Italian Journal of Zoology, March 2007; 74(1): 83–99

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Interstitial harpacticoids from groundwater in Tuscany (Central Italy): *Parastenocaris reidae* sp. nov., *Nitocrella ensifera* sp. nov., and notes on the morphology of *Parastenocaris* cf. *glacialis* Noodt (Crustacea: Copepoda)

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(Received 13 April 2006; accepted 25 July 2006)

Abstract
Two new interstitial harpacticoids were recently collected from groundwater in Tuscany (Central Italy). The Parastenocarididae *Parastenocaris reidae* sp. nov. was collected from both phreatic and hyporheic habitats of River Serchio, Lucca province, together with specimens of *Parastenocaris* cf. *glacialis* Noodt. We describe and discuss the affinities of the former species, and give a preliminary description of the latter. *Nitocrella ensifera* sp. nov. was collected from the phreatic habitat of Fiora River, Grosseto, and from a well in the same area; this new species is described and its affinities are discussed.

Keywords: Parastenocaris, Nitocrella, *interstitial habitat, Harpacticoida, hyporheos, groundwater*

Introduction

During the last decade, we have been investigating groundwater copepods from phreatic and hyporheic habitats of several rivers in Central Italy (Cottarelli et al. 2002; Cottarelli & Berera 2003; Berera et al. 2003). Harpacticoid and cyclopoid copepods represent a dominant component of groundwater communities in shallow aquifers (Galassi 2001; Cottarelli & Berera 2003). The first results regarding the study of Fiora, Orcia, and Serchio rivers were published elsewhere (Cottarelli et al. 2002; Berera et al. 2003; Cottarelli & Berera 2003), and they provided the faunistic and ecological description of interstitial copepod assemblages.

In the present work we describe and discuss two taxa of Parastenocarididae, which belong to the genus *Parastenocaris* Kessler, collected from Serchio River: *Parastenocaris reidae* sp. nov. (previously listed as *Parastenocaris* sp. A in Cottarelli et al. 2002), and *Parastenocaris* cf. *glacialis* (previously listed as *Parastenocaris* cfr. *glacialis* in Cottarelli et al. 2002 and in Berera et al. 2005); these taxa are new to science or rarely collected in Italy. We present the most important morphological characters of *P.* cf. *glacialis* and underline the differences with the description of *P. glacialis* by Pesce et al. (1995), which is the most recent description available in literature.

We also describe and discuss the Ameridae *Nitocrella ensifera* sp. nov. (previously listed as *Nitocrella* sp. 2 in Cottarelli & Berera 2003), collected from the Fiora River. The genera *Parastenocaris* and *Nitocrella* Petkovski include species almost exclusive to groundwater habitats; *Nitocrella ensifera* sp. nov. shows unusual morphological peculiarities never recorded before for the genus.

Materials and methods

Specimens were collected using the Karaman–Chappuis method (Delamare Deboutteville 1960) along the river banks, and with a Cvetkov phreatobiological net (Cvetkov 1968) in wells.

Specimens were fixed in 5% buffered formalin solution, sorted and mounted on slides in Faure's medium. Drawings were made at 1250 ×, with an oil

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ISSN 1125-0003 print/ISSN 1748-5851 online
DOI: 10.1080/11250000601022605

immersion lens, using a drawing tube mounted on a Zeiss Axioskop® phase-contrast microscope.

Eight females and one male of *Parastenocaris reidae* sp. nov., and one female and one male of *Nitocrella ensifera* sp. nov. were prepared for scanning electron microscopy. They were fixed for 24 h in 10% formalin solution, washed twice in cacodylate buffer (pH 7.2), post-fixed in 1% osmium tetraoxide in the same buffer, dehydrated in a graded ethanol series, critical-point-dried in a Balzers Union® CPD 020 apparatus, and coated with gold in a Balzers Union® MED 010 sputter coater. Observations were performed with a 1,200 JEOL JEM® EX II scanning electron microscope.

The following abbreviations are used, when required, throughout the text and figures: Enp= endopod; Exp=exopod; P1–P5=thoracic appendages. The nomenclature and descriptive terminology follow Huys and Boxshall (1991).

Specimens are deposited at the Museo Civico di Storia Naturale di Genova (MCSNG); at the Museo Civico di Storia Naturale di Verona (MCSNV); at the Natural History Museum, London (NHM); at the National Museum of Natural History, Smithsonian Institution (USNM). The remaining material is located at the senior author's collection at the Department of Environmental Sciences, ''della Tuscia'' University, Viterbo (DSAUT). The stubs prepared for SEM are deposited at the Interdepartmental Center for Electron Microscopy, Tuscia University (CIME).

Taxonomic account

Family Parastenocarididae Chappuis 1940
Genus *Parastenocaris* Kessler 1913
Parastenocaris reidae sp. nov.

Material examined

Turrite Secca stream (Garfagnana, Lucca province, Tuscany, Italy), right tributary joining the Serchio river at about 30 km from the spring and 68 km from the rivermouth, location ''Isola Santa'', 850 m asl, about 13 km upstream, coordinates 44 03.125 N 010 14.171 E, from parafluvial phreatic habitat in mixed gravel and sand bank. Holotype: male, dissected and mounted on slide labelled: ''*Parastenocaris reidae* holotype'' (MCSNG 53219a). Paratypes: female, dissected and mounted on slide labelled: ''*Parastenocaris reidae* paratype, female no. 1'' (MCSNG 53219b); four females, mounted on different slides labelled: ''*Parastenocaris reidae* paratype, female no. 2–5'' (DSAUT). Material collected by V. Cottarelli, 20 May 1998.

Serchio river (Garfagnana, Lucca province, Tuscany, Italy), location ''Piaggione'', 160 m asl, about 54 km from the spring and 44 km from the rivermouth, coordinates 43 57.665 N 010 31.053 E, from hyporheic habitat in a mid-river sand bar on the left side of the river. Paratypes: three males, each dissected and mounted on different slides labelled: ''*Parastenocaris reidae* paratype, male no. 1–3'' (MCSNV 633, NHM 2006.1271, USNM 1091070, respectively; three males, each dissected and mounted on different slides labelled: ''*Parastenocaris reidae* paratype, male no. 4–6'' (DSAUT); one female mounted on slide labelled: ''*Parastenocaris reidae* paratype, female no. 7'' (MCSNV 632). Two females, each dissected and mounted on different slides labelled: ''*Parastenocaris reidae* paratype, female no. 8, 9'' (NHM 2006.1272, and USNM 1091072, respectively). Material collected by V. Cottarelli, 27 June 1998.

Serchio river (Garfagnana, Lucca province, Tuscany, Italy), location ''Sillano'', 730 m asl, about 6 km from the spring and 92 km from the rivermouth, coordinates 44 12.489 N 010 18.204 E, from hyporheic habitat in a mid-river sand bar on the left side of the river. Three dissected and one whole females, mounted on different slides labelled: ''*Parastenocaris reidae* paratype, female no. 10–13'' (DSAUT). Material collected by V. Cottarelli, 31 May 1998. Ten females and one male, prepared for scanning electron microscopy, on one stub labelled: ''*Parastenocaris reidae* sp. nov. Fiume Serchio'' (CIME). Material collected by M. C. Bruno, 22 October 2004.

Description of male

Length averaged among holotype and six paratypes, from rostrum to distal apex of caudal rami: 0.34 mm. Body vermiform, slender, unpigmented, eyeless. Hyaline frills of cephalotorax, thoracic and abdominal somites and urosome smooth. Cephalotorax with round dorsal hyaline integumental window; four abdominal somites with oval dorsal integumental windows. Anal somite (Figure 1A): with paired sensilla on dorsal side, two lateral rows of small proctodeal spinules, and ventral spinules near caudal rami. Anal operculum (Figure 1A): with straight, smooth distal margin, protruding slightly beyond insertion line of caudal rami. Caudal rami (Figures 1A, 3F): longer than last abdominal somite; length to width ratio: 3.6. Anterolateral accessory seta (I), anterolateral seta (II) and posterolateral seta (III) short, subequal. Outer terminal seta (IV) short (length seta/length caudal ramus: 0.8), unipinnate. Inner terminal seta (V) without breaking planes,

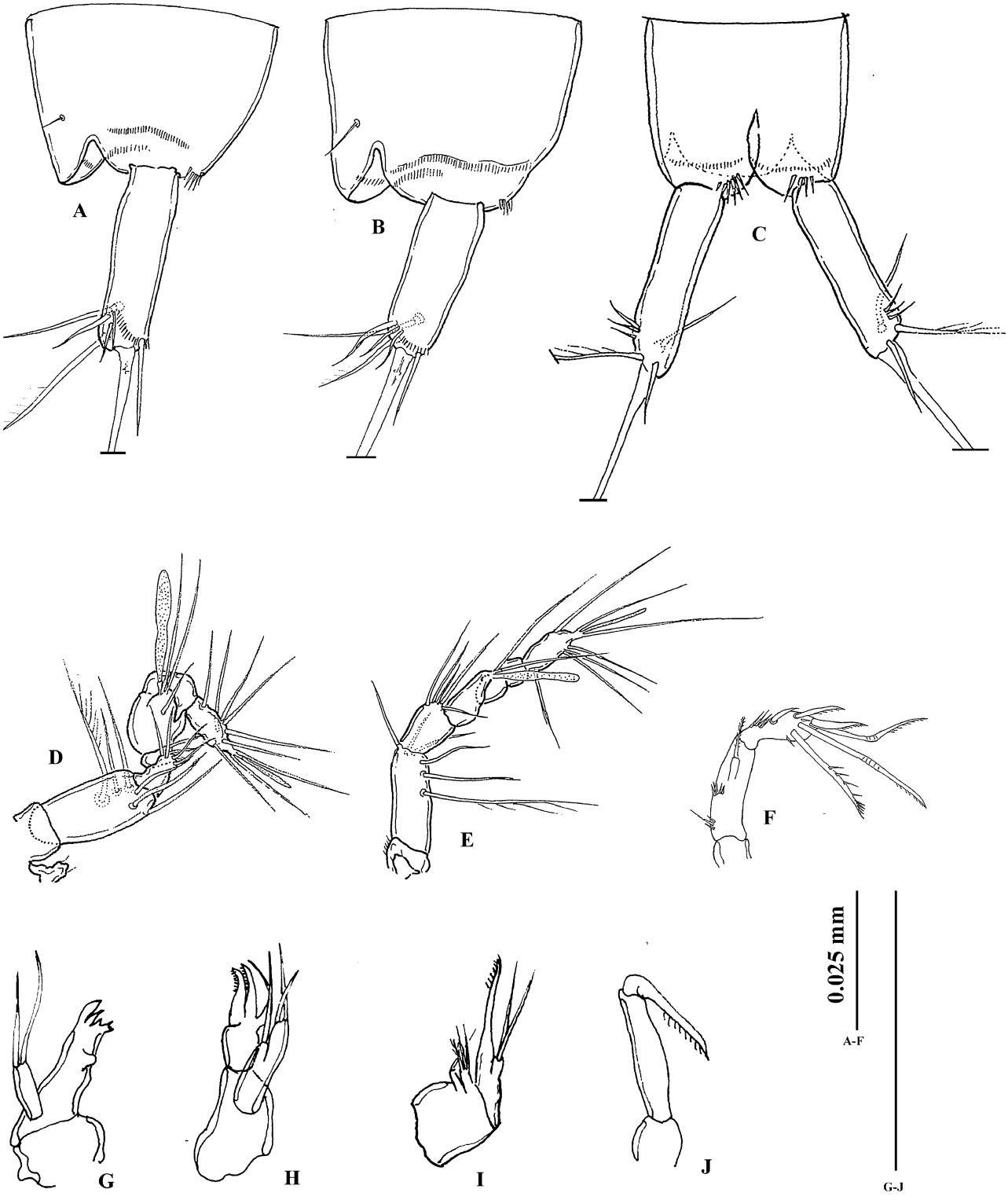

Figure 1. *Parastenocaris reidae* sp. nov. A, D, F, G, H, I, J: male. B, C, E: female. **A**: anal somite, anal operculum, caudal rami, lateral view. **B**: anal somite, anal operculum, caudal rami, lateral view. **C**: anal somite, anal operculum, caudal rami, ventral view. **D**: rostrum and antennule. **E**: rostrum and antennule. **F**: antenna. **G**: mandible. **H**: maxillule. **I**: maxilla. **J**: maxilliped.

unipinnate. Terminal accessory seta (VI) smooth (length seta/length caudal ramus 0.5). Dorsal seta (VII) articulate (length seta/length caudal ramus: 0.6). All setae inserted on the distal third of the caudal ramus.

Rostrum (Figure 1D): small, reaching the end of first segment of antennule, with two apical sensilla.

Antennule (Figure 1D): geniculate, eight-segmented. First segment bare, second segment with six setae, one plumose. Third segment with four distal setae; fourth segment represented by a U-shaped sclerite, with two setae. Fifth segment enlarged, with a distal tubercle with two setae and one long aesthetasc on the apex, reaching the end of the antennule, and one subapical short seta. Sixth segment partially merged with the fifth one near the tubercle, with no armature, small and cylindrical. Seventh segment bare, with a tooth-like expansion that matches with the enlarged fifth segment. Eighth segment with seven setae and apical acrothek consisting of two setae of different lengths and one slender, short aesthetasc.

Antenna (Figure 1F): coxa unarmed; allobasis with two transversal rows of spinules on medial margin. Exopod one-segmented, not defined at base, with one short pinnate apical spine. Endopod bearing two geniculate and one transformed setae (Figure 3D), and two distal spines. The transformed seta is smooth in the first half, curved and one-side pinnate in the second half (Figure 3D). Some spinules along the distal margin, near the insertion of the apical setae. On the lateral margin one smooth and slightly curved spine with four short transversal spinules near its insertion.

Mandible (Figures 1G, 3A, 3B): cutting edge of coxal gnathobase with two strong teeth and a row of smaller teeth; one-segmented palp, with two distal setae.

Maxillule (Figures 1H, 3A, 3B): praecoxal arthrite with two transformed spines (Figure 3B) and one subapical curved seta. Coxa with one distal seta, basis with two apical setae.

Maxilla (Figures 1I, 3A, 3B): syncoxa with two endites, the proximal one with one seta, the distal one with two normal ad one transformed setae. Allobasis prolonged in an apical claw, on the medial side a proximal transversal row of spinules and a large pore (Figure 3B). Endopod with two setae.

Maxilliped (Figures 1J, 3A 3B): prehensile. Syncoxa small and unarmed; basis slim and elongate, unarmed; endopod represented by distally unipinnate claw.

P1 (Figure 2A): basis with a thin lateral seta, some spinules near the endopod insertion, and a pinnate medial spine. Exopod three-segmented, shorter than endopod. Third segment with two geniculate and one pinnate apical setae, and a subapical pinnate seta. Endopod two-segmented, Enp-2 with a long geniculate seta and one short pinnate seta on the apex.

P2 (Figure 2C): coxa with row of spinules and one pore; basis with one row of spinules and without lateral seta. Exopod three-segmented, armature shown in figure. Endopod reaching half of Exp-1, represented by a small cylindrical segment, with one apical seta surrounded by three shorter ones.

P3 (Figure 2E): elongated, basis with one row of spinules and one long seta along the margin between coxa and basis, and one pore. Endopod represented by one thin and pointed seta. Exp-1 distally slender, at about 1/5 of length a tubercle on the medial margin; at about 1/3 of lateral margin three spinules. At 2/3 of length, a row of spinules on the lateral margin, and a tubercle on medial margin. Distal thumb represented by a long leaf-like segment apically bent and with denticled margins. Exp-2 fused with exp-1 and prolonged into a short, bulbous apophysis with hyaline membrane on the tip.

P4 (Figure 2H): basis with a long seta, and a row of spinules. Exopod long, three-segmented, first segment slightly curved inward, chaetotaxy shown in figure 2H. Exp-1 and Exp-3 with fringed extensions on medial distal corner. Endopod (Figure 2I) with six spinules near its insertion, represented by a cylindrical segment, with serrate longitudinal hyaline membrane along the medial margin, and pinnate apical spine surrounded by thin spinules.

P5 (Figure 2L): represented by an almost trapezoidal, elongated plate, with a pore at 2/3 of the medial margin. A medial subapical tooth-like and slightly curved expansion, four apical setae of different lengths, the lateral-most is the longest, the middle ones short, the medial-most long.

Description of female

Length averaged among 4 paratypes, from rostrum to distal apex of caudal rami: 0.36 mm. Cephalothorax with round dorsal hyaline integumental window; genital double somite, and succeeding two abdominal somites with oval dorsal integumental windows. Genital somite and first abdominal somite fused, forming genital double-somite (Figures 2N, 3E). Genital field located in the proximal 1/2 of the genital double-somite (Figures 2N, 3E). Anal somite, anal operculum, rostrum, antenna, mouthparts, maxilliped, P1 exopod and endopod, P2 exopod, as in the male. Caudal rami (Figures 1B, 1C): similar to those of the male, length to width ratio

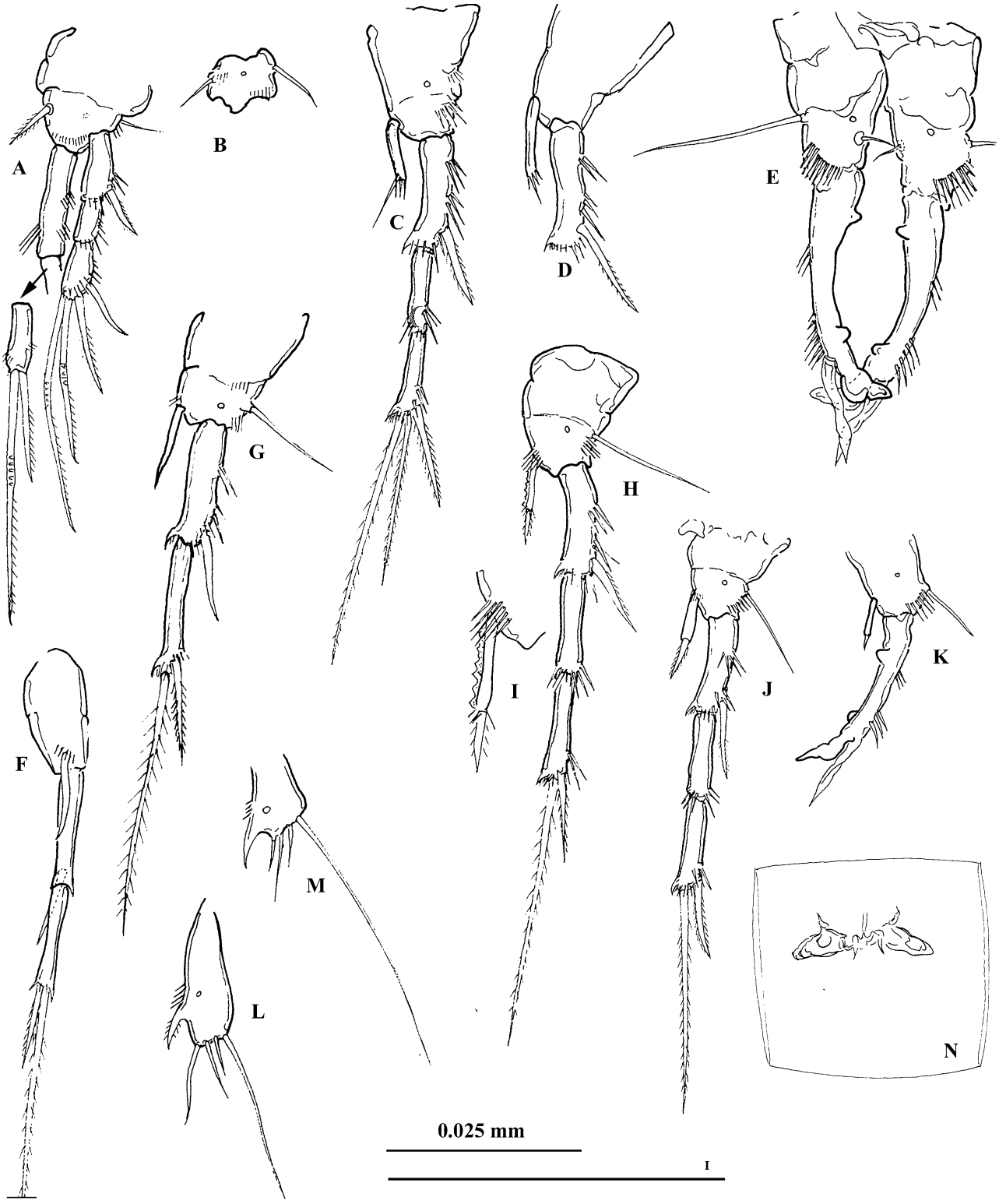

Figure 2. *Parastenocaris reidae* sp. nov. A, C, E, H, I, K, L: male. B, D, F, G, J,M, N: female. **A**: P1 (endopod disarticulated). **B**: basis P1. **C**: P2. **D**: P2 basis, endopod and Exp-1. **E**: P3. **F**: P3, medial view. **G**: P3. **H**: P3. **I**: P3 endopod. **J**: P4. **K**: P3, aberrant morphology. **L**: P5. **M**: P5. **N**: genital double somite and genital field, ventral view.

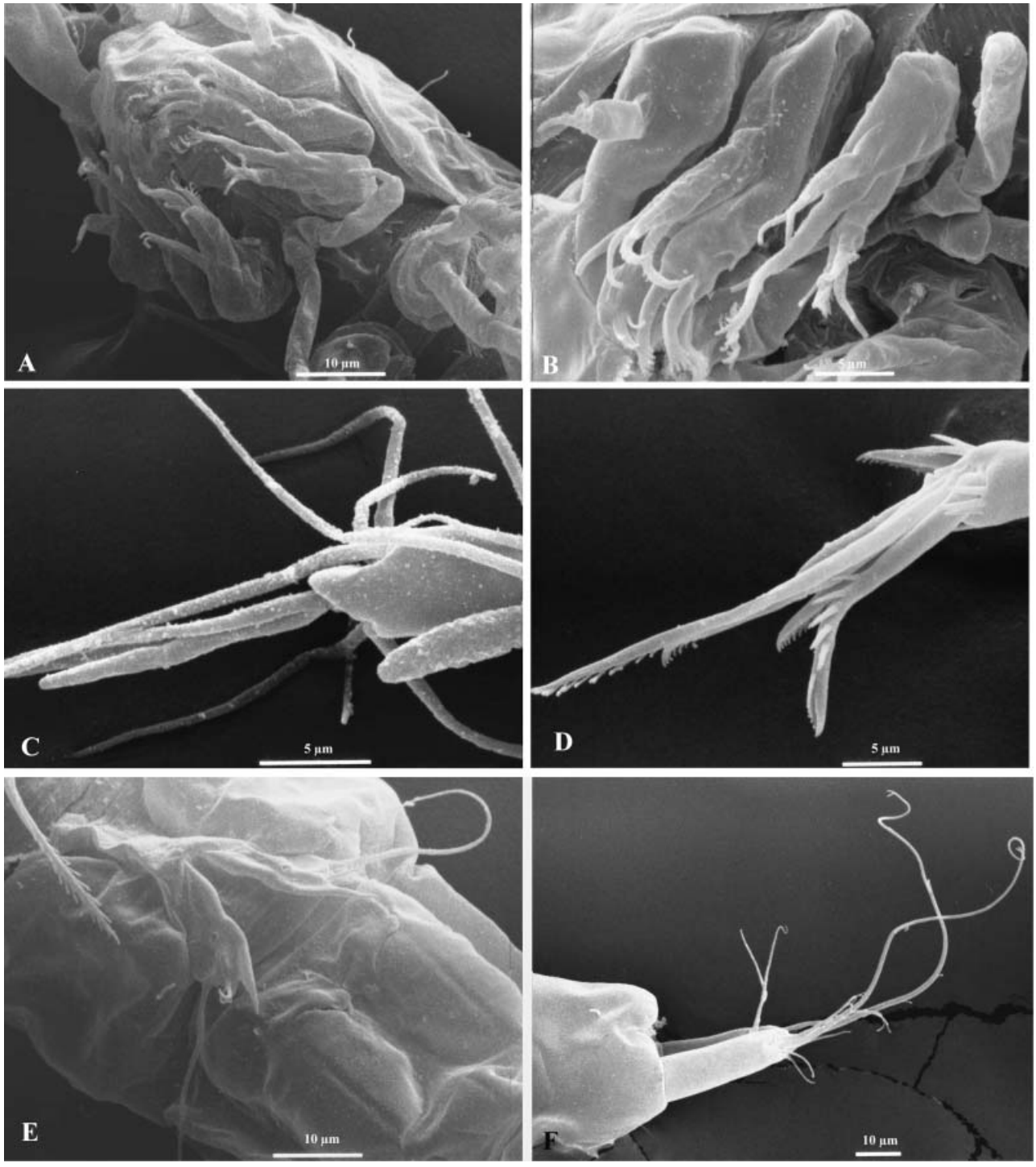

Figure 3. *Parastenocaris reidae* sp. nov. A, B, D, F: male. C, E: female. **A**: mandible, maxillule, maxilla, maxilliped, P1, ventral view. **B**: mandible, maxillule, maxilla, maxilliped, ventral view. **C**: seventh segment of antennule, apical acrothek. **D**: antennal endopod, apical setae. **E**: P5, genital double somite and genital field, ventral view. **F**: anal somite, anal operculum, caudal rami, lateral view.

3.5. Lateral terminal seta proportionally shorter than in male.

Antennule (Figure 1E): seven-segmented, with aesthetasc on fourth segment almost reaching the end of segment seven. First segment with row of short spinules. Number of setae beginning at proximal segment: 0, 6, 5, 2+aesthetasc, 1, 0, 5+acrothek. Apical acrothek represented by two setae of different lengths and one slender, short aesthetasc.

P1 basis (Figure 2B): ornamentation as in the male, but the medial spine is thin and bare.

P2 endopod (Figure 2D): similar to that of the male, but longer.

P3 (Figures 2F, 2G): exopod two-segmented, Exp-1 with one apical spine, Exp-2 with fringed extensions on medial distal corner, one spine and one pinnate seta on the apex. Endopod reduced to a thin, curved and pointed segment, reaching 2/3 of Exp-1, with a row of spinules near its insertion.

P4 (Figures 2J): basis with lateral seta and row of spinules, and one pore. Exopod similar to that of the male. Exp-3 with fringed extension on medial distal corner. Endopod represented by a small cylindrical segment reaching 1/2 of Exp-1, with one apical, short spine, and some spinules at its insertion.

P5 (Figure 2M): similar to the male's, but more markedly trapezoidal in shape; the medial tooth-like expansion is apical, pointed, curved, and stronger than in male; the medial-most apical seta is the shortest.

Variability

No significant variability has been observed on morphological characters, except one male paratype (Figure 2K) with aberrant P3 endopod, represented by a cylindrical segment with one apical short spine.

Etymology

The new species is named after our dear friend Dr Janet W. Reid, Virginia Museum of Natural History, in appreciation of her outstanding contribution to the study of copepods, and Parastenocarididae in particular, and for her continuous and friendly support to M.C.B. research in the USA. Specific epithet in singular feminine genitive.

Family Parastenocarididae Chappuis 1940
Genus *Parastenocaris* Kessler 1913
Parastenocaris cf. *glacialis* Noodt 1952

Material examined

Turrite Secca stream (Garfagnana, Lucca province, Tuscany, Italy), same location as *Parastenocaris reidae* sp. nov. One male, mounted on slide labelled: ''*Parastenocaris* cf. *glacialis* Noodt, 1952 male''. One male and one female, mounted on one slide labelled: ''*Parastenocaris* cf. *glacialis* Noodt, 1952 female and male''. Material collected by V. Cottarelli, 20 May 1998.

All material is deposited at the Department of Environmental Sciences, ''della Tuscia'' University, Viterbo (senior author's collection).

Description

Cephalotorax of both sexes with round dorsal hyaline integumental window; genital double-somite and succeeding abdominal somites of female, first, second third and fourth abdominal somites of male with oval dorsal integumental windows. Genital field of female (Figure 4P) located in the proximal 1/2 of genital double-somite; opercula visible in Figure 3P. Furcal rami (Figure 4A, B): length/width ratio about 4.1 in male, and 3.8 in female. Lateral terminal seta 1.7 and 1.5 times longer than terminal accessory seta, respectively, in males and females.

Male antennule (Figure 4C): eight-segmented, first segment bare; second segment with five normal and one plumose seta; third segment small, with four setae; fourth segment reduced to a U-shaped sclerite, with two setae. Fifth segment enlarged, with a subapical seta and one apical tubercle with two setae and one short aesthetasc. Sixth segment bare; seventh segment short, with lateral tooth-like expansion. Eighth segment with seven setae and acrothek represented by two subequal setae and one slender, short aesthetasc.

Mandible (Figure 4D): coxal gnathobase with subapical thin seta; one-segmented palp, with two distal setae.

Maxillule (Figure 4E, 4F): praecoxal arthrite with three claw-like elements and one subapical curved seta. Coxa with one distal seta, basis with two apical setae.

Maxilla (Figure 4G): syncoxa with two endites; one small seta on the proximal endite; distal endite with one normal and one leaf-like pinnate setae. Allobasis prolonged into an apical pinnate claw. Endopod reduced to a small tubercle with one seta.

P1 basis (Figure 4H) with two short setae near the endopod insertion in the male (Figure 4I), only one longer and stronger seta in the female (Figure 4J).

Male P3 (Figure 4K): basis with a lateral longitudinal row of spinules, and one pore. Endopod represented by one thin and pointed seta. Exp-1 distally slender, at about 1/5 of length a tubercle on medial margin and a row of spinules on lateral margin. At 2/3 of length, a row of short spinules on lateral margin, and a tubercle on medial margin. Distal thumb represented by a leaf-like segment with blunt tip, shorter than Exp-3. Exp-2 fused with exp-1 and prolonged into a short, bulbous and inwardly curved apophysis.

Male P4 (Figure 4L): with row of cuticular spinules of variable number from four to six even in the same individual. Female endopod (Figure 4M) with short apical seta surrounded by few spinules.

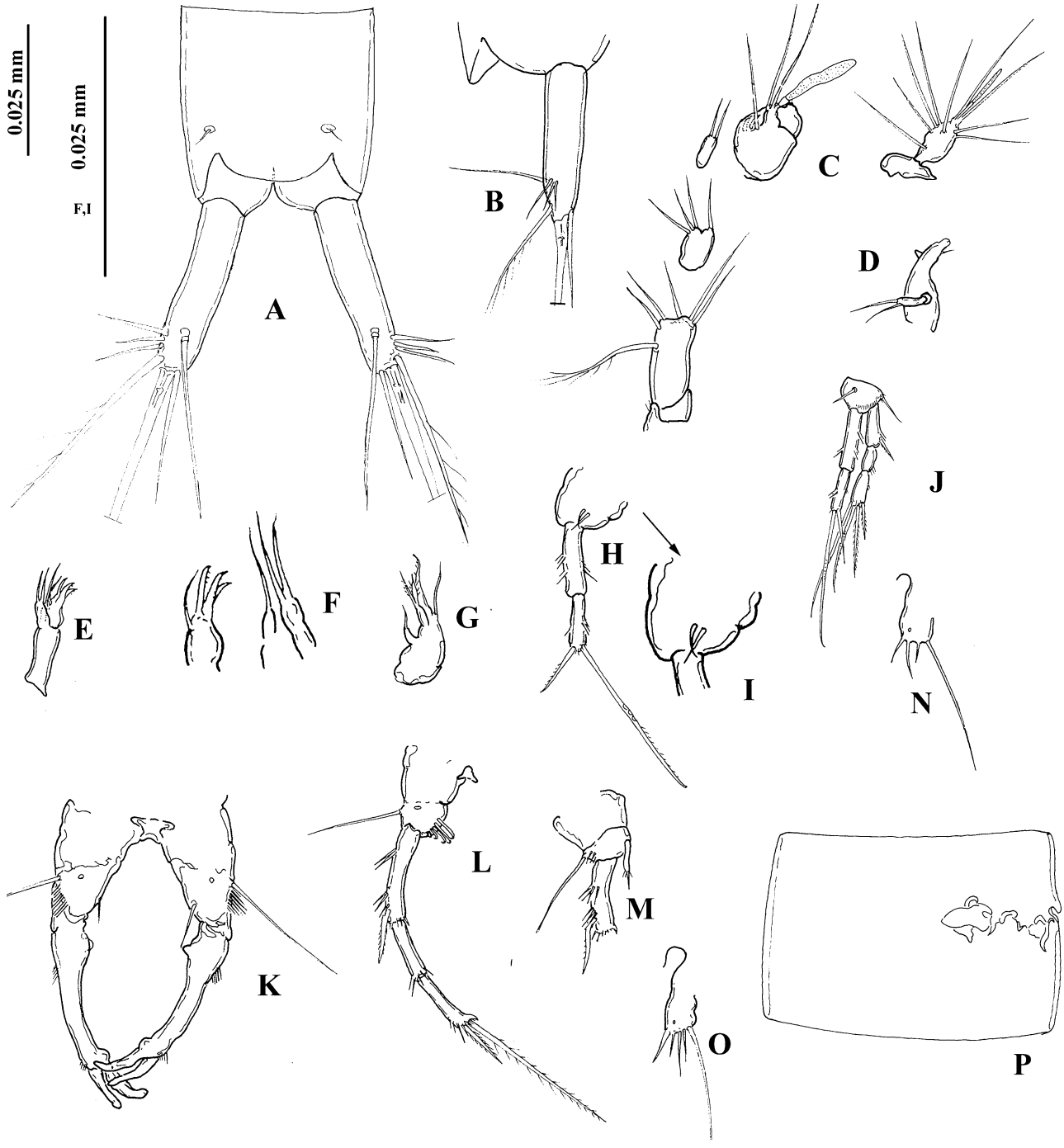

Figure 4. *Parastenocaris* cf. *glacialis*. A, C, D, E, F, G, H, I, K, L, N: male. B, J, M, O, P: female. **A**: anal somite, anal operculum, caudal rami, dorsal view. **B**: anal somite, anal operculum, caudal ramus, lateral view. **C**: antennule (disarticulated). **D**: mandible. **E**: maxillule. **F**: maxillule, disarticulated. **G**: maxilla. **H**: P1, medial view. **I**: P1, detail of ornamentation of inner basis. **J**: P1. **K**: P3. **L**: P4. **M**: P4 basis, enp and Exp-1. **N**: P5. **O**: P5. **P**: genital field, lateral view.

P5 with a medial pore in both sexes (Figure 4N, 4O); female P5 (Figure 4O) with four apical setae.

Family Ameiridae Monard, 1927; Lang, 1948

Genus *Nitocrella* Chappuis,1923 *sensu* Petkovski, 1976

Nitocrella ensifera sp. nov.

Material examined

Well along the road Manciano-Farnese, (Grosseto province, Tuscany, Italy) at 5 m depth, coordinates 42 39.225 N 011 36.831 E. Holotype: male, dissected and mounted on slide labelled: "*Nitocrella ensifera* holotype" (USNM 109173). Paratypes: one

female, dissected and mounted on slide labelled: ''*Nitocrella ensifera* paratype, female no. 1'' (USNM 109175); five females, three of which dissected, mounted on different slides labelled: ''*Nitocrella ensifera* paratype, female no. 2–6'' (DSAUT); seven males, 3 of which dissected and mounted on different slides labelled: ''*Nitocrella ensifera* paratype, male no. 1–7'' (DSAUT). One female, one male, prepared for scanning electron microscopy, on one stub labelled: ''*Nitocrella ensifera* sp. nov. Manciano-Farnese'' (CIME). Material collected by V. Cottarelli and A. Santacroce, 17 April 1996.

Fiora river (Grosseto province, Tuscany, Italy), locality ''Sovana'', 340 m asl, about about 28.2 km from the spring and 29.2 km from the rivermouth, coordinates 42 39 688N, 011 37 259 E, from parafluvial phreatic habitat. Paratypes: two females, each mounted on different slides labelled: ''*Nitocrella ensifera* paratype, female no. 7, 8'' (DSAUT); one male mounted on slide labelled: ''*Nitocrella ensifera* paratype, male no. 8'' (DSAUT). Material collected by V. Cottarelli, 12 March 2003.

Description of male

Length averaged among 5 paratypes, from rostrum to distal apex of caudal rami: 0.42 mm. Body slender, cephalosome smooth with no dorsal hyaline window, hyaline frills of all abdominal somites smooth, body surface variolated. Each abdominal somite with one row of ventral spinules extending laterally. Second, third, and fourth abdominal somite ornamented with one row of spinules anterior to hyaline frill (Figure 7C). Anal somite (Figure 5A) with two dorsal sensilla, and two dorsal pores (Figure 5A); four short rows of ventral spinules and four ventral pores (Figure 7C); one row of spinules around insertion of each ramus, and one row of thin hair-like setules on each side of the anal operculum. Rows of proctodeal spinules (Figure 7C). Anal operculum (Figures 5A, 7C) slightly convex, with seven spinules on the distal margin. Caudal ramus (Figures 5A, 7C) subconical, short; length/width: 1. Posterolateral seta (III) longer than terminal accessory seta (VI), and as long as anterolateral seta (II). Anterolateral accessory seta (I) thin. Two small spinules near insertion of terminal accessory seta, two spinules close to anterolateral seta, and one spinule near insertion of posterolateral seta (III). Dorsal seta (VII) 2.2 times as long as caudal ramus, inserted near distomedial corner. Inner terminal seta (V) 2.4 as long as outer terminal seta (IV), both terminal setae with breaking planes (Figure 7C).

Rostrum (Figure 5B) small and triangular, with two sensilla.

Antennule (Figures 5B, 7B) 10-segmented; first segment with one seta and a transversal row of spinules; second and third segments with eighth setae each. Fourth segment small, with one seta. Fifth segment with four normal ventral setae and distal tubercle with two setae and one aesthetasc of same lengths, reaching past end of ninth segment; dorsally three pinnate spiniform (arrowed in figure) and one normal setae. Sixth segment bare, partially fused with the fifth one. Seventh segment with one normal and one transformed seta. Eighth segment with one distal seta; ninth segment with three setae; last segment with seven setae.

Antenna (Figure 5C) basis unarmed, exopod one-segmented, with two transversal rows of spinules and two transformed and one pinnate apical setae. Endopod two-segmented, second segment bearing on the apex four geniculate setae, and two setae fused together in the proximal part (Figure 5C). Two smooth spines and a longitudinal spinule row on lateral margin.

Labrum (Figure 5D): with lateral row of spinules.

Mandible (Figures 5E, 7E): coxal gnathobase elongate, cutting edge with two strong teeth and row of small teeth; one plumose seta near dorsal corner. Palp two-segmented: basis naked, endopod with five apical setae.

Maxillule (Figure 5F): arthrite of precoxa with three curved unipinnate apical spines and six naked subapical spines. Coxa with three setae, one is geniculate; basis with four setae. Endopod reduced to a tubercle with one slender seta.

Maxilla (Figures 5G, 7D): syncoxa with one endite with one apical normal and one transformed seta. Allobasis with terminal pinnate claw and one lateral seta. Endopod reduced to a tubercle with two apical setae of same length.

Maxilliped (Figure 5H): prehensile; syncoxa with one plumose seta, one pore and one transversal row of spinules on the medial margin. Basis with row of spinules; endopod with strong unipinnate claw.

Major ornamentation of P1–P4 as follows:

P1	basis 1–1	exp 0–1; 0–1; 1,2,1	enp 1–0; 0–0; 1,2,0
P2	basis 0–1	exp 0–1; 1–1; 0,2,2	enp 0–0; 0,2,0
P3	basis 0–1	exp 0–1; 1–1; 0,2,2	enp 0–0; 0,2,0
P4	basis 0–1	exp 0–1; 1–1; 1,2,2	enp 0–0; 0,2,0

Distal spines on Exp-1 and Exp-2 of P2 and P3 strong.

P1 (Figure 5I): basis with two pores visible with SEM (Figure 7A), one strong lateral spine. Medial corner with transformed spine which is extremely long, curved, ending in an enlarged and bilobed

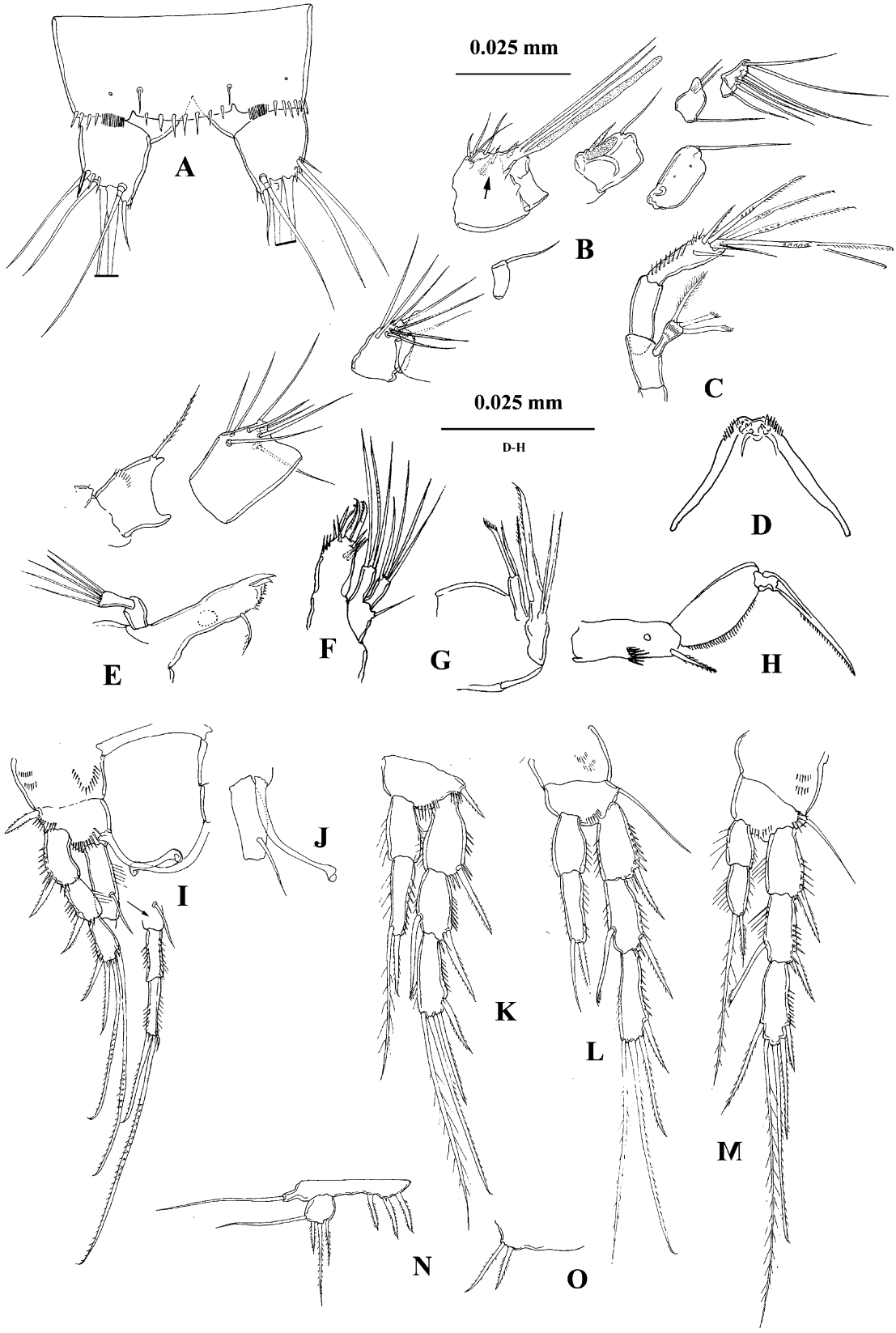

Figure 5. *Nitocrella ensifera* sp. nov., male. **A**: anal somite, anal operculum, caudal rami, dorsal view. **B**: antennule (disarticulated), ventral view. **C**: antenna. **D**: labrum. **E**: mandible. **F**: maxillule. **G**: maxilla. **H**: maxilliped. **I**: P1. **J**: transformed basal medial seta and Enp-1. **K**: P2. **L**: P3. **M**: P4. **N**: P5. **O**: P6.

extension (Figures 5J, 7A). Exopod and endopod with three-segmented rami. Endopod longer than exopod, Enp-1 almost as long as Exp-1 and Exp-2 together; Enp-2 and Enp-3 subequal; Enp-3 with one pinnate, one long geniculate, and one slender apical setae. Exp-3 with two pinnate spines on lateral corner, and two geniculate apical setae.

P2–P4 with three-segmented exopods and two-segmented endopods (Figures 5K, 5L, 5M). Exp-2 of P2–P4 with one medial subapical transformed seta.

P2 (Figure 5K): endopod reaching the end of Exp-2. Enp-2 with one subapical pinnate seta, and one apical pinnate spine as long as about 1/2 of seta.

P3 (Figure 5L): endopod reaching to 1/2 of Exp-2. Enp-2 with one terminal pinnate spine and one slightly longer seta.

P4 (Figure 5M): endopod reaching half of Exp-1 and Exp-2 together. Enp-2 with one apical pinnate medial seta and pinnate spine as long as 1/3 of seta.

P5 (Figure 5N): not coalescent; baseoendopod little reduced, with basipodal outer seta, medial lobe with three subequal pinnate setae. Exopod rounded, with two apical pinnate setae, one shorter pinnate spine, and one long and slender lateral seta.

P6 (Figure 5O): a small plate with two setae, medial seta shorter.

Description of female

Length averaged among 4 paratypes, from rostrum to distal apex of caudal rami: 0.48 mm. Body slender, cephalosome smooth with no dorsal hyaline window, hyaline frills of all abdominal somites smooth. Genital and first abdominal somite clearly distinct on both dorsal and ventral surfaces (Figures 6H, 7G), genital somite with one lateral row of spinules (Figure 7G). Genital field (Figures 6H, 7G) located at half of the genital somite. Copulatory pore small, inserted in a small depression; the opercula derived from the sixth legs bear two setae of different length.

First abdominal somite with one row of spinules anterior to hyaline frill. Ornamentation of two last abdominal somites as in male (Figure 6B). Anal somite (Figures 6A, 6B, 7F) as in male. Anal operculum (Figure 6B) slightly convex, with 11 marginal spines. Caudal rami (Figures 6A, 6B, 7F) similar to that of the male; posterolateral seta longer than terminal accessory seta, and almost as long as anterolateral seta. Dorsal seta 1.6 times as long as caudal ramus; medial terminal seta 2.1 as long as lateral terminal seta. Rostrum, A2, mouthparts; P2–P4 basis and exopod as in male.

Antennule (Figure 6C) eight-segmented; segment four with aesthetasc, reaching past end of segment eight. Number of setae beginning at proximal segment: 1, 7, 6, 3+aesthetasc, 2, 3, 4, 4+4 apical setae.

P1 (Figure 6D): basis with medial and lateral strong spines. Endopod and exopod similar to those of male.

P2 (Figure 6E): Enp-2 apical spine as long as 1/4 than the apical seta.

P3 (Figure 6F): Enp-2 with apical seta very long and spine as long as 1/4 of the apical seta.

P4 (Figure 6G): Enp-2 with apical spine as long as 1/2 of the apical seta.

P5 (Figure 6I): both P5 fused medially. Baseoendopod more developed than in male, with same ornamentation. Exopod oval, same ornamentation as in male but the inner pinnate spine and the lateral pinnate seta are proportionally longer, and with short spinules along medial corner.

Etymology

The specific name from the Latin adjective *ensiferus* meaning ''sword-bearing'', alluding to the peculiar shape of the transformed seta on the male P1 basis. The specific epithet is an adjective in feminine singular.

Accompanying fauna

The accompanying fauna of *P. reidae* sp. nov. and *P.* cf. *glacialis* was represented by specimens of the following copepod species:

(1) Station Sillano.

Harpacticoida: *Bryocamptus (Rheocamptus) zschokkei* (Schmeil 1893), *Bryocamptus (Rheocamptus) pygmaeus* (Sars 1863), *Bryocamptus (Limocamptus) echinatus* (Mrázek 1893), *Canthocamptus (Canthocamptus) staphylinus* (Jurine 1820), *Elaphoidella bidens* (Schmeil 1894), *Epactophanes richardi* Mrázek 1893, *Moraria (Moraria) poppei* (Mrázek 1893), *Parastenocaris* sp. (listed as ''*Parastenocaris* sp. B'' in Cottarelli et al. 2002).

Cyclopoida: *Diacyclops* sp. 1; *Eucyclops agilis* (Koch 1838), *Paracyclops chiltoni* (Thomson 1882), *Speocyclops* sp. 1.

(2) Station Turrite Secca.

Harpacticoida: *Halectinosoma* sp. 1, *E. richardi*; *M. (M.) poppei*, *B. (B.) pygmaeus*, *B. (B.) zschokkei*.

Cyclopoida: *Diacyclops* sp. 1, *Speocyclops* sp. 1.

(3) Station Piaggione.

Harpacticoida: *Nitokra hibernica* (G.S. Brady 1880), *Nitocrella stammeri* (Chappuis 1938), *E. richardi*, *E. bidens*, *E. agilis*.

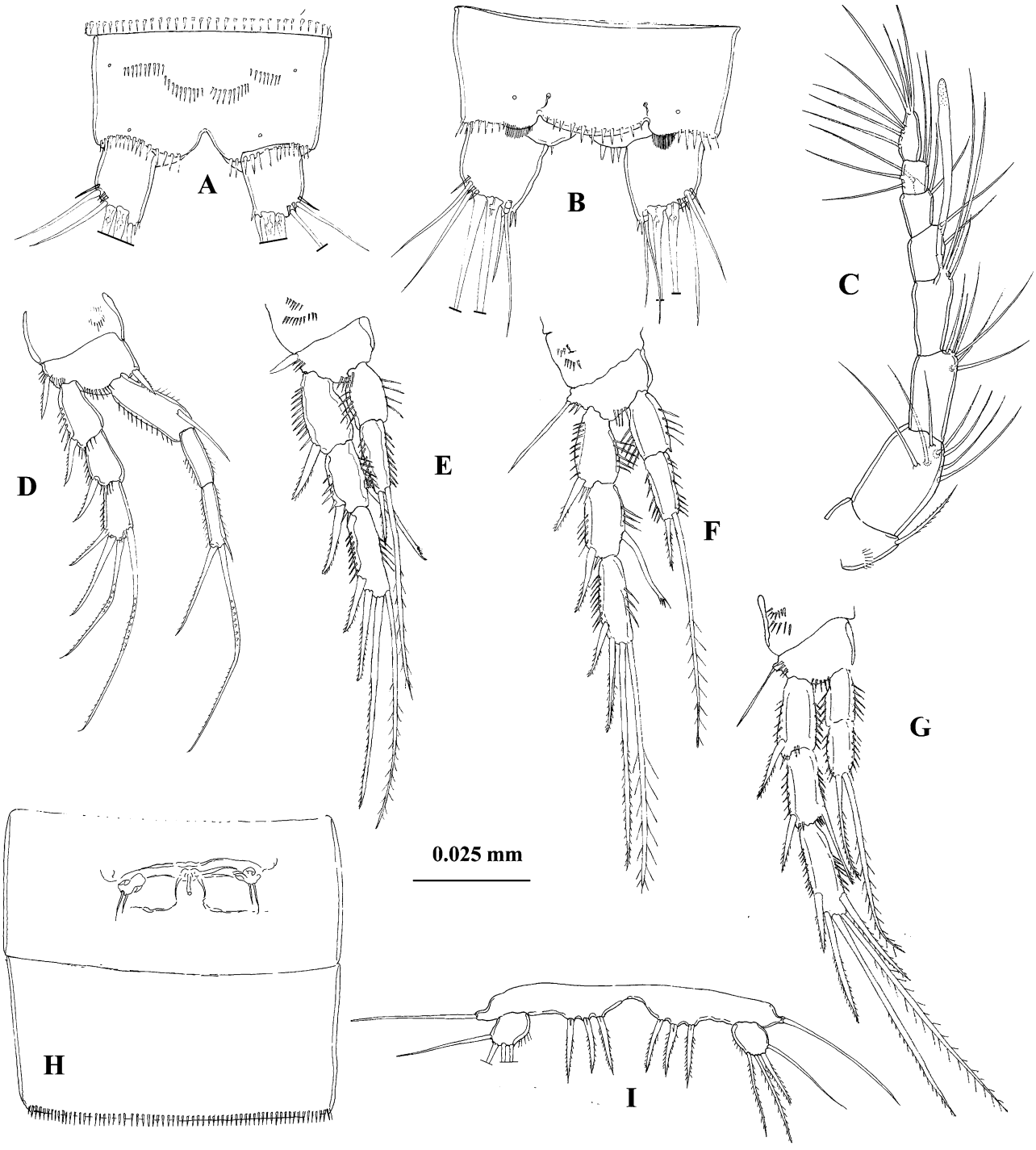

Figure 6. *Nitocrella ensifera* sp. nov., female. **A**: anal somite, caudal rami, ventral view. **B**: anal somite, anal operculum, caudal rami, dorsal view. **C**: antennule. **D**: P1. **E**: P2. **F**: P3. **G**: P4. **H**: genital field, genital and first abdominal somite, ventral view. **I**: P5.

Cyclopoida: *Acanthocyclops sp.* 1, *Diacyclops* sp. 2, *Microcyclops varicans* (G. O. Sars 1863), *Thermocyclops* sp. 1.

Another interesting component of the interstitial communuty of Sillano site is represented by a Hydridae (Cnidaria, Hydrozoa, Hydroida), collected with numerous depigmented individuals, all exclusively from the hyporheic habitat. It is well known that the biodiversity of groundwater has a truncated nature (Gibert & Deharveng 2002) because primary

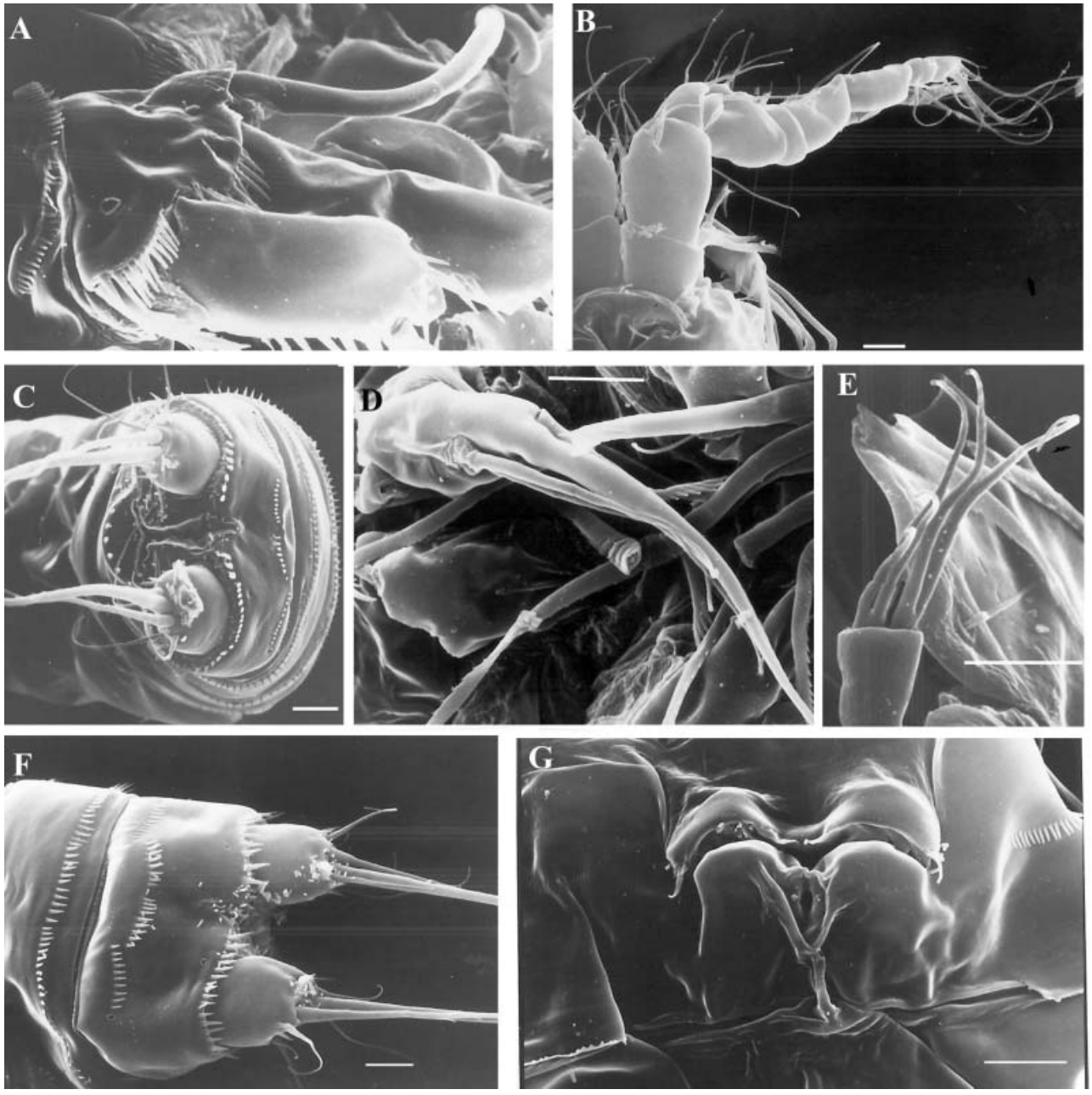

Figure 7. *Nitocrella ensifera* sp. nov. A, B, C, D, E: male. F, G: female. **A**: P1 basis and transformed seta. **B**: rostrum and antennule, dorsal view. **C**: last abdominal somite, anal somite, anal operculum, proctodeal spinules, caudal rami, posterior view. **D**: maxilla. **E**: mandible: last segment of palp and cutting edge of coxa. **F**: last abdominal somite, anal somite, caudal rami, ventral view. **G**: genital field. Scales: A, D, E: 5 μm; B, C, F, G: 10 μm.

producers are lacking, and strict predators are very few. The Hydridae appeared to be specialised in preying on microcrustaceans: two specimens had an almost undamaged and well-visible *Speocyclops* in their coelenteron, and a third specimens ingested a female of *P. reidae* sp. nov.

The accompanying fauna of *N. ensifera* sp. nov. was represented by specimens of the following copepods:

Harpacticoida: *Elaphoidella* sp. 1, *Parastenocaris amalasuntae* Bruno and Cottarelli 1998.

Cyclopoida: *Diacyclops* sp. 3, *Speocyclops* sp. 2.

Discussion

Parastenocaris is the type-genus of the family Parastenocarididae, and shows high taxonomic ''uncertainties'', due to the large number of species

assigned to it, and to the often incomplete older descriptions, with drawings of poor quality. Several authors recently underlined these problems (Schminke & Notenboom 1990; Reid 1995, Cottarelli & Bruno 1997; Martínez-Arbizu 1997; Berera & Cottarelli 2003; Galassi & De Laurentiis 2004) and discussed how the characters traditionally used in the taxonomy of the genus are no longer adequate to define true phylogenetic affinities.

Among the species-groups instituted by Lang (1948) for the genus, the *minuta* species-group includes 51 species in continental and insular Europe (Galassi & De Laurentiis 2004). According to Rouch (1990) and Galassi (1997) these species share a similar morphology of the male P3 and Enp-P4 and, but they actually differ in several phylogenetic informative characters; the *minuta* species-group was recognised as polyphyletic by Galassi and De Laurentiis (2004). Berera and Cottarelli (2003) defined the *hera* group on the basis of morphological, biogeographical and ecological characters; this new group included eight species previously assigned to the *minuta*-group. Even if the existing species-groups are questionable, we think they should be still used pending the revision of the genus *Parastenocaris*, and the formal assessment of natural species-groups.

Within the *minuta*-group, *Parastenocaris reidae* sp. nov. is distinguishable from the remaining species by the unique combination of structure and ornamentation of male P3 and Enp-P4, and of P5 and caudal rami in both sexes. The medial seta on P1 basis of the male is not modified and it is not accompanied by a chitinous ''protrusion'' (see Galassi & De Laurentiis 2004), it is longer and stronger than the corresponding seta of the female, as it occurs, for instance, in *P. silvana* Cottarelli, Bruno and Berera 2000. The remaining affinities are represented by the simple male P4 endopod, the caudal rami of both sexes cylindrical and longer than the last abdominal somite, with setae inserted towards the apex, and the P5 of both sexes with a medial tip. These features resemble to those of *P. silvana* and partly to those of *P. tryphida* Cottarelli and Bruno 1993 and *P. nertensis* Rouch 1990. The male P3 is not well characterized in several species of the *minuta*-group; in *P. reidae* sp. nov. it is slim and elongated, partially resembling the male P3 of *P. nertensis*.

Both the morphology and position of the genital field were recently listed among the characters that should be taken into account when assessing affinities within the family (Reid 1995; Galassi & De Laurentiis 2004). The genital field can be best examined from SEM images; comparison of images of *P. reidae* sp. nov. with the few images or drawings available for other species (Glatzel 1991; Reid 1995; Cottarelli & Bruno 1997; Galassi 1997; Martínez-Arbizu 1997; Bruno & Cottarelli 1998, 1999; Galassi & De Laurentiis 2004) showed marked differences between genera (i.e. *Parastenocaris* and *Simplicaris*), among species-groups, and also within a species-group (such as *P. reidae* sp. nov., *P. crenobia* Galassi 1997, *P. pasquinii* Cottarelli 1972 within the *minuta*-group).

Parastenocaris cf. *glacialis* is the second species collected in Garfagnana, Tuscany; these specimens correspond only in part to the original description and the subsequent ones given by Kiefer (1960, 1961), Kulhavy & Noodt (1968), and Pesce et al. (1995). The final definition of the taxonomic status of *P.* cf. *glacialis* requires observation of new and more abundant material of *P.* cf. *glacialis* and of *P. glacialis* collected from the type locality. The most important morphological characters of *P.* cf. *glacialis* and differences from the descriptions of *P. glacialis* available in literature are as follows:

(1) Integumental windows on cephalotorax and first three abdominal somites are present in *P.* cf. *glacialis*; versus absent in *P. glacialis* according to Noodt (1955); no longer detectable in diaphanized specimens (Galassi, personal communication), and not recorded (neither as present nor as absent) in Pesce et al. (1995).
(2) Caudal rami: more elongated than in the specimens described by Pesce et al. (1995), where the length/width ratio was about 3 in males and 2.6–2.75 in females. Lateral terminal seta is 1.7 and 1.5 times longer than terminal accessory seta, respectively, in males and females, whereas it was reported as twice as long for both sexes in Pesce et al. (1995).
(3) Mouthparts not previously described; they fit the basic structure known for the genus, with the exception of the proximal maxillar endite, which bears only one apical seta, a condition rarely found in species of *Parastenocaris*.
(4) Basis of male P4 with 4–6 cuticular spinules, the number varies even in the same individual; 5 spinules are reported in the original description and in Kiefer (1960), 4 in Kulhavy and Noodt (1968), and in Pesce et al. (1995); the exopod of the male P4 is longer and more curved than in Pesce et al. (1995).
(5) Female P5 with 4 apical setae, whereas it was reported with 3 in the original description and in Kulhavy and Noodt (1968), and 4 in Pesce et al. (1995); the lateral-most seta is longer than previously reported. The pore is present

in other species of *Parastenocaris* (e.g. *P. pasquinii*, *P. amalasuntae* Bruno & Cottarelli 1998, *P. silvana*).

(6) Basis of P1 with two medial setae in the male and only one longer seta in the female. Pesce et al. (1995) report only one medial seta for both male and female, confirmed by Galassi (personal communication). In all the other species of *Parastenocaris* the male has a longer and stronger seta. The male of *P.* cf. *glacialis*, moreover, has a second true seta on the medial side, almost identical to the first one. This peculiar feature could represent a new ornamentation pattern never recorded before in *Parastenocaris* (see Galassi & De Laurentiis 2004 for a more detailed discussion). However, as two males only were collected, a deeper interpretation of this feature will be given, pending the availability of additional material.

As for Parastenocarididae, the systematics of Ameridae is currently in revision. After the older revisions by Lang (1965), and Petkovski (1976), more recently several other authors (Cottarelli & Forniz 1993; Galassi & De Laurentiis 1997; Conroy Dalton & Huys 1998; Galassi et al. 1999; Fiers & Iliffe 2000; Lee & Huys 2002; Karanovic 2004) underlined how the systematics traditionally based on swimming legs segmentation can lead to results sometimes misleading and not informative, if characters with greater phylogenetic significance (such as setation patterns, and morphology and ornamentation of mouthparts and of other cephalic appendages) are not taken into account (Cottarelli & Forniz 1993; Galassi et al. 1999). Within this framework, ''central to this confusion stands the genus *Nitocrella* which has served as a taxonomic repository for freshwater Ameiridae'' (Lee & Huys 2002). As a consequence, the phylogenetic value of the *hirta*, *chappuisi* and *vasconica* groups of *Nitocrella* proposed by Petkovski (1976) on the basis of the number of setae/spines present on the P4 Exp-3, requires confirmation. However, Petkovski's species-groups are, according to us and to Karanovic (2004), still useful for a first taxonomic screening of the species, which must be followed by discussion and comparison of other characters with higher phylogenetic relevance.

The new species can be easily distinguished from all the other *Nitocrella* and also from all the Ameridae by the remarkable autapomorphy represented by the uniquely transformed medial seta of the male P1 basis. *Nitocrella ensifera* sp. nov. has 5 spines/setae on the P4 Exp-3, and therefore can be included in the *chappuisi* species-group. Within this group, the new species shares the highest number of characters with *Nitocrella stammeri*, reported for several localities in Italy (Cottarelli & Fasano 1979; Pesce 1985; Cottarelli et al. 1996). The two species have a similar structure and chaetotaxy of legs P1–P4 in both sexes (with the exception of the above-mentioned modified seta on male P1 basis); the same number of segments of male A1 [the male A1 of *N. stammeri* is 10-segmented and not 8-segmented as previously reported, for instance, by Cottarelli & Fasano (1979)]; similar A2, with distinct basis; female genital somite not fused with the first abdominal somite in both species; similar ornamentation of anal operculum and abdominal somites; similar shape and size of caudal rami which nonetheless differ in length between the two species. The genital field is similar for the two species but the two setae near the operculum are of same length in *N. stammeri*, whereas the lateral one is longer in *N. ensifera* sp. nov. The Italian populations of *N. stammeri* and *N. ensifera* sp. nov. differ in the following morphological features:

(1) Mandibular palp with one distal seta on the basis in *N. stammeri* versus naked in *N. ensifera* sp. nov.; endopod with 5 apical setae in the new species, and 4 in *N. stammeri.*
(2) Maxillule differing in the ornamentation of coxa, basis, and endopod, which bear, respectively, 3, 4, and 2 setae in *N. ensifera* sp. nov., and 3, 2, and 1 setae in *N. stammeri.*
(3) Maxilla with two endites with 2 setae each in *N. stammeri*, and one endite with 2 setae in *N. ensifera* sp. nov.
(4) P1–P4 very similar in the two species, but the P2–P3 endopods are proportionally shorter in the new species, and the apical setae of the P2–P4 endopods differ in length between the two species.
(5) P5 with the same number of setae/spines in the two species, but the setae differ in length between the two species; the male P5 exopod of *N. ensifera* sp. nov. is oval, whereas it is smaller and almost rectangular in *N. stammeri.*

Rouch (1985) redescribed of *N. stammeri* from specimens collected in Andalusia (Spain), but the ornamentation and morphology of mandible and maxillule he described do not correspond to those of the Italian populations of *N. stammeri*, and of *N. ensifera* sp. nov. It is likely that *N. stammeri* is in fact a group of cryptic species, as suggested also by the wide distribution of *N. stammeri.*

Nitocrella ensifera sp. nov. also has some affinities with *N. achaiae* Pesce 1981, in the morphology and

ornamentation of P1–P4, and the morphology of A2 and the unfused genital and first abdominal somites. It is likely that *N. achaiae* has a 10-segmented male A1, because this seems to be the common feature in *Nitocrella* and in other Ameiridae genera (Galassi et al. 1999; Lee & Huys 2002; Karanovic 2004). The shape and ornamentation of P5 are similar in the two species as well. *N. ensifera* sp. nov. differs from *N. achaiae* in the size of P2–P4 endopods, the ornamentation of the anal operculum, chaetotaxy and morphology of caudal rami. The mouthparts can not be compared because they were not described for *N. achaiae*.

Notes on distribution and ecology

The already-mentioned state of flux of the systematic of *Parastenocaris* complicates the interpretation of the biogeography of this genus. Nonetheless, *P. reidae* sp. nov. is closely related to species from Sardinia, Corsica, and the Pyrenean Massif, suggesting that their origin dates back to the fragmentation of the Sardinian–Corsica microplate, during the Early Miocene, 13 MYA (Bernini & Avanzati 1980; Cottarelli et al. 2000, 2002). The origin of this taxon is related to the fragmentation of this land unit, which may have led to vicariance events, and consequently to speciation.

Parastenocaris cf. *glacialis* and *P. glacialis* appear to have similar ecology: they can be considered cold-stenotherm relicts (Husmann 1975; Pesce et al. 1995).

Nitocrella is a stygobitic ameirid genus with 11 species recorded mostly from peninsular Italy, and with few species from Sicily, Sardinia and small Tyrrhenian islands (Berera et al. 2005). Some Italian *Nitocrella* have wide distributions: for instance *N. stammeri* is circum-Mediterranean, *N. psammophila* Chappuis 1954 is alpine, *N. achaiae* is reported for Greece and Italy. Other species have very narrow distributions and are recorded for only one site (Berera et al. 2005), such as *N. fedelitae* Pesce 1985, *N. juturnae* Cottarelli 1975, *N. stochi* Pesce & Galassi 1986. These ''spot'' distributions can be due to narrow ecological requirements of the species that are associated to peculiar microhabitats (see Galassi & De Laurentiis 1997), but can also be the consequence of missing records from undersampled regions, as it occurs for several other stygobitic taxa. Until further data are available, *N. ensifera* sp. nov. can be considered endemic to Tuscany. Its origin can be related to vicariance phenomena which affected the ancestral population from which both *N. ensifera* sp. nov. and its sister-species *N. stammeri* may have derived.

N. ensifera sp. nov. lives in several groundwater habitats such as the phreatic waters in the parafluvial of the Fiora River, and the deeper phreatic aquifer in the same area.

Acknowledgements

We want to thank Dr Annalisa Santacroce for collecting part of the material. Dr Annarita Taddei (Interdepartmental Center for Electron Microscopy, ''della Tuscia'' University) helped us to prepare specimens for SEM, and took the pictures. Dr Diana M. P. Galassi (University of L'Aquila, Italy) provided useful information on *Parastenocaris* cf. *glacialis*.

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