliar stages.** The nauplii of *P. setouchiensis* were briefly figured and described by Kitazima (1985). The Brazilian nauplii (Figs 18 A–F) are like the Japanese nauplii. We add a few details:



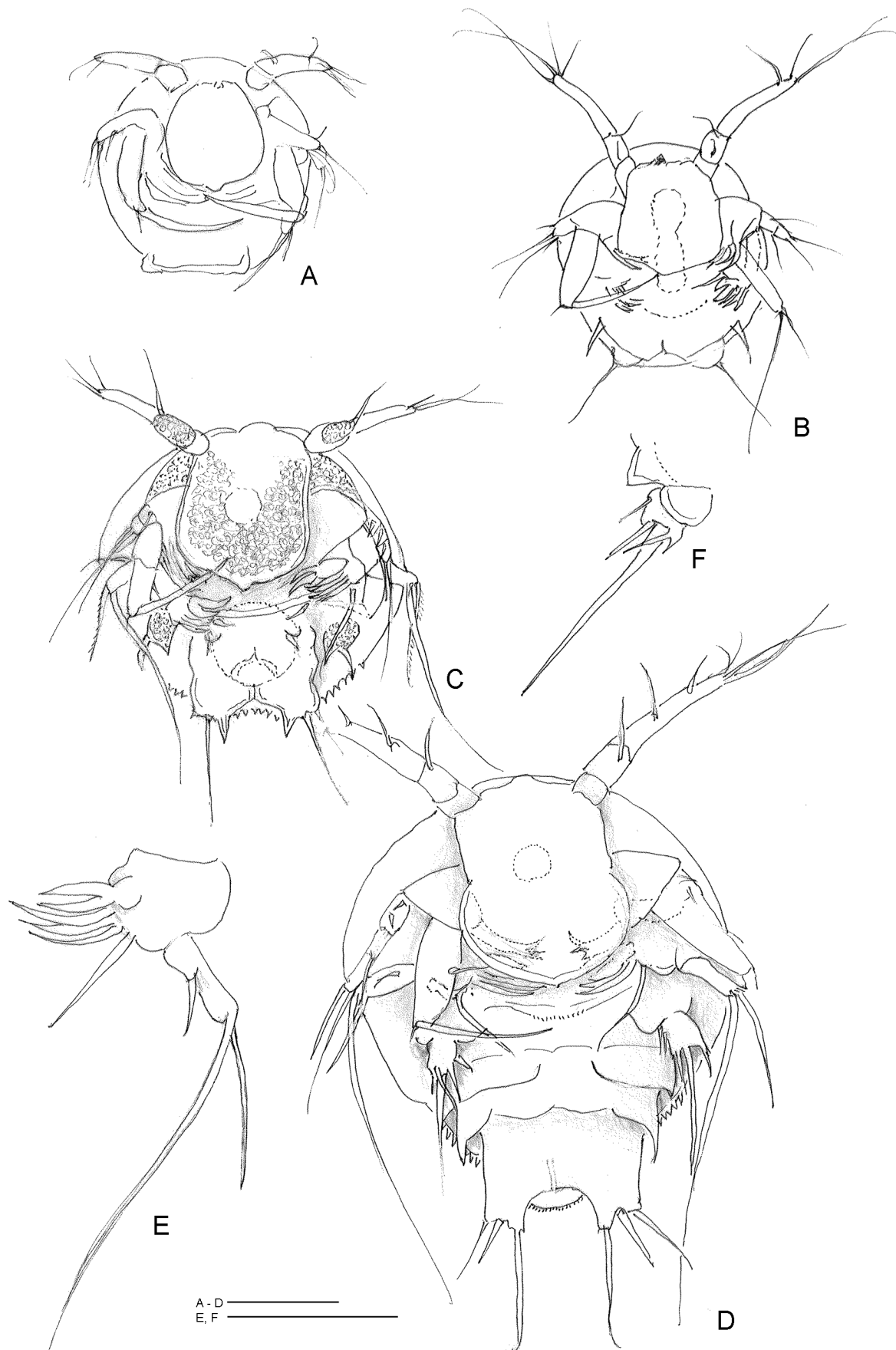
**FIGURE 17.** *Phyllopodopsyllus setouchiensis* Kitazima, 1981. (A) caudal rami, dorsal; (B) caudal ramus, lateral. Scale bars: 50  $\mu\text{m}$ .

NI (Fig. 18A) prae-eclosion, 98  $\mu\text{m}$  long, dorsal shield almost round, without ornaments. Labrum more or less round, extending from between antennule insertions to about middle area of ventral body wall. Antennular segmentation not very clear, with one seta proximally and three setae distally. Antenna with exopod 1-segmented bearing three setae; endopod 1-segmented with an inner marginal seta and two terminal setae, one hook-like, another, very small, inserted at the basis of the bigger one. Mandible, not clearly visible, with 1-segmented exopod, bearing two long terminal setae, inner one about twice the length of the other one.

NII (Fig. 18B) body round, about the same size as NI, differs from NI as follows: last antennular segment thinner Antennal basis: bears a curved process, terminally bifid, turned towards the labrum, added to the other already present. Exopod 1- or 2-segmented with two setae on first and three setae on second segment. Mandible with endopod bearing two fat pointed setae and three thin setules on basis and a seta on coxa. Maxillule anlage present in the form of a strong pointed seta situated laterally on each side of the ventral body wall. On each side of the anal indentation, there is a small rounded process limiting the distal edge of the anal region. The rounded processes carry a terminal seta each.

NIII (Fig. 18C): Body 132  $\mu\text{m}$  long, covered by a dorsal shield finely serrate marginally. Body divided by ventral suture line into frontal and back ventral body wall. Antennules with 3 segments, not always clearly delimited. First two segments very short, third segment twice the length or longer than the two first summed together. First segment glabrous; second segment has 1–2 setae; third segment bears 2 terminal, and a lateral, pre-terminal seta. Antenna: exopod 2- or 3-segmented: first segment with two setae second with three. Mandible: as in





**FIGURE 18.** *Phyllopodopsyllus setouchiensis* Kitazima, 1981 (nauplii). (A) NI prae-eclosion, ventral; (B) NII, ventral; (C) NIII, ventral; (D) NVI, ventral; (E) NVI mandible; (F) NVI maxillule. Scale bars: 10  $\mu$ m.

NII. Maxillule: a lateral lobe below ventral suture line bearing a pointed process posteriorly and an outer curved pointed spine. Between the two maxillule lobes the ventral body wall protrudes from the suture line backwards with a square shape bearing anteriorly and laterally two little round processes, with a setule each, and posteriorly, medially, the anal area indentation. This area shows posteriorly, on each side, a minute pointed process, a small spine and a seta.

NVI (Fig. 18D) differs from NIII as follows: body length 199  $\mu\text{m}$ , last antennular segment with six setae, antennal coxa basis bears a well-developed masticatory process, a 3-segmented exopod, with two setae on first segment, one seta on second, and two setae on last segment (in Fig. 18 D represented on left side of nauplius). Mandible (Fig. 18E) basis with a thin blade-like process directed medially; exopod with a short and a very long bipinnate seta; endopod with 2 blade-like (scissor-shaped) processes and three setae. Maxillule (Fig. 18F) 2-segmented, with 5 spines on distal segment. Anlagen or primordia of maxillae and maxillipeds represented by lateral pointed processes. Posterior region of the nauplius divided into 2 almost square pointed terminal processes, bearing 3 setae each (the inner seta, the longest) and the dorsal shield or scutum with denticles along the posterior margin and the anal operculum with a fine setulose margin.

**Remarks.** *Phyllopodopsyllus setouchiensis* is a widespread and variable species. Kitazima (1981) called attention to its variability showing the drawings of the holotype female P4 compared to the same appendage of the paratype collected in the same region. The Brazilian specimens are characterized by thinner furcal rami (Figs 17A–B) and seta V insertion on the caudal rami is slightly different from the Japanese specimens. Described from the Inland Sea of Japan (Kitazima 1981) it was also collected in Costa Rican waters, both in the Caribbean Sea and in the Pacific Ocean (Mielke 1989, 1992). According to this author “the populations of Japan, Hawaii (Kunz 1984) and Costa Rica belong to one and the same species”. Following Mielke’s (1992) reasonings, we also consider the Brazilian specimens as belonging to the same species in spite of these slight differences. Brazilian specimens were identified as *P. setouchiensis* because the leg setal formulae, the genital structures and the nauplii (represented by Kitazima 1981, 1985) were the same in Japanese and Brazilian species.

### ***Phyllopodopsyllus aegypticus* Nicholls, 1944**

(Fig. 19)

**Synonymy.** *Phyllopodopsyllus gertrudi* Kunz, 1983 (fide Karanovic *et al.* 2001)

**Material examined.** São Sebastião, Cigarras Beach (23°43'47.36''S, 45°23'53.16''W): Ten females and 10 males, 03 Mar. 2005, in the intertidal zone; G. Lotufo coll. São Sebastião, Sitio de Calhetas Beach (23°49'51''S, 45°31'26''W): Ten females and 10 males; 20 copepodites and 30 nauplii, 01 Jan 2009, from rock pool; L. Tomita Simões coll.

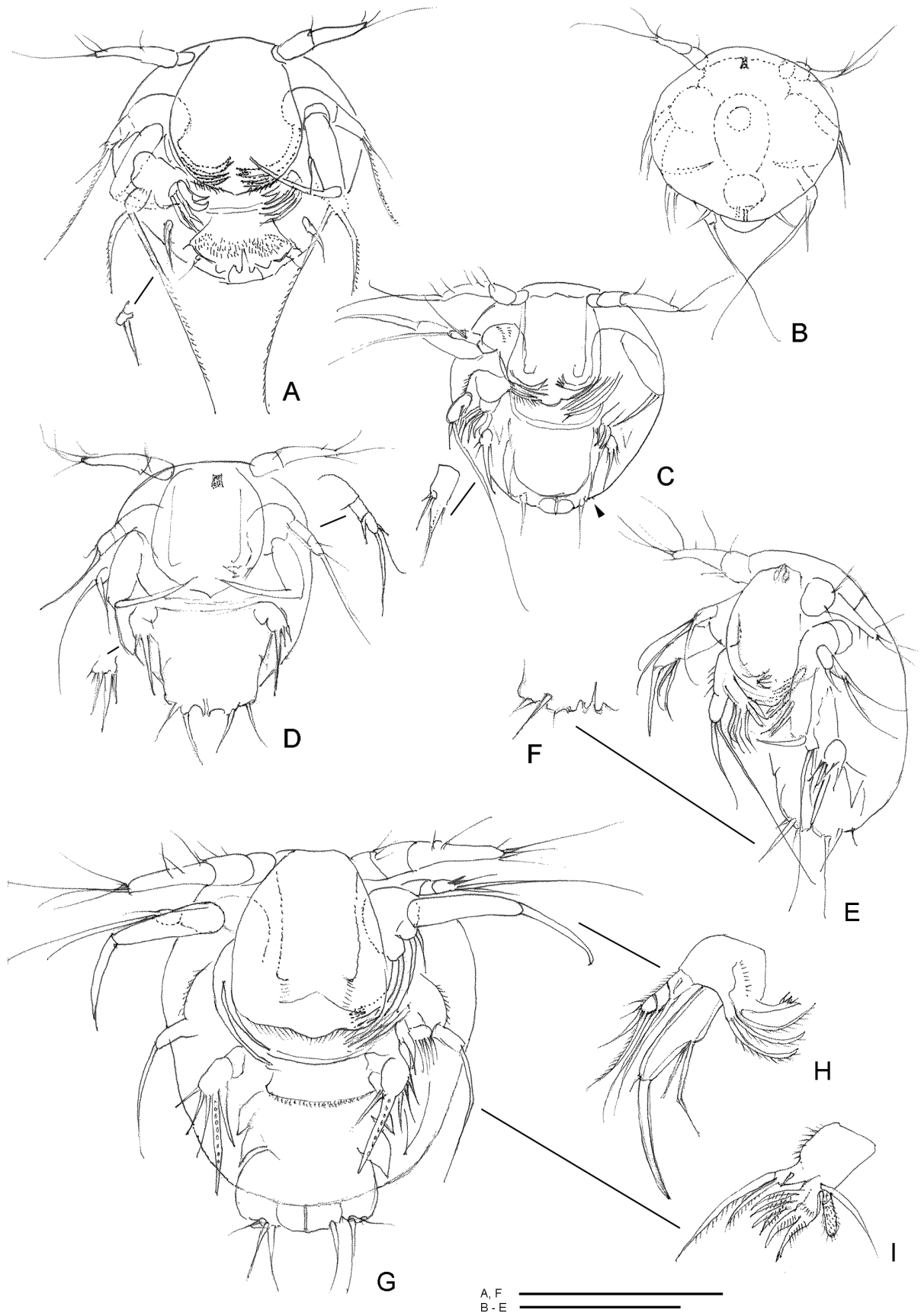
**Description of naupliar stages NII–NVI.** The naupliar stages obtained were NII–VI.

NII (Figs 19A–B) 100  $\mu\text{m}$  long. Body round, slightly produced posteriorly, reddish-brown coloured, very transparent. Only a small part of posterior region is not covered by dorsal shield as shown in Fig. 19B. Labrum wide, oval shaped, setulose along posterior margin, arising from near anterior margin between bases of antennules, extending caudally across the ventral surface of the nauplius. Posterior to labrum a ventral butterfly-shaped area, heavily setulose, bears a pair of small setae on each side, of which the outer pair protrude beyond the dorsal shield (Fig. 19B). Posteriorly and medially there is an indentation, the anal indentation and suture. Antennule 3-segmented, extending beyond dorsal shield, the first segment bare; the second with one seta, the third bearing 3–4 setae.

Antenna: Coxobasis with two processes medially directed. Exopod 1- or 2-segmented with 3 terminal setae, of which one very long. Endopod strongly developed, longer than exopod, with a long terminal claw, a small medial seta and a very small seta terminally. The coxal processes bear each 2 confluent pinnate setae directed medially to the labrum. The most anterior seta is terminally pinnate.

Mandible: Coxobasis, rectangular in outline, with a row of setules. Coxa with a digitiform, setulose process difficult to perceive below the geniculate setae. Endopod, not distinctly two-segmented, with three geniculate setae proximally and 2 modified setulose setae forming a scissor-like structure distally. A long row of setules ornaments the basis near the insertion points of the setae. Mandibular exopod with 2 setae, one over-reaching the end of the nauplius and the other shorter.

Maxillule: a small protuberance with a short little spine and a minute setule.



**FIGURE 19.** *Phyllopodopsyllus aegypticus* Nicholls, 1944 (nauplii). (A) NII and detail of maxillule, ventral; (B) NII, dorsal; (C) NIII and detail of maxillule, ventral; (D) NIV and detail of antennal exopod and maxillule, ventral; (E) NV, latero-ventral; (F) NV right ventral furcal region; (G) NVI, ventral; (H) NVI antenna; (I) NVI mandible. Scale bars: 10  $\mu$ m.

NIII (Fig. 19C): Body slightly more elongated. Differs from NII mainly in the length of the posterior terminal setae. The maxillule anlage now shows four terminal setae: one longer and stronger, 2 very thin ones, lateral to the strong one and a minute setule. Two long pointed processes are added (arrowed), one on each side of the ventral wall, between the maxillule and the former butterfly structure. Sometimes the pointed process only appears in later naupliar stages.

NIV (Fig. 19D) differs from NIII as follows: 125 µm long, body with 2 terminal setae on each side posteriorly, maxillule bearing thicker setae on outer and inner sides of the main thick seta. Antennule with one seta added to third segment.

NV (Fig. 19E) differs from NIV as follows: 159 µm long, body with three terminal setae on each side posteriorly. The pointed process (Fig. 19F) of NIII is now shorter and wider proximally.

NVI (Fig. 19G) differs from NV as follows: 171 µm long, body with three pairs of anlagen of the next pairs of appendages. The detail of the antenna (Fig. 19H) shows the increase in the number of setae and segments on the exopod. The detail of the mandible (Fig. 19I) shows pinnate setae, basis and mandibular finger-like process of the appendage.

**Remarks.** *Phyllopodopsyllus aegypticus* was described from Ghardaga in the Red Sea and was found again off the Nicobar Is. and South Andaman (Wells & Rao 1987), in Hawaii (Kunz, 1984) and in Costa Rica (Mielke 1992). Its occurrence in Brazilian waters is perhaps another indication that the genera of the Tetragonicipitidae have a tendency to wide distribution, such as already observed for *P. setouchiensis*.

The naupliar stages of *P. aegypticus* differ from most of the other tetragonicipitid nauplii occurring in the region by the absence of pointed outer protuberances on the margins of the dorsal shield (Figs 19A–C). It is, in general shape, similar to the nauplius of *P. setouchiensis*.

## Genus *Laophontella* Thompson & Scott, 1903

*Laophontella horrida* (Por 1964)  
(Fig. 20)

**Material examined.** São Sebastião Is., Parcel da Praia Grande (23°51'30''S 45°25'00''W): One female, 2 males, 2 copepodites. 14 Feb. 2007. Oliveira J. M. coll. São Sebastião, Guaecá Beach, Itaçucê (23°50'00''S 45°26'62''W): One nauplius. 08 Feb. 2006. Pepato, A. coll.

**Description of nauplius.** The dorsal shield of the nauplius (Fig. 20A) 470 µm long, covered with furrows and striations. Two medial lateral processes are followed by two more bifid lateral processes and one posterior small pointed process and these complete the ornamentation of the dorsal shield. The furcal region of the body is prolonged into two bifid projections onto which the posterior setae are inserted. The dorsal shield is strongly furrowed and rugose externally.

Labrum: wide and finely setulose distally.

Labium (part of the ventral wall, just below the labrum) bears 2 patches of setules posteriorly.

Antennule (Figs 20A–B) long and thin, but also rugose proximally, and distinctly divided into 3 segments of which the last bears 10 setae.

Antenna. Exopod with 4 setae, and segmented. Endopod with 2 lateral setae and distal claw. Coxa with a masticatory process bearing 3 thick setae: inner seta distally divided into a 3-pointed fork with a minute inner seta, a middle seta distally denticulated, and an outer glabrous seta. Basis: a process with 3 setae.

Mandible: Coxabasis externally spinulose, innerly confluent with endopod. This bears a bifid seta, 3 pointed setae, and 2 foliaceous setae. Exopod 2-segmented with a pinnate seta on first segment and 2 long setae on second segment (one longer seta, twice the length of the other).

Maxillule: maxilla and maxilliped are represented by pointed lateral body projections as in Fig. 20B.

**Remarks.** *Laophontella horrida* (Por, 1964) has a subspecies *L. h. dentata* Mielke, 1992 which occurs in Costa Rica. Males and female found agree in their general aspect but not in details with Mielke's specimens. The furca and the antennules are like Por's (1964) illustrations of the species

Por (1964) figured a nauplius together with *L. horrida*. It agrees in general aspect with the nauplius which we found in samples collected in São Sebastião Channel waters where the adults occurred. It has the characteristic long, thin antennules of the other Tetragonicipitidae nauplii.



**FIGURE 20.** *Laophontella horrida* (Por, 1964) (nauplii). (A) NIV, dorsal; (B) NIV, ventral. Scale bars: 25  $\mu$ m.

## Discussion

If we follow Wells' species identification key (2007), *P. iuanamai* is classified as *P. bradyi* (T. Scott, 1892), but it can be easily distinguished from this species by the longer first segment of A1, the different form and setation of the female P5, the number of setae in some mouth appendages, the less protuberant inner bulge of the female caudal ramus.

*Phyllopodopsyllus iuanamai* is closely related to *P. opistoceratus* Geddes 1968, from which it differs by the 2-segmented endopod on the Mx, P3 enp-1 with internal seta and seta V enlarged (bulbiform) at insertion site on furca of *P. iuanamai* and not bulbiform in *P. opistoceratus* (see Geddes 1968). Furcal rami are shorter in Brazilian specimens than in northern species. It is interesting to note how northern Atlantic species of the same genus are closely related to Brazilian South Atlantic species. *P. iuanamai* can be considered a new species based on A1 and A2 setal formulae, P3 enp-2 with 3 setae and Mx endopod 2-segmented.

Relationship also seems to exist in species of the same genus but present in South American waters of the Atlantic and Pacific. *Phyllopodopsyllus kunzi* differs from *P. pseudokunzi* by the shape of the rostrum and by the setal formulae of P2–P4. P5 in *P. kunzi* has no spiny processes, which are present in *P. pseudokunzi*. *Phyllopodopsyllus kunzi* has a small pointed protuberance on the second segment of A1; *P. pseudokunzi* has a large hook on the same segment of A1. In some aspects this species is close to *P. danielae* Bodin, 1964, such as A1 8-segmented with unguiform process on segment 2; P3 exp-3 with 4 elements; P4-enp 2 with 3 setae (see Bodin 1964). It can easily be differentiated from *P. danielae* by the shape of the furca (similar to *P. kunzi*) and armature of the legs.

The nauplii of the Tetragonicipitidae show some apomorphies when compared to nauplii of other families (compare with Dahms 1990). The most striking one is the long thin antennule usually short and fat in nauplii belonging to other families. Some of the tetragonicipitid nauplii have pointed processes on the rim of the dorsal shield. These are also present in *Laophonte cornuta* nauplii (Brian 1925). *Laophonte cornuta* has spinulose antennules, processes absent in the nauplii here studied. The tetragonicipitid antennules are characteristically very simple, with no ornaments except setae.

Nauplii are good indexes of relationship among species, and even higher taxa (see Dahms 1990, 2004). Of the Tetragonicipitidae nauplii here studied, the less ornamented one belongs to *P. aegypticus*. The adult of this species also shows the less ornamented antennules. If we compare the identified tetragonicipitid nauplii, those of *P. setouchiensis* are very similar to those of *P. aegypticus*. The dorsal shield of *P. iuanamai* shows two lateral pointed processes as those of *Laophontella horrida*, but it differs from *Laophontella*. This nauplius has two posterior lateral processes and the whole dorsal shield is more ornamented than in *P. iuanamai*. The *P. pseudokunzi* nauplius presents the most divergent appendages, when compared to the other tetragonicipitid nauplii here described. *Phyllopodopsyllus pseudokunzi* adult is also the most apomorphic (the vertical insertion of the furcal rami on the anal segment). When more nauplii are identified to species level it will be possible to trace relationships between species, genera, even families with greater precision with the use of naupliar characteristics.

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