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# Morphological and molecular analyses of epikarstic Parastenocarididae (Copepoda: Harpacticoida) from two Sicilian caves, with description of a new *Stammericaris*

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## Abstract

We describe *Stammericaris destillans* **sp. nov.**, and re-describe *Stammericaris trinacriae* (Pesce, Galassi and Cottarelli 1988) based on new material. The two species were collected from epikarstic drips and pools on the floor of two different caves: a karstic (Molara Cave) and a gypsum (Entella Cave) cave, respectively, both located in Sicily, Italy. We also report the presence of previously undescribed structures for *Stammericaris amyclaea* (Cottarelli 1969) and *Stammericaris orcina* (Chappuis 1938). Phylogenetic analyses of the mitochondrial COI and ribosomal 18S genes attributed the new species to *Stammericaris* Jakobi 1972, therefore the structure of the male P4 endopod of *S. destillans* is interpreted as an autapomorphy; other morphological features (structure of male antennule and P3, of female P3; inner ornamentation of P1 basis, armature of caudal rami and shape and armature of P5 of both sexes) correspond to those typical of the genus. Hence, we slithgly amended the generic diagnosis. [zoobank.org:pub:4CC84A0C-C511-4388-9728-41647E58097A]

**Key words:** 18S rDNA gene, COI gene, crustacean, evaporitic cave, stygofauna

## Introduction

In Sicily, stygobiotic copepods are mainly found in hyporheic, parafluvial, and phreatic habitats (Pesce and Galassi 1987, 1988, Pesce *et al*. 1987, 1988; Pesce 1988), but little is known about the diversity and ecology of Sicilian cave fauna. Knowledge of the biodiversity in these sensitive ecosystems is particularly relevant for their management and protection.

Species of the family Parastenocarididae Chappuis, 1940 are highly specialized for life in groundwater and are exclusive to this habitat, with very few exceptions (Galassi and De Laurentiis 2004; Corgosinho *et al*. 2017). In the last few years, the taxonomy of this species-rich family has been modified. The large genus *Parastenocaris* Kessler 1913 has been split with some of its species attributed to new genera or redefined. Among the latter are several genera initially described by Jakobi (1972), such as *Proserpinicaris* Jakobi 1972 (subfamily Fontinalicaridinae Schminke 2010) and *Stammericaris* (subfamily Parastenocaridinae Chappuis 1940). These two genera retain all the morphological characteristics of their subfamily and therefore are easily identifiable based on traditional taxonomy (see Schminke 2010 for details). *Proserpinicaris* was recently redefined by Karanovic *et al*. (2012), who attributed 20 species to this genus, and defined it as Palearctic, with the center of diversity in southern Europe. Totakura *et al*. (2014) described two more *Proserpinicaris* from India, widening its distribution. The genus *Stammericaris* was recently redefined by Schminke (2013) and combined with *Phreaticaris* Jakobi (1972) by the same author. Hence,

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*Stammericaris* currently includes the following nine species: *S. acherusia* (Noodt 1955), *S. amyclaea*, *S. diversitatis* (Cottarelli and Bruno 2012), *S. lorenzae* (Pesce, Galassi and Cottarelli 1995), *S. orcina*, *S. pasquinii* (Cottarelli 1972), *S. phreatica* (Chappuis 1936), *S. stammeri* (Chappuis 1937), and *S. trinacriae*. All these were described from Italy, except for *S. stammeri* and *S. phreatica*, which were found in Spain/France and Romania/ Czech Republic, respectively.

We recently discovered a new species of *Stammericaris* in the epikarstic drip water of the Molara Cave (Palermo Province, Sicily). Here we provide a complete morphological description based on optical as well as scanning electron microscopy (SEM). Although the current species of *Stammericaris* and *Proserpinicari*s were described based on traditional morphological characteristics, some of the older descriptions do not adhere to current standards of taxonomic classification. Therefore, here we provide a complete and detailed re-description of *S. trinacriae* (syn: *Parastenocaris trinacriae*), originally described from a few specimens collected in 1986-1987 from two wells in Trapani (Sicily, Italy) at depths of 4.5 and 10.5 m (Pesce and Galassi 1987; Pesce *et al*. 1988). Our redescription of *S. trinacriae* is based on 1) individuals collected from a very abundant population found in water dripping from the fractured upper layers of the Entella Cave (the first gypsum cave of Sicily to be investigated for groundwater copepod fauna), and 2) the re-examination of the holotype and one female paratype from the type series. Additionally, based on our specimens, we provide previously undescribed genus-specific morphological characters of *S. amyclaea* and *S. orcina*. Because some of the diagnostic morphological characters of some Parastenocarididae are equivocal, we used a phylogenetic approach to complement traditional taxonomic classification of selected species of this family, including *S. trinacriae* and one new species (after Karanovic and Cooper 2011a, b; Zagoskin *et al*. 2014; Karanovic *et al*. 2015).

The aims of this paper are: 1) to describe and taxonomically define a new species of Parastenocarididae; 2) to redescribe *S. trinacriae*; 3) to assess the phylogenetic relationships between the genera *Stammericaris* and *Proserpinicaris*, and among some species of *Stammericaris;* 4) to amend the diagnosis of the genus *Stammericaris*.

## Material and methods

**Site description and sampling methods.** The 'Grotta di Entella' (Entella Cave; 37°46'45.15"N 13°06'46.93"E; Fig. 1), one of the largest in Sicily, is found in an area with a Mediterranean climate ('Csa' in the Köppen climate classification; see Cottarelli *et al*. 2012 for details), and opens at 370 m a.s.l. under a gypsum epikarstic layer about 200 m thick dating back to the Messinian (ca. 6 MYA; Catalano 1986). Water in the cave percolates from the epikarst to form cave-roof drips and pools; water also seeps in through the cave floor from a series of faults and collects in some of the pools. For this study, monthly from October 2012 to November 2013 (seven pools) and from November 2013 to April 2014 (11 pools), all water was collected from each pool with a syringe. Drip water was also collected in January 2013 from eight constant cave-roof drips in the upper reaches of the cave following Brancelj (2003).

The karstic 'Grotta della Molara' (Molara Cave; 38°08'47.30"N, 13°18'17.57"E; Fig. 1) opens at 90 m a.s.l. under an epikarstic layer 20 m thick in coral limestone of the Meso-Cenozoic (24-65 MYA; Di Maggio *et al*. 2012). Cave water is due exclusively to percolation of rainfall, forming cave-roof drips that form permanent pools in the clay soil of the cave floor. Water samples were collected monthly as above from March 2012 to April 2014 from three small pools of 2, 3, and 35 cm depth with different sediment types (2-6 cm diameter pebbles on a silt-clay layer, brown clay, 1-10 cm diameter pebbles on a silt-clay layer, respectively), and from three cave-roof drips, one near each pool. Pools 1 and 2 were located in areas with more intense drip.

**Morphological methods.** All specimens were sorted alive under a stereomicroscope, and placed individually in 70% ethanol or 100% ethanol and stored at room temperature until further morphological and/or molecular analysis. Before morphological analyses, specimens were rinsed in distilled water, dissected and mounted in Faure's or glycerine jelly medium solution between two cover slips to allow observations from both sides. Illustrations were made at different magnifications up to a maximum of 1250x, using drawing tubes mounted on a Zeiss Axioskop phase-contrast microscope and a Polyvar Reichert-Jung interferential-contrast microscope. The morphology of seven females and one male of *S. trinacriae* were also analysed with a JEOL JSM 6010LA scanning electron microscope. Praparation for SEM included dehydration of specimens in progressively higher ethanol solutions (20%, 30%, 50%, 70%, 80%, 90%, 100% ethanol at 4 °C for 10 minutes each step), critical point drying in a Balzers Union H CPD 020 apparatus and gold coating in a Balzers Union HMED 010 sputter coater

following standard methods. The stubs were deposited at the Interdepartmental Center for Electron Microscopy, Tuscia University.

Specimens of the type series are deposited at the Natural History Museum, London (NHMUK), in VC's collection at the Department for Innovation in Biological, Agro-food and Forest Systems, Tuscia University (DIBAF), or at RG's collection at the Department of Biological, Geological and Environmental Sciences, Catania University (DSBGA). The taxonomic descriptions and the authority of the new species are the sole responsibility of MCB and VC. Authorship of the new species is attributed to should be cited as "Bruno and Cottarelli in Bruno *et al*., 2017 (ICZN 2000, Recommendation 51E).

The following abbreviations are used throughout the text and figures: enp: endopod; exp: exopod; A1: antennule; A2: antenna; P1-P5: first to fifth pereiopod; P6: rudimentary sixth pereiopod. The nomenclature and descriptive terminology follow Huys and Boxshall (1991).

**DNA extraction, PCR amplification and sequencing.** We focused our phylogenetic analysis on the following species of Parastenocarididae: *S. trinacriae*, *S. destillans*, *S. diversitatis*, *S. pasquinii*, *Proserpinicaris amalasuntae* (Bruno and Cottarelli 1998). Two outgroups for the molecular phylogenies were chosen from the family Canthocamptidae Brady, 1880: *Bryocamptus (Rheocamptus) stillae* Cottarelli and Bruno 2012, and Leptopontiidae Lang, 1948: *Bererariа* sp., collected in Italy (Table 1). Specimens of additional species of *Stammericaris* were not available due to the imprecise description of the type localities (i.e., for the older species: *S. stammeri*, *S. orcina, S. phreatica*, *S. acherusia*) or habitat alteration (e.g., *S. amyclaea* was collected twice (Cottarelli 1969; Cottarelli *et al*. 1994) near the mouth of a small stream in an aera transformed into a commercial harbor, Cottarelli V. and Bruno M.C., pers. com.). The specimens were identified morphologically without dissection under a 100x magnification using a MOTIC SMZ-168 stereoscope, and were stored individually in 100% ethanol at -20°C until DNA extraction. Prior to DNA extraction, ethanol preserved samples were re-hydrated and rinsed 10 times with MilliQ water. The QIAamp DNA Investigator kit (QIAGEN, Hilden, Germany) was used for whole DNA extraction following the protocol 'Isolation of Total DNA from Nail Clippings and Hair'.

Since Karanovic *et al*. (2015) advocated the use of a multi-gene approach for reconstructing phylogenetic relationships in copepods, we used the mitochondrial (cytochrome C oxidase subunit 1 gene, COI) and the nuclear 18S rDNA gene (18S) for our analyses. A 623 base pair (bp) fragment of the COI gene was amplified using the primers LCO1490 and HCO2198 (Folmer *et al*. 1994). Due to the low success of DNA amplification using these primers and the amplification of COI pseudogenes in *P. amalasuntae*, four internal primers (StammF1: TARCWGGRGTGTGRGCAGGA; StammF2: GGSTTTGGYAAYTGGCTAGT; StammR1: ATRGCRAAATCYACTGACGC; StammR2: AASCTBCTRTTYAARTTACG) were designed by us to amplify 595 bp of the COI gene of *Stammericaris* and *P. amalasuntae*. Amplifications were carried out with a Veriti 96 Well Thermo Cycler (Applied Biosystems) in 25 µl volumes containing 2 µl DNA, 2.5 mM $MgCl_2$, 0.8 mM dNTPs, 1x PCR buffer (Applied Biosytems), 10 pmol of each primer and 1.25 U of AmpliTaq Gold 360 (Applied Biosystems). PCR cycles consisted of initial denaturation at 95°C for 10 min, followed by 45 cycles at 95 °C for 45 s, 48°C for 40 s, 72 °C 60 s, and a last step at 72 °C for 7 minutes. A 1756 bp fragment of the 18S rDNA gene was amplified following Tang *et al*. (2012) with minor modifications. Amplifications were carried out as above. All PCR products were purified using the Illustra ExoProStar 1-Step (GE Healthcare UK Limited) and sequenced using the ABI prism Big Dye Terminator Cycle sequencing kit (Applied Biosystems). Sequencing was carried out using the ABI 3730xl DNA analyser (Applied Biosystems). Sequences were edited and assembled using Sequencher 5.1 (Gene Codes Corporation, USA).

**Phylogenetic analyses.** All COI sequences of Parastenocarididae available in GenBank were downloaded (accessed March 2016): *Dussartstenocaris idioxenus* Karanovic and Cooper, 2011 (accession number: JN039168), *Kinnecaris lined* Karanovic and Cooper, 2011 (JN091677.1, JN091678.1), *K. linel* Karanovic and Cooper, 2011 (JN039167.1, JN039162.1), *K. linesae* Karanovic and Cooper, 2011 (JN091680.1, JN091681.1), *K. uranusi* Karanovic and Cooper, 2011 (JN039172.1, JN039171.1, JN039169.1), *Kinnecaris* sp. (JN091679.1) and *Parastenocaris jane* Karanovic, 2006 (JN039164.1). All of these specimens were collected in Australia. No 18S rRNA sequences were available.

For all COI sequences, functionality was verified using the Expasy – Translate tools (http://web.expasy.org/translate/) to exclude potential nuclear pseudogenes with mitochondrial origin (Lopez *et al*. 1994). For all COI and 18S sequences (Genbank plus those generated in this study), we identified haplotypes using FaBox (Villesen 2007). For COI haplotypes, we also estimated the rates of variation among sites at each nucleotide position using

the Monte Carlo test with 500 replicates (setting estimate position-by-position rates (ML) to the HKY+G+I model; Hasewaga *et al*. 1985). This analysis indicated that the evolutionary rate variation among sites of the COI gene was saturated. Thus, we combined the COI and partial 18S fragments of the same individual into a 2212 bp fragment; however, this also meant that the Australian Parastenocarididae sequences were excluded from further phylogenetic analysis since 18S sequences were not available. Raw genetic distances between haplotypes were calculated with MEGA 5.2 (Tamura *et al*. 2011). For phylogenetic analyses, separately for 18S and COI, we selected the best-fit model of nucleotide substitution as GTR+I+G using jModeltest2 (Darriba *et al*. 2012) on the CIPRES Science Gateway (Miller *et al*. 2010) under the Bayesian Inference Criterion, which was implemented in phyML ver. 3.1 (Guindon *et al*. 2010), and in MrBayes ver. 3.1.2 (Huelsenbeck & Ronquist 2001). Node support values were based on the approximate likelihood ratio test (aLRT) values for ML, and on posterior probabilities for BI. *Bererariа* sp. and *Bryocamptus stillae* were used as outgroups. In MrBayes, two partitions for the two genes were defined and the set of parameters were estimated independently for each partition, and analysis was run for 20 million generations, sampling every 100$^{th}$ generation, with a burning of 40 000 trees (20 %). The phylogenetic trees were visualized using FigTree v1.3.1. (Rambaut 2009).

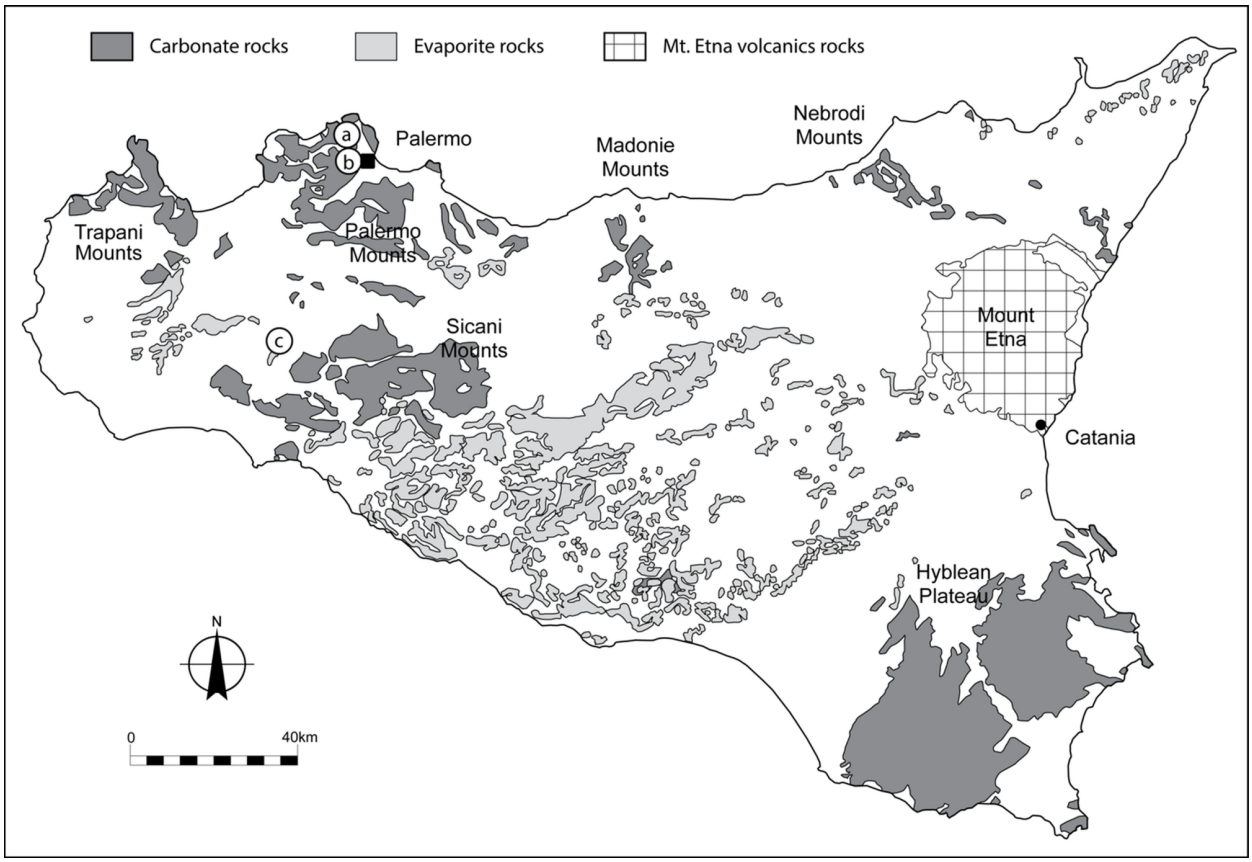


**FIGURE 1.** Map of carbonatic and evaporitic rocks located in Sicily: A, Conza Cave; B, Molara Cave; C, Entella Cave.

## Taxonomic results

### Subphylum Crustacea Brünnich, 1772

### Class Maxillopoda Dahl, 1956

### Subclass Copepoda H. Milne Edwards, 1840

### Order Harpacticoida G.O. Sars, 1903

**Family Parastenocarididae Chappuis 1940**

**Subfamily Parastenocaridinae Chappuis 1940**

**Genus *Stammericaris* Jakobi, 1972**

***Stammericaris destillans* sp. nov.**
(Figs. 2–5, 11, 12)
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**Type locality.** Italy, Sicily, Palermo province, Molara Cave (cadastral number: Si Pa 53), two rimstone pools (pools 2, 3) and two drip funnels.

**Type material**. Holotype: male, dissected, mounted on one slide labelled: "*Stammericaris destillans* holotype: male" (NHMUK 2017. 58), pool number 1, 25/X/2012. Paratypes: one female, dissected, mounted on slide labelled: "*Stammericaris destillans* paratype: female" (NHMUK 2017. 59), pool number 3, 04/IV/2014; one female, undissected, mounted on slide labelled: "*Stammericaris destillans* paratype: female" (NHMUK 2017. 60), pool number 3, 25/X/2012; one male, dissected and mounted on slide labelled: "*Stammericaris destillans* paratype: male" (NHMUK 2017. 61), pool number 3, 04/IV/2014; one male, undissected, mounted on slide labelled: "*Stammericaris destillans* paratype: male" (NHMUK 2017. 62), pool number 2, 04/IV/2014. All material collected by RG and MTS.

**Etymology.** The species epithet is the present active participle of the Latin verb *destillo* ("drip or trickle down), because the specimens dripped with water from the cave epikarst into the rimstone pools.

**Description.** Male (holotype). Body unpigmented, nauplius eye absent. Total body length, measured from tip of rostrum to posterior margin of caudal rami (excluding caudal setae) from 280 to 315 µm, mean 301 m (n = 5). Habitus (Fig. 2A) cylindrical and slender, without any demarcation between prosome and urosome; prosome to urosome ratio: 0.75. Free pedigerous somites without any lateral or dorsal expansions, all connected by well-developed arthrodial membranes. Integument weakly sclerotized, without cuticular pits, ornamented with sensilla on all somites except preanal one; second and fourth free pedigerous somites and second urosomite with proximal dorsal pore. Cuticular windows on urosomites not present (Fig. 2A). Cephalothorax (Fig. 2A) representing about 19.3 % of total body length (measured from tip of rostrum to end of caudal ramus), cephalosome with dorsal cuticular window and medial pore. Anal somite (Figs. 3A, B) with pair of large dorsal sensilla at base of anal operculum, pair of cuticular ventral pores (one pore on each side) near the insertion of caudal rami. Anal operculum (Figs. 3A, B) well-developed, with straight distal margin. Anal sinus wide open, with spinular rows. Spermatophore as in Fig. 12A.

Caudal rami (Figs. 3A, B): shorter than anal somite, approximately cylindrical, slightly divergent; length/width ratio: 3.5, with distal pore between setae IV and V. Anterolateral accessory seta (I), and posterolateral seta (III) subequal in length, anterolateral seta (II) longer than seta I and III (length seta/length caudal ramus: 0.34), all three setae inserted together distally at 3/4 length of the caudal ramus. Outer terminal seta (IV) short and pinnate (length seta/length caudal ramus: 0.53); inner terminal seta (V) without fracture plane. Terminal accessory seta (VI) short (length seta/length caudal ramus: 0.40) and smooth. Dorsal seta (VII) articulate, inserted distally at 3/4 length of the caudal ramus as setae I-III.

Rostrum (Fig. 3C): small, almost reaching distal margin of first antennulary segment, ornamented with two dorsal sensilla.

Antennule (Fig. 3C): prehensile, eight-segmented pocket-knife type *sensu* Schminke (2010). First segment short with transversal row of spinules, second segment longest, with six setae, the longest seta plumose. Third segment with four distal bare setae; fourth segment reduced to a small sclerite with two short setae. Fifth segment enlarged, distal tubercle with two equal setae and one large aesthetasc, reaching almost to the end of eighth segment, one short seta inserted at base of tubercle; a cylindrical protrusion at the base of tubercle, carrying a spear-like spiniform seta (marked with asterisk in Fig. 3D). Sixth segment bare, partially fused to previous one. Seventh segment bare, distal anterior corner protruding as a triangular, pointed apophysis. Eighth segment with seven setae and apical acrothek represented by two subequal setae and a slender shorter aesthetasc, approximately as long as segment. Armature formula: 1-[0], 2-[1 uniplumose + 5 bare], 3-[4 bare], 4-[2 bare], 5-[3+ 1tr +ae], 6-[0], 7-[0], 8-[7 bare + (2 + ae)].

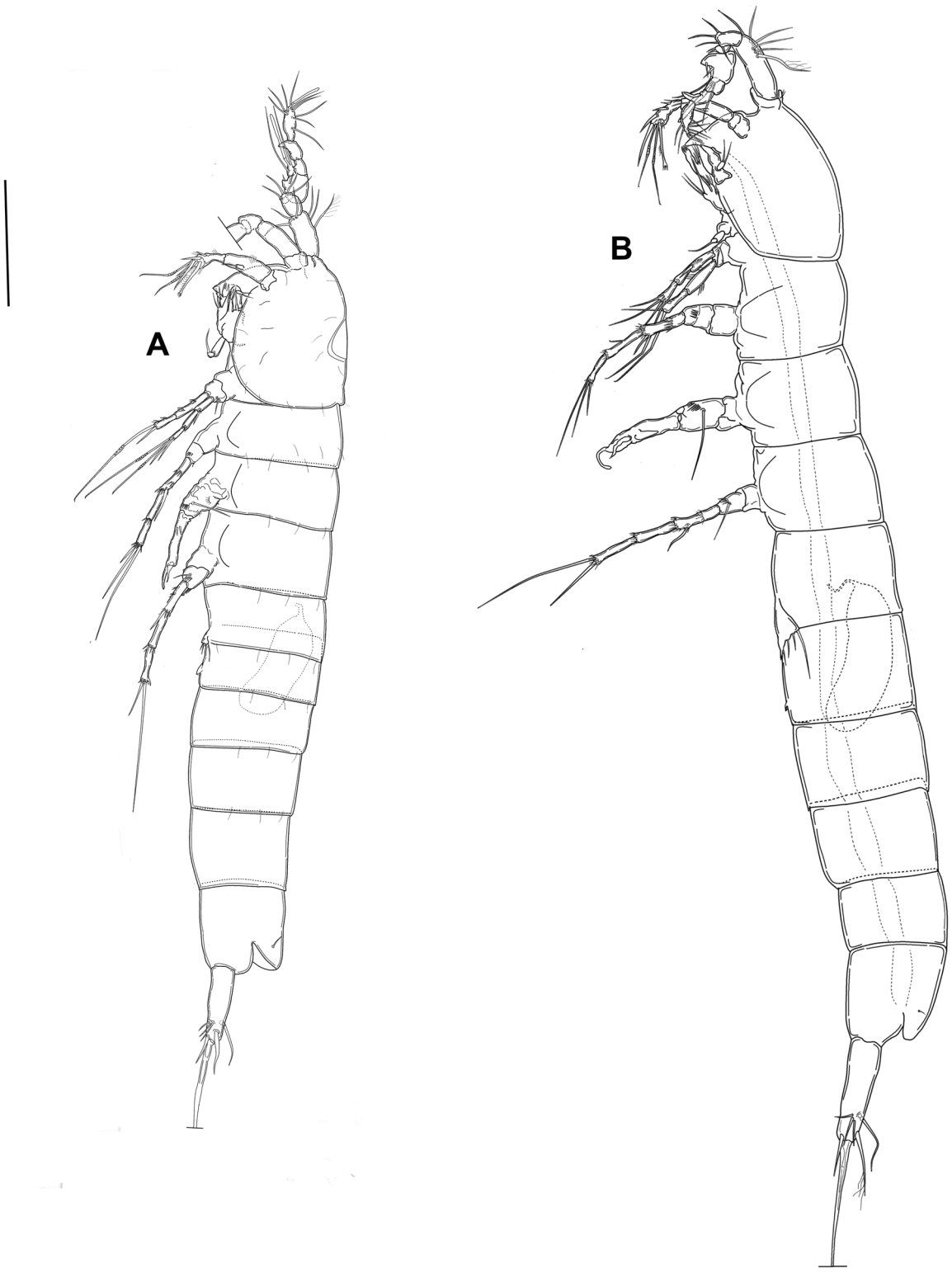


**FIGURE 2.** A, *Stammericaris destillans* **sp. nov.**: A, male, habitus, lateral view; B, *Stammericaris trinacriae* (Pesce, Galassi and Cottarelli, 1988), male, habitus, lateral view. Scale bar: 50 micrometers.

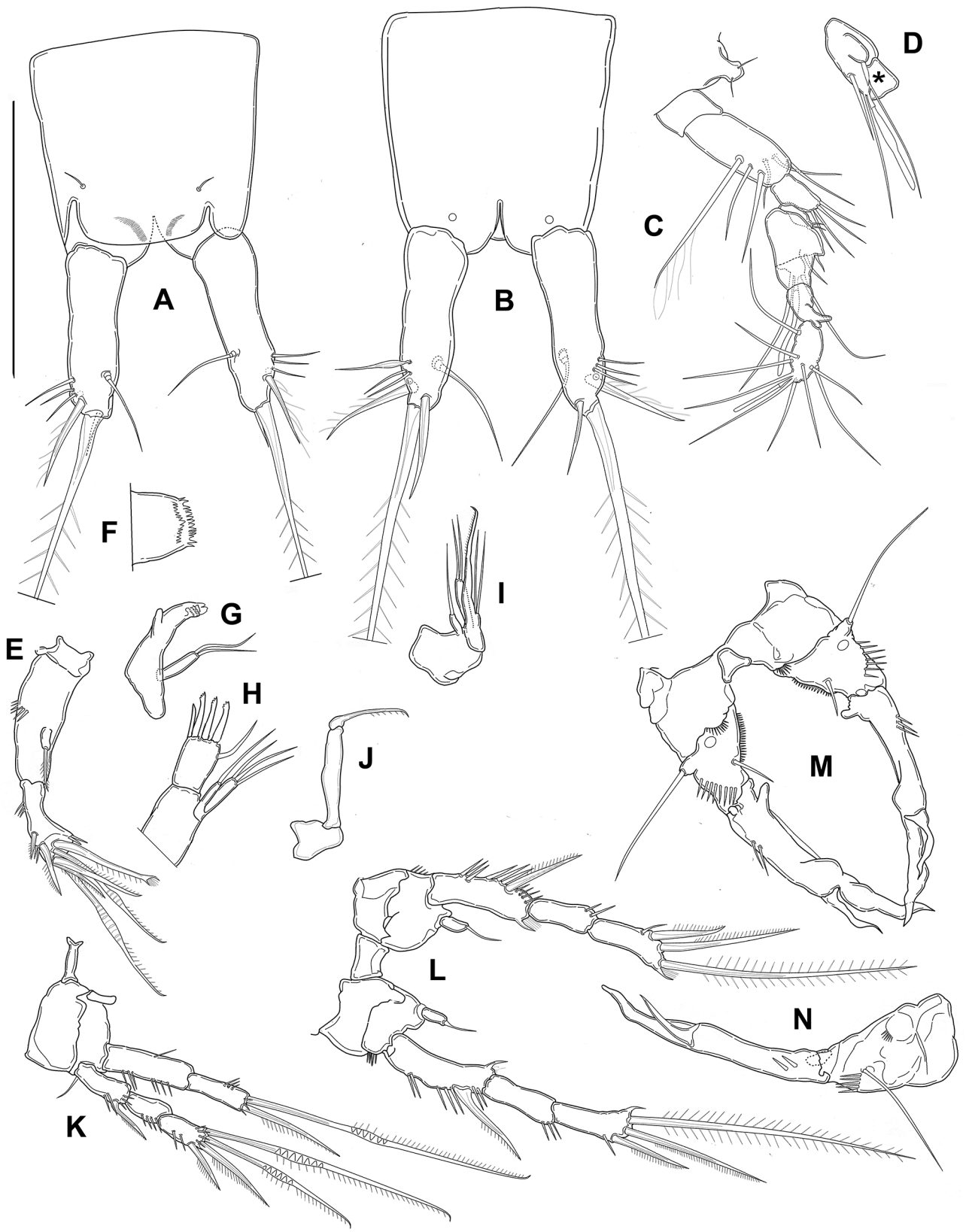


**FIGURE 3.** *Stammericaris destillans* **sp. nov.**, male: A, anal somite, anal operculum and caudal rami, dorsal view; B, anal somite, anal operculum and caudal rami, ventral view; C, rostrum and antennule, dorsal view; D, fifth antennular segment, ventral view; E, antenna; F, labrum; G, mandible; H, maxillule; I, maxilla; J, maxilliped; K, P1; L, P2; M, P3; N, P3, outer view. Scale bar: 50 micrometers.

Antenna (Fig. 3E): coxa unarmed; allobasis with transverse row of spinules on inner margin. Exopod represented by a small segment merged with allobasis, with pinnate apical seta. Endopod bearing two short subdistal, and five longer distal elements, two of them geniculate, all elements with long spinules near their insertions.

Labrum (Fig. 3F): large and approximately rectangular, with convex and smooth anterior surface, cutting edge with apical row of slender denticles.

Mandible (Fig. 3G): coxal gnathobase bare, cutting edge with apical teeth. One-segmented palp, with two distal setae of equal length.

Maxillule (Fig. 3H): praecoxal arthrite with three apical curved robust spines apically denticled, one large spine, one subdistal curved seta. Coxal endite long, with one apical seta. Basis cylindrical, with three distal bare setae of subequal length. Endopod and exopod absent (fused to basis without trace).

Maxilla (Fig. 3I): syncoxa with two endites, proximal endite short, with one thin seta; distal endite cylindrical, longer, armed apically with two subequal thin bare setae; allobasis prolonged into apical pinnate claw; endopod represented by small segment fused at the base, with two long setae of equal length.

Maxilliped (Fig. 3J): prehensile. Syncoxa small and unarmed; basis slim and elongate, unarmed; endopod represented by distally unipinnate claw.

P1 (Fig. 3K): with smooth and small intercoxal sclerite; basis large, armed with single slender seta on outer margin and a hook with rounded tip on the inner margin of basis; ornamented with transverse row of minute spinules at base of outer seta. Exopod three-segmented, slightly shorter than endopod, second segment shortest; exp-1 with thin and slightly curved pinnate seta on outer distal corner; exp-3 with two geniculate and one normal pinnate apical setae, and one subapical pinnate seta. Endopod two-segmented; enp-1 as long as the first two segments of the corresponding exopod, with two transversal rows of spinules on the outer margin, one spinule at 2/3 of the inner margin. Enp-2 thinner and shorter than enp-1, with three spinules at 2/3 of the inner margin; long, geniculate pinnate seta, and shorter pinnate seta on apex.

P2 (Fig. 3L): with smooth intercoxal sclerite, twice as wide as tall. Basis unarmed, with row of four spinules on outer margin. Exopod three-segmented, exp-1 longest, with transversal row of three spinules at 1/3 of outer margin, longitudinal row of three spinules proximal to strong distolateral pinnate spine, row of four smaller spinules along outer distal margin; second and third segments of same length, exp-3 armed with subapical outer unipinnate spine, apical bipinnate seta and unipinnate spine. Endopod one-segmented, about 1/3 the length of the corresponding exp-1, represented by cylindrical segment, with apical seta about as long as segment.

P3 (Figs. 3M, N): intercoxal sclerite narrow and tall, trapezoidal, unornamented, with slightly concave distal margin. Coxa with distal spinule row. Basis robust, with long, slender, smooth outer seta, basal pore and transverse spinule row above outer seta, inner row of transversal spinules. Endopod reduced to short seta. Exp-1 of characteristic shape, enlarged near origin of distal apophysis; inner margin with thin and long conical proximal tubercle fused to exopod. Exp-2 fused with exp-1, without ornamentation, prolonged into long apophysis slightly bent inwards, with pointed tip. Distal thumb represented by thin and pointed segment, reaching to 1/2 of the apophysis.

P4 (Figs. 4A, B): intercoxal sclerite smaller than in P1 or P2, with concave, smooth distal margin. Basis armed with single slender seta on outer margin; ornamented with row of spinules at base of outer seta; two spiniform processes of different size, slightly curved inwards aligned along inner margin, the larger one close to endopod. Exopod three-segmented, slender, all segments approximately of the same length; exp-1 slightly curved inwards, with distolateral pinnate spine; exp-3 armed with outer pinnate spine and very long apical pinnate seta, spine length less than 1/3 of seta length. Endopod one-segmented, length about 1/2 than corresponding exp-1, represented by a cylindrical element distally enlarged in three pointed protrusions, the middle one strongest and apically curved outwards.

P5 (Fig. 4D): fused to intercoxal sclerite, represented by two trapezoidal cuticular plates with inner-distal corner produced into small pointed tip, one large pore at midlength of each plate. Armature on free distal margin, from inner to outer: three bare setae of different length, innermost shortest, middle one longest, and long basipodal seta.

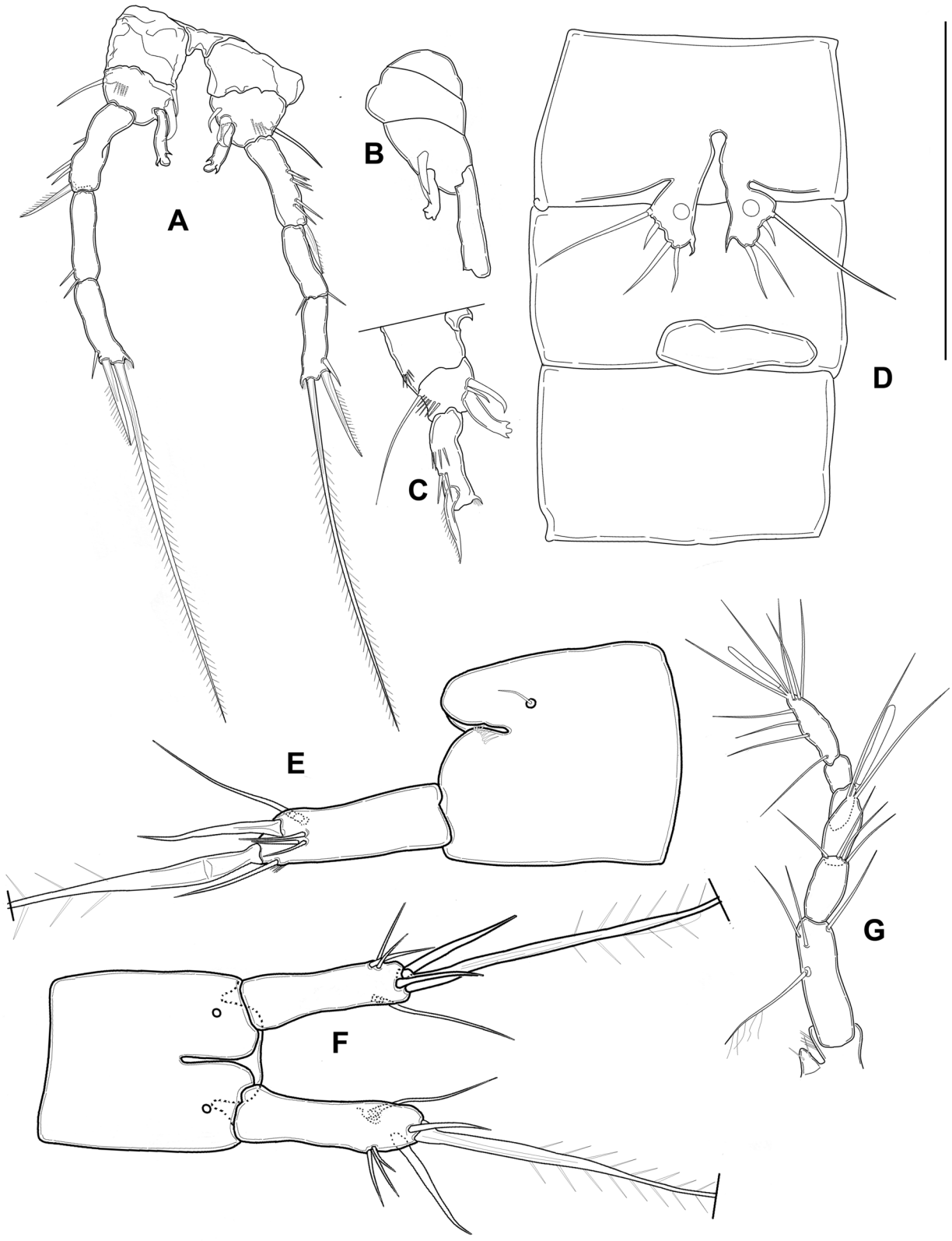


**FIGURE 4.** *Stammericaris destillans* **sp. nov.**: A, male, P4, dorsal view; B, male, P4 basis and endopodite, lateral view; C, male, P4 basis and endopodite, lateral view, variability; D, male, P5, P6, first to third urosomites, ventral view; E, female, anal somite, anal operculum and caudal ramus, lateral view; F, female, anal somite, anal operculum and caudal ramus, ventral view; G, female, antennule. Scale bar: 50 micrometers.

P6 (Fig. 4D): vestigial, fused into simple cuticular plate, unornamented and unarmed.

Female. Body length, excluding caudal setae, from 260 to 315 m, mean 289 m (n = 5), ornamentation of cephalothorax (Fig. 11A), somites, pigmentation, and lack of nauplius eye as in male, except genital and first urosomite fused into double-somite. Prosome/urosome ratio: 0.86. Genital double-somite (Fig. 5F) without any trace of subdivision. Genital field (Figs. 5F, 12A) broader than tall, occupying anterior ventral half of genital double-somite; single genital aperture covered by fused vestigial sixth legs; median copulatory pore located medially at 1/3 of double-somite length. Anal operculum and anal sinus as in male.

Caudal rami (Fig. 4E, F, 12B): shape, ornamentation and armature similar to those of male, length/width ratio: 3.5.

Rostrum, antenna, oral appendages, maxilliped, as in male.

Antennule (Figs. 4G, 11A): seven-segmented, aesthetasc on fourth segment longer and thinner than in male, reaching below end of seventh segment. First segment bare. Second segment longest. Apical acrothek represented by two setae of different length and slender aesthetasc. Armature formula: 1-[0], 2-[1 pinnate +3 bare], 3-[4 bare], 4-[2 + ae], 5-[0], 6-[0], 7-[6 bare + (2 + ae)].

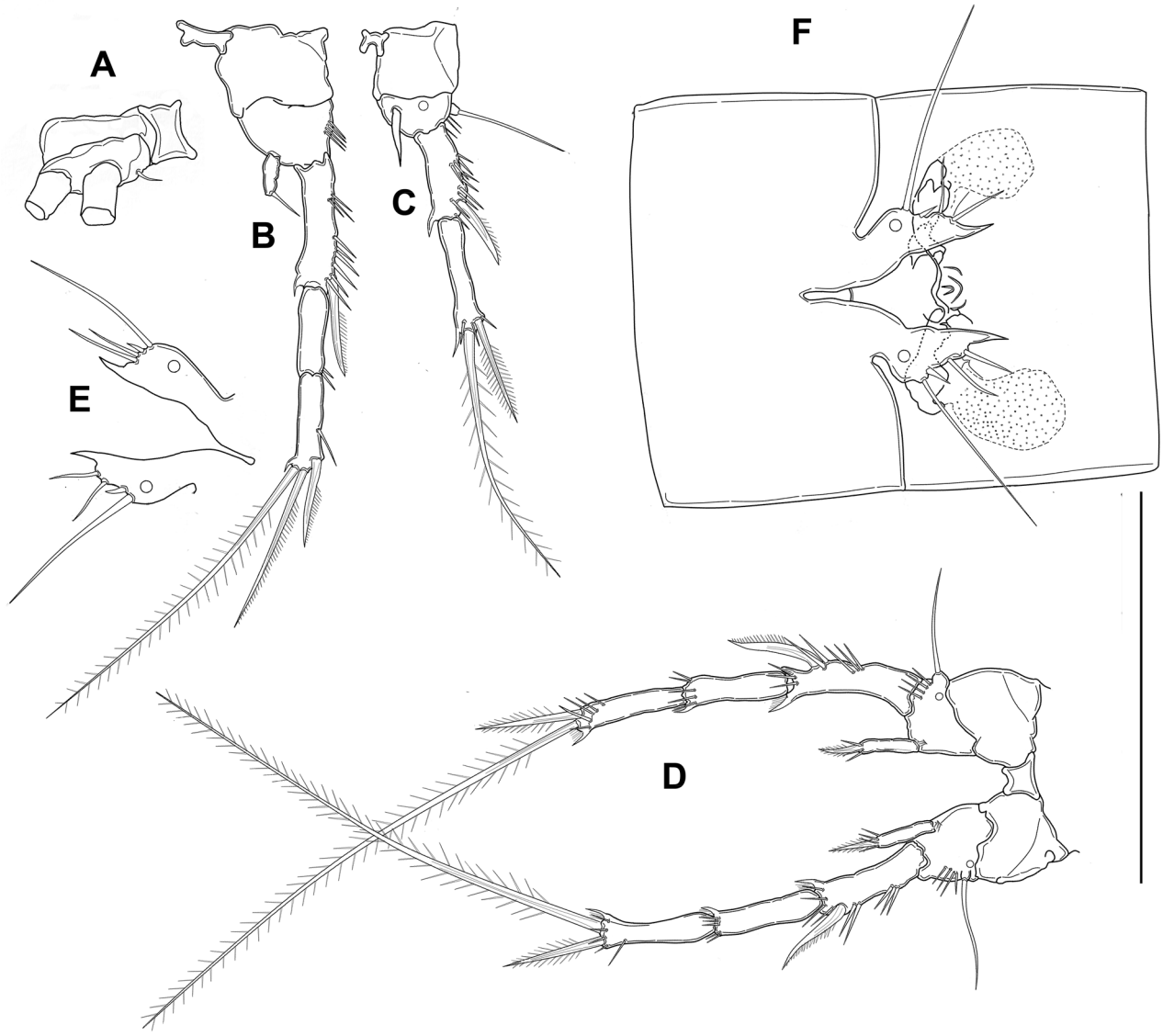


**FIGURE 5.** *Stammericaris destillans* **sp. nov.,** female: A, P1 basis, detail; B, P2; C, P3; D, P4; E, P5; F, first urosomite, P5, genital double-somite and genital field, P6, ventral view. Scale bar: 50 micrometers.

P1 (Figs. 5A, 11B): basis, ornamentation and armature as in male except with inner curved seta apically transformed instead than hook (Fig. 11B); exopod and endopod similar to the male's ones in shape, ornamentation and armature.

P2 (Figs. 5B, 11C): intercoxal sclerite longer and narrower than in male, basis as in male but with spinular row below endopod insertion (Fig. 11C). Exp-1 less inwardly curved than in male, and subapical outer spine inserted more distally; remaining ornamentation and armature of exopod as in male. Endopod similar in shape and ornamentation to that of the male, but proportionally slightly longer.

P3 (Figs. 5C, 11D): intercoxal sclerite small, with concave margin, bare. Basis with outer seta and spinular row and pore near seta insertion, spinular row below endopod insertion; exopod two-segmented: exp-1 slightly shorter than exp-2, with distolateral pinnate spine and transversal row of four spinules at 1/2 of the outer margin; exp-2 with subapical outer pinnate spine and apical pinnate seta, spine slightly longer than 1/3 of seta. Endopod represented by a thin and pointed segment, slightly longer than half of corresponding exp-1, (at about 4500x magnification (SEM) apically pinnate).

P4 (Figs. 5D, 11E, 12A): intercoxal sclerite, basis as in male, with pore near outer seta insertion; exopod as in male. Endopod with spinular row near insertion, represented by a thin cylindrical segment, about the length of corresponding exp-1, ending in a short pinnate seta fused with endopod, with four spinules near insertion.

P5 (Figs. 5E, F, 11F, 12A): fused to intercoxal sclerite, represented by two cuticular plates slightly longer than in the male, with inner-distal corner produced into long and strong pointed tip, one large pore at midlength of each plate. Armature on free distal margin, from inner to outer: two bare setae of same length, one very small seta, and long basipodal seta.

P6 (Fig. 5F): vestigial, fused into simple cuticular plate, covering gonopore, unornamented and unarmed.

**Variability.** Three female paratypes with seta V of caudal rami proximally enlarged. In one male paratype the spiniform process on P4 basis closest to the endopod is smaller than the far most one (Fig. 4C).

### ***Stammericaris trinacriae*** **(Pesce, Galassi, Cottarelli, 1989)**

(Figs. 2, 6–9, 13, 14)

**Locality.** Italy, Sicily, Palermo province, Entella Cave (cadastral number: Si Pa 328), from different rimstone pools.

**Material examined**. Twenty males, 10 of which completely dissected, each mounted on one slide labelled *Stammericaris trinacriae,* male (NHMUK 2017. 63 - 72, DIBAF), pool number 6, 23/X/2012; three males, dissected, mounted each on one slide labelled *Stammericaris trinacriae* male (NHMUK 2017. 73 -75), pool number 3, 23/X/2012; one male, undissected, mounted on one slide labelled *Stammericaris trinacriae* male (NHMUK 2017. 76), pool number 1, 23/X/2012; one male, undissected, mounted on one slide labelled *Stammericaris trinacriae* male (NHMUK 2017. 77), pool number 7, 23/X/2012; one female, dissected, mounted on one slide labelled *Stammericaris trinacriae* female (NHMUK 2017. 78), pool number 19, 05/I/2014; 10 females, five of which dissected, each mounted on one slide labelled *Stammericaris trinacriae* female (DIBAF) pool number 6, 23/X/2012; one female, undissected, mounted on one slide labelled *Stammericaris trinacriae* female (NHMUK 2017. 79), pool number 1, 23/X/2012; one female, dissected, mounted on one slide labelled *Stammericaris trinacriae* female (NHMUK 2017.80), pool number 8, 23/X/2012; one female, dissected, mounted on one slide labelled *Stammericaris trinacriae* female (NHMUK 2017. 81); 132 specimens preserved in 90% ethanol in vial labelled *Stammericaris trinacriae*, pool number 3, 27/XII/2012 (NHMUK 2017. 82-91); 24 specimens preserved in 90% ethanol in vial labelled *Stammericaris trinacriae*, pool number 15, 01/III/2014 (NHMUK 2017. 92-101); 10 specimens preserved in 90% ethanol in vial labelled *Stammericaris trinacriae*, pool number 15, 05/I/2014 (NHMUK 2017. 102 -111); three females (05/XII/2013, pool 15); two females and one male (05/IV/2014, pool 19) prepared for scanning electron microscopy, on two stubs (CIME). Remaining material (not examined): all remaining specimens (3146 males, 3718 females, 517 copepodites) from same locality, preserved in vials with 70% ethanol and glicerin (DIBAF). All material collected by RG and MTS. Male holotype and female paratype loaned from L. Pesce's collection, University of L'Aquila (Italy).

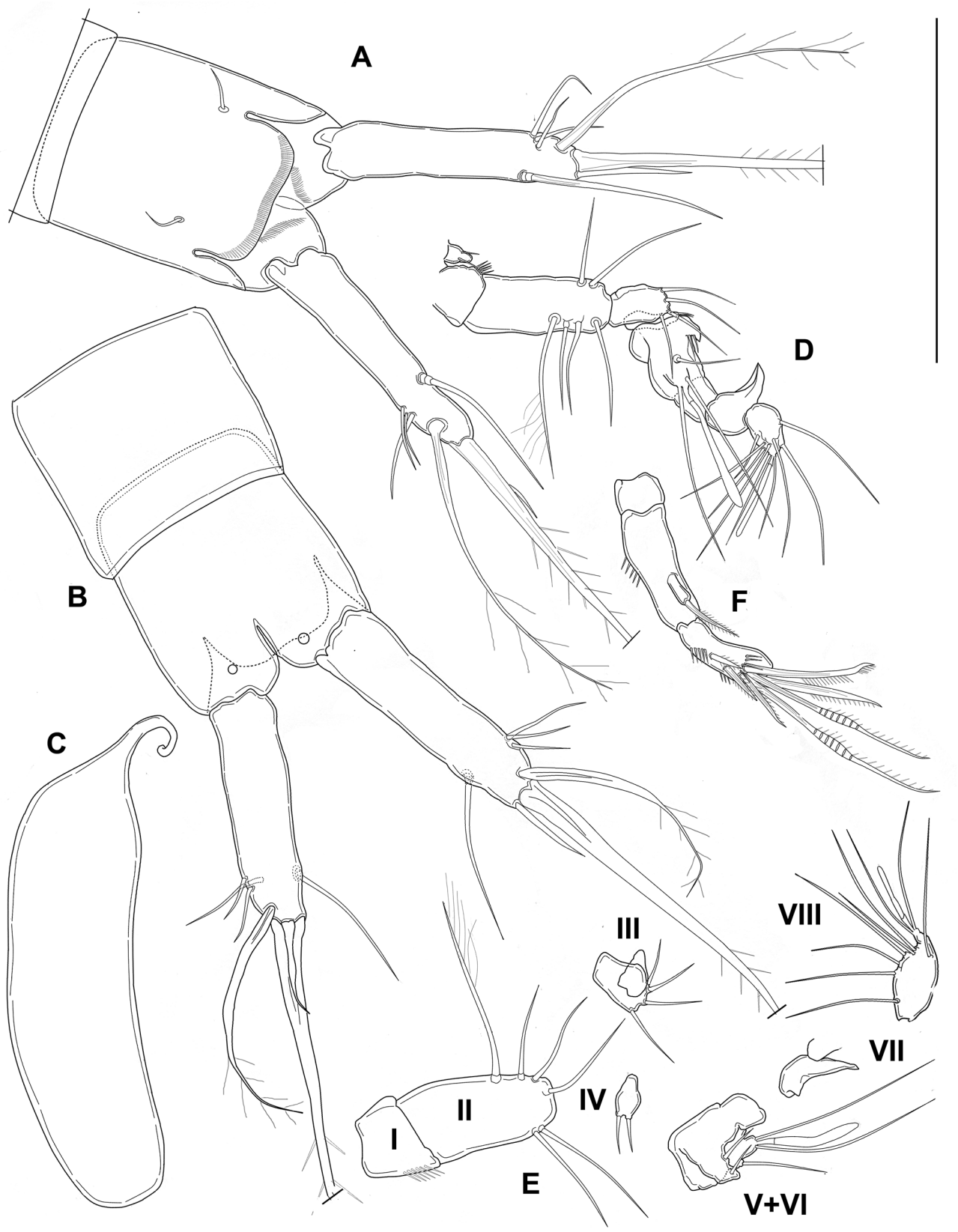


**FIGURE 6.** *Stammericaris trinacriae* (Pesce, Galassi and Cottarelli 1988), male: A, anal somite, anal operculum and caudal rami, dorsal view; B, fourth and fifth urosomites, anal somite, anal operculum and caudal rami, ventral view; C, spermatophore; D, rostrum and antennule, dorsal view; E, antennule, disarticulated (antennular segments marked with roman numerals); F, antenna. Scale bar: 50 micrometers.

**Description.** Male. Body unpigmented, nauplius eye absent. Total body length, measured from tip of rostrum to posterior margin of caudal rami (excluding caudal setae) from 366 to 438 μm, mean 411 μm (n = 10). Habitus (Figs. 2B, 12C) cylindrical and slender, without any demarcation between prosome and urosome; prosome to urosome ratio: 0.69. Free pedigerous somites without any lateral or dorsal expansions, all connected by well-developed arthrodial membranes. Integument weakly sclerotized, without cuticular pits, ornamented with sensilla on all somites except preanal one. Cephalothorax (Figs. 2A, 12D) and urosomites without cuticular windows (Fig. 2B). Cephalothorax (Fig. 2B) representing almost 15.7 % of total body length (measured from tip of rostrum to end of caudal ramus). Anal somite (Figs. 6A, B) with pair of large dorsal sensilla at base of anal operculum, pair of cuticular ventral pores (one pore on each side) near the insertion of caudal rami. Anal operculum (Figs. 6A, B) well-developed, with slightly concave distal margin. Anal sinus wide open, with spinular rows (Fig. 6B). Spermatophore (Fig. 6C) about 1.5 times as long as genital somite.

Caudal rami (Figs. 6A, B, 13A): longer than anal somite, approximately cylindrical, slightly divergent; length/width ratio: 5.1, with distal pore between setae IV and V. Anterolateral accessory seta (I), and posterolateral seta (III) subequal in length, anterolateral seta (II) longer than seta I and III (length seta/length caudal ramus: 0.36); all three setae inserted together distally at 4/5 length of the caudal ramus. Outer terminal seta (IV) pinnate (length seta/length caudal ramus: 1.29); inner terminal seta (V) apically pinnate, without fracture plane. Terminal accessory seta (VI) short (length seta/length caudal ramus: 0.40) and smooth. Dorsal seta (VII) articulate, inserted distally at 4/5 length of the caudal ramus as setae I–III.

Rostrum (Fig. 6D): small, triangular, almost reaching distal margin of first antennular segment, ornamented with two dorsal sensilla.

Antennule (Figs. 6D, 6E, 12D): prehensile, eight-segmented, pocket-knife type *sensu* Schminke (2010). First segment short with transversal row of spinules, second segment longest, with six setae, the longest seta uniplumose. Third segment with four distal bare setae; fourth segment reduced to a small sclerite with two short setae (Fig. 6E). Fifth segment enlarged, with inner protrusion, which is approximately conical and folded at the tip, carrying two small spiniform setae; distal tubercle with two equal setae and one large aesthetasc, restricted at midlength, reaching to half of eighth segment, one longer seta inserted at base of tubercle. Sixth segment bare, fused to previous one. Seventh segment bare, distal anterior corner protruding as a triangular, pointed apophysis. Eighth segment with seven setae and apical acrothek represented by two subequal setae and a slender shorter aesthetasc, approximately as long as segment. Armature formula: 1-[0], 2-[1 uniplumose + 5 bare], 3-[4 bare], 4-[2 bare], 5-[5 + ae], 6-[0], 7-[0], 8-[7 bare + (2 + ae)].

Antenna (Figs. 6F, 12D): coxa unarmed; allobasis with transverse row of spinules on inner margin. Exopod represented by a small segment, with very long pinnate apical seta. Endopod bearing two short lateral, one short subdistal, and four longer distal elements, two of them geniculate; all elements with long spinules near their insertions.

Labrum (Fig. 7A): large and rounded, with convex and smooth anterior surface, narrow cutting edge, cutting edge with apical row of slender denticles.

Mandible (Fig. 7B): coxal gnathobase bare, with pore, cutting edge with apical teeth and small supabical pinnate seta. One-segmented palp, with two distal setae of subequal length.

Maxillule (Fig. 7C): praecoxal arthrite with three distal, curved robust spines apically denticled, one subdistal curved seta, one distal spinule row. Coxal endite short, with one apical thin seta reaching to the end of arthrite. Basis cylindrical, with two distal bare setae of same length. Endopod and exopod absent (fused to basis without trace).

Maxilla (Fig 7D): syncoxa with two endites, proximal endite short, with one thin seta; distal endite cylindrical, longer, armed apically with one enlarged pinnate seta, two long bare setae; allobasis prolonged into apical pinnate claw; endopod represented by small segment fused at the base, with two long setae of equal length.

Maxilliped (Fig. 7E): prehensile. Syncoxa small and unarmed; basis slim and elongate with spinular row along inner margin; endopod represented by distally unipinnate claw.

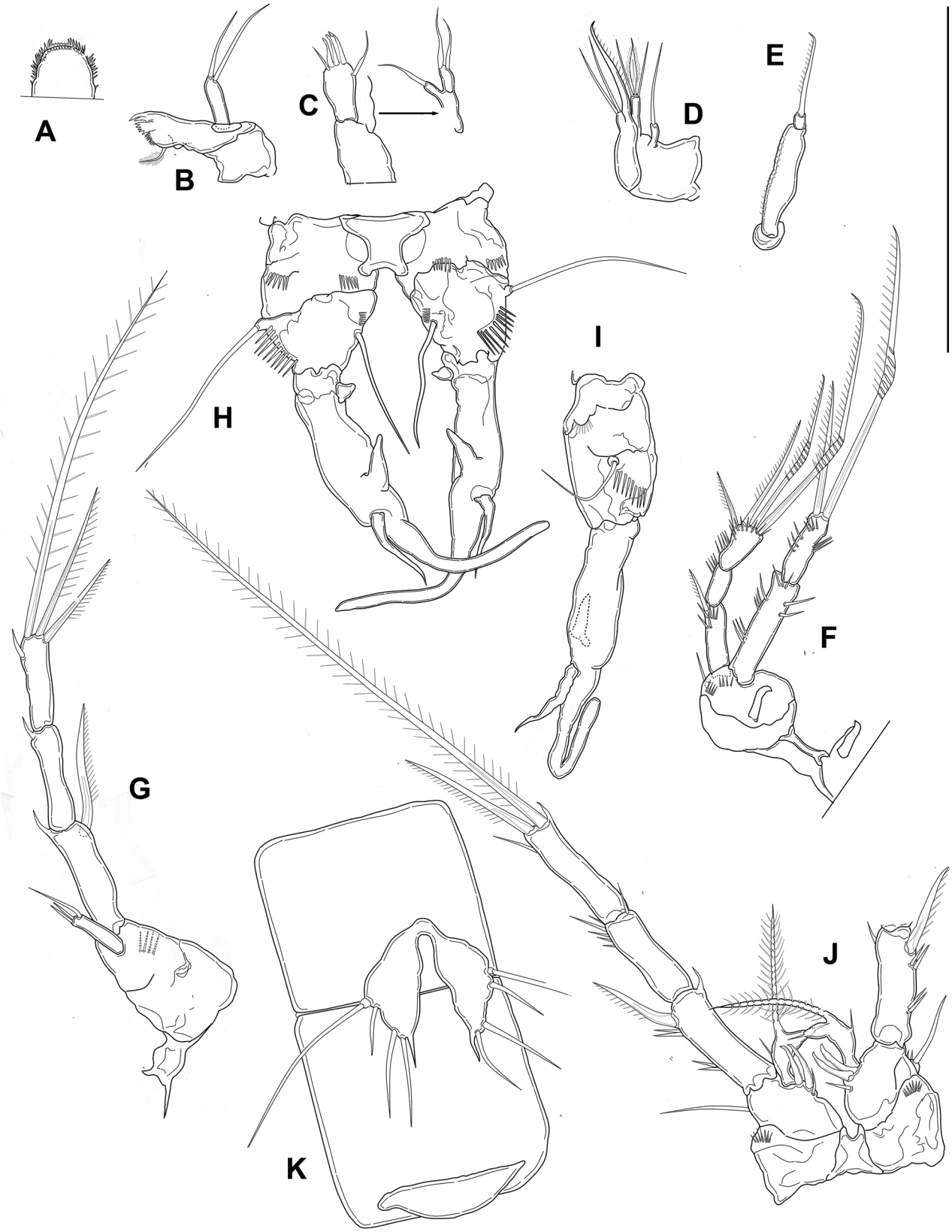


**FIGURE 7.** *Stammericaris trinacriae* (Pesce, Galassi and Cottarelli 1988), male: A, labrum; B, mandible; C, maxillule; D, maxilla; E, maxilliped; F, P1; G, P2; H, P3; I, P3, lateral view; J, P4; K, P5, P6, first and second urosomites, ventral view. Scale bar: 50 micrometers.

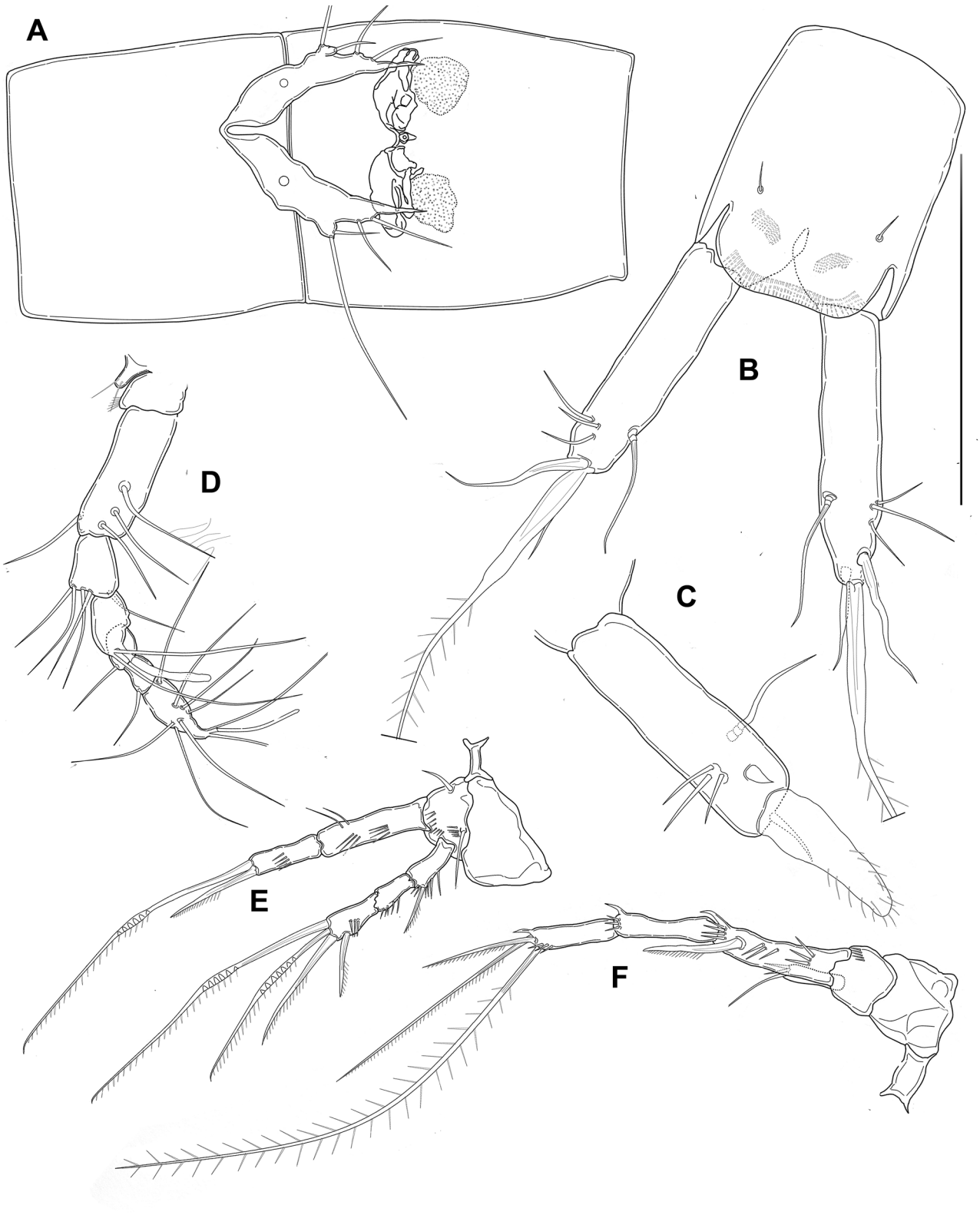


**FIGURE 8.** *Stammericaris trinacriae* (Pesce, Galassi and Cottarelli 1988), female: A, first urosomite, P5, P6, genital double-somite and genital field, ventral view; B, anal somite, anal operculum and caudal rami, dorsal view; C, caudal ramus, lateral view (variability); D, antennule; E, P1; F, P2. Scale bar: 50 micrometers.

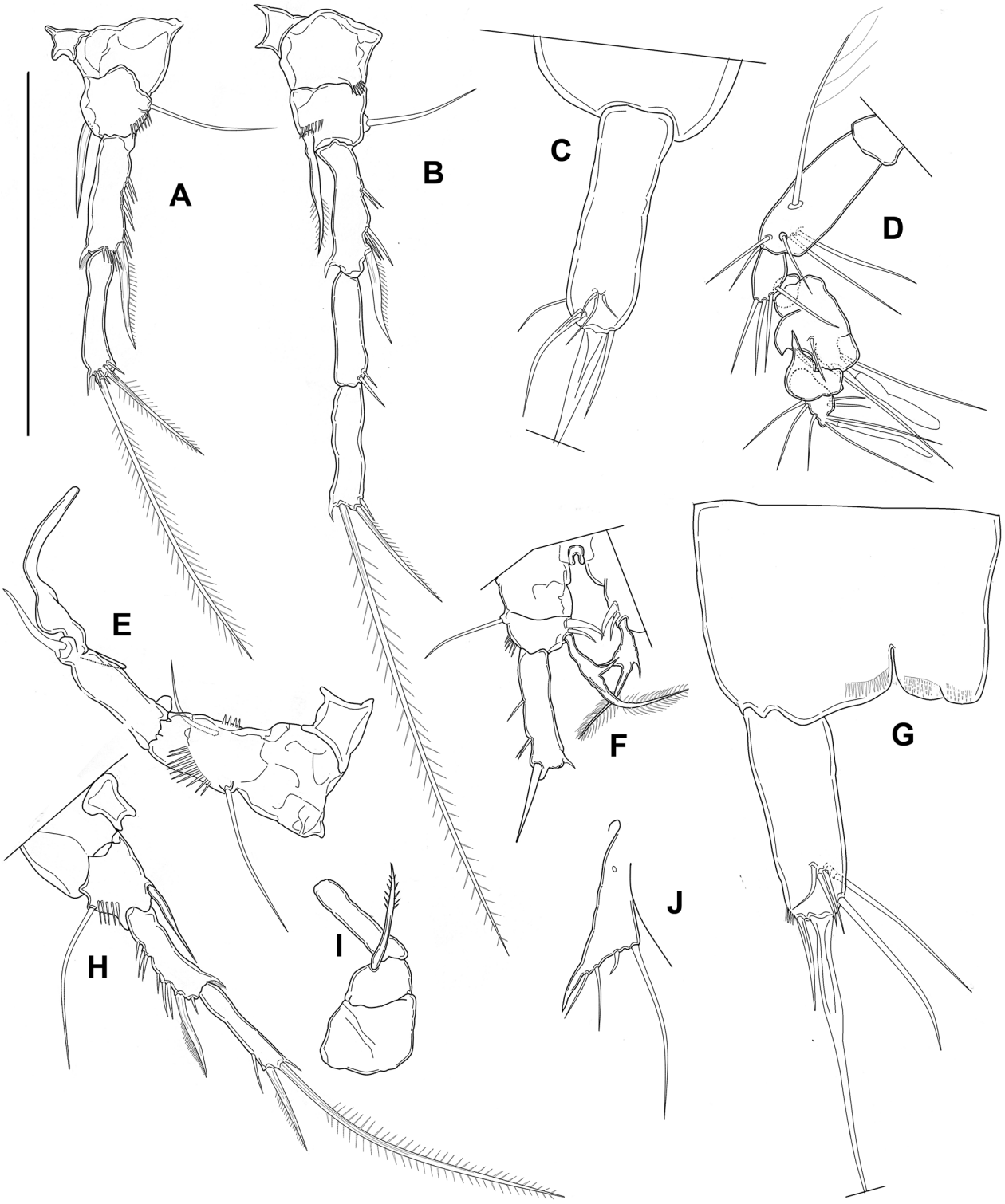


**FIGURE 9.** *Stammericaris trinacriae* (Pesce, Galassi and Cottarelli 1988): A, female, P3; B, female, P4; C, male holotype, caudal ramus, lateral view; D, male holotype, antennule; E, male holotype, P3; F, male holotype, P4 basis and endopodite; G, female paratype, typical series, caudal ramus, lateral view; H, female paratype, typical series, P3; I, female paratype, typical series, P4 endopodite; J, female paratype, typical series, P5. Scale bar: 50 micrometers.

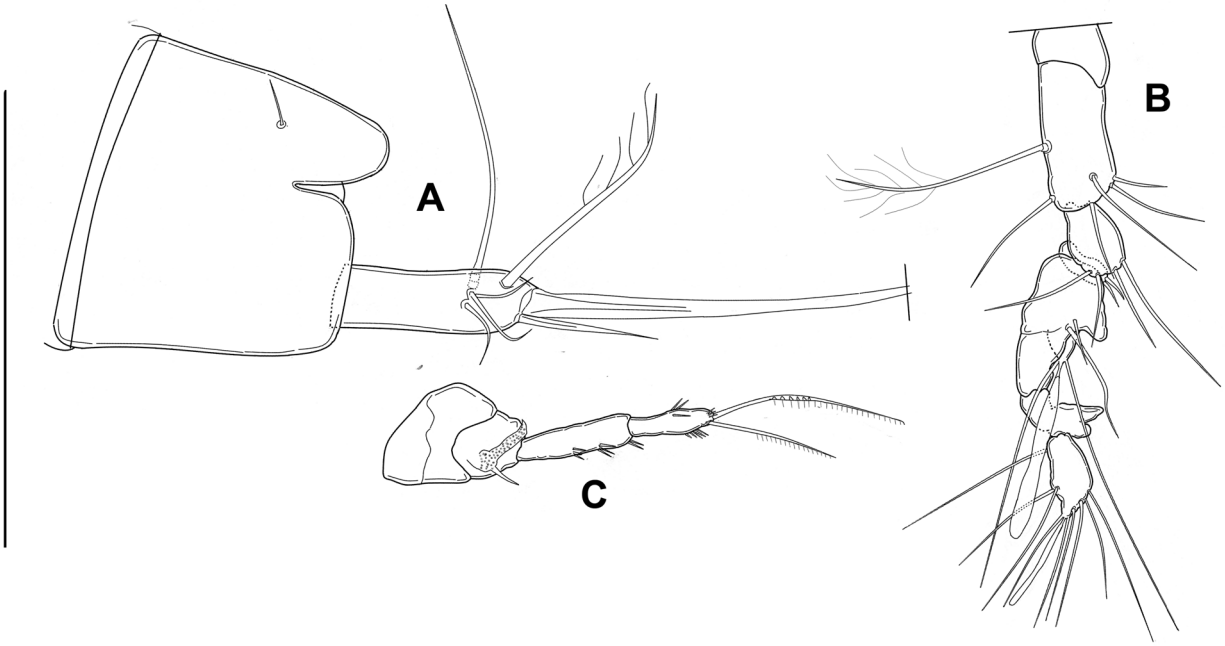


**FIGURE 10.** *Stammericaris amyclaea* (Cottarelli 1969), male holotype: A, anal somite, anal operculum and caudal rami, dorsal view; B, antennule; C, P1 basis and endopod, inner view. Scale bar: 50 micrometers.

P1 (Figs. 7F, 12D, 12E): with smooth and small intercoxal sclerite; basis armed with single slender seta on outer margin and a hook on the inner margin of basis; ornamented with transverse row of minute spinules at base of outer seta; transverse spinular row along distal margin on anterior surface, between exopod and endopod. Exopod three-segmented, slightly shorter than endopod, second segment shortest; exp-1 with thin and slightly curved pinnate seta on outer distal corner; exp-3 with two geniculate and one normal pinnate apical setae, and one subapical pinnate seta. Endopod two-segmented; enp-1 as long as the first two segments of the corresponding exopod, with two transversal rows of spinules on the outer margin, two spinules at 3/4 of the inner margin. Enp-2 thinner than enp-1 and as long as  of enp-1, with longitudinal row of spinules on the outer margin and two spinules at 3/4 of the inner margin; long, geniculate pinnate seta, and shorter pinnate seta on apex.

P2 (Fig. 7G): with smooth and large intercoxal sclerite, twice as wide as long. Basis unarmed, with spinular row on outer margin. Exopod three-segmented, exp-1 with strong distolateral pinnate spine and transversal spinular row at  of the outer margin (Fig. 12D); first and second segments of same length, exp-3 shortest; exp-3 armed with unipinnate subapical outer spine, apical unipinnate spine and bipinnate seta. Endopod one-segmented, about 1/3 the length of the corresponding exp-1, represented by cylindrical segment, with apical seta about as long as segment, and two apical short spinules about  the length of apical spinule.

P3 (Figs. 7H, 7I, 12F): intercoxal sclerite taller than wide, trapezoidal, unornamented, and with slightly concave distal margin. Basis robust, with long, slender, smooth outer seta and outer row of transversal spinules, inner row of transversal spinules below endopod insertion. Endopod reduced to long thin seta. Exp-1 length/width ratio: 2.9; inner margin with conical proximal tubercle, and long, downward-pointed distal process. Exp-2 fused with exp-1 and prolonged into very long apophysis bent inwards, with blunt round tip, exp-2 and apophysis twice as long as exp-1. Distal thumb represented by thin and pointed segment, reaching to almost 1/2 of the apophysis.

P4 (Fig. 7J): intercoxal sclerite smaller than in P2 or P3, with concave, smooth distal margin. Basis armed with single slender seta on outer margin; ornamented with row of spinules at base of outer seta, and one short seta and two spinules of same length and slightly curved outwards aligned along inner margin. Exopod three-segmented, slender, all segments approximately of the same length; exp-1 slightly curved inwards, with distolateral pinnate spine; exp-3 armed with outer pinnate spine and long apical pinnate seta, spine length less than ¼of seta length. Endopod one-segmented, longer than corresponding exp-1, represented by a basal plate distally enlarged into an inner pointed protrusion ending in a fused spinule; a second spinule is inserted at half of the outer margin. The endopod extends on the distal outer corner into a long bipinnate process (fused seta).

P5 (Figs. 7K, 13B): fused to intercoxal sclerite, represented by two trapezoidal cuticular plates with inner-distal corner produced into short and thin pointed tip. Armature on free distal margin, from inner to outer: three bare setae of different length, outermost shortest, and long basipodal seta.

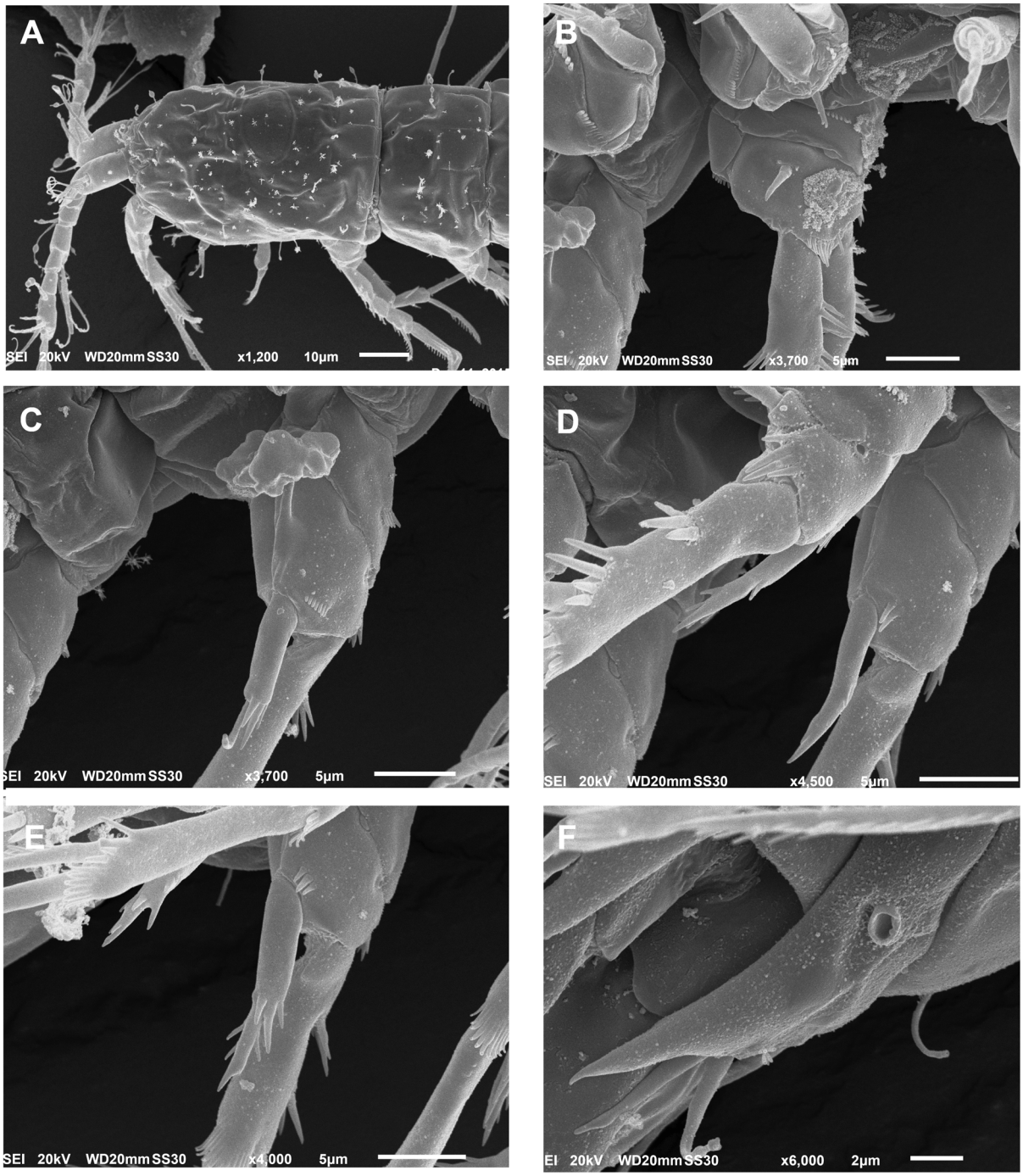


**FIGURE 11.** *Stammericaris destillans* **sp. nov.,** female: A, antennule, cephalothorax and first pedigerous somite, dorsal view; B, P1 basis, inner view; C, P2 basis and endopod, inner view; D, P3 basis and endopod, anterior view; E, P4 basis and endopod, inner view. F, P5.