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Two new species of *Nitocrella* from groundwaters of Italy (Crustacea, Copepoda, Harpacticoida)

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ABSTRACT

Two new species of *Nitocrella* Chappuis, 1923, *N. pescei* sp. n. and *N. kunzi* sp. n., from the Presciano spring system (Tirino River, Abruzzo, central Italy) are described. Both species fit into the *hirta*-group of *Nitocrella*, as defined by Petkovski (1976). From an ecological point of view, they are true stygobiont species, linked to different biotopes of the crenal habitat.

KEY WORDS: Harpacticoida - *Nitocrella* - Springwaters - Stygobiont.

ACKNOWLEDGEMENTS

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INTRODUCTION

The genus *Nitocrella* Chappuis, 1923 belongs to the primarily marine family Ameiridae Monard, 1927, which secondarily invaded fresh water. After Petkovski's (1976) revision, it includes 53 species, almost always occurring in subterranean fresh water and brackish environments. From a biogeographic point of view, this genus shows a discontinuous and relatively wide distributional range. The sparse data from North America and Africa, as well as the lack of records from South America and Australia, mean that much more detailed investigations are needed in regions and habitats not well known.

The fine-scale approach to the study of copepod distribution in the subterranean microhabitats of the Presciano spring system (Abruzzo, central Italy) shows a greatly diversified fauna, with different displacements of the species along both vertical and longitudinal profiles (Galassi *et al.*, 1997; Galassi & De Laurentiis, in press). Samples taken from different sites of this crenal system revealed the presence of two new species of the genus *Nitocrella*, herein described respectively as *N. pescei* sp. n. and *N. kunzi* sp. n.

MATERIALS AND METHODS

Quantitative replicate samples were taken by pumping 20 litres of water with a Bou-Rouch pump (1967) at two different depths (70 cm and 150 cm, respectively) below the bottom and filtering through a 60-µm mesh net. Qualitative Surber (1937) samples in surface-subsurface microhabitats were taken by filtering the topmost layer of sediments near the bottom, and washing submerged vegetation (epibenthic, epiphytic, inbenthic biotopes). Specimens were preserved in 7% formalin solution. Dissected specimens were mounted in polyvinyl lactophenol. Drawings and measurements were made using a Leitz Laborlux phase contrast microscope, with the aid of a camera lucida. Because body length measurements are often affected by the telescoping action of the body somites, an additional method for measuring the total length of the specimens was used, according to Clément & Moore (1995). The terminology proposed by Huys & Boxshall (1991) is adopted. Abbreviations used in the text are: ae, aesthetasc; P1-P6, first to sixth thoracopods; exp, exopod; enp, endopod, benp, baseoendopod.

TAXONOMIC ACCOUNT

Order HARPACTICOIDA Sars, 1903

Family AMEIRIDAE Monard, 1927

Genus *Nitocrella* Chappuis, 1923

Nitocrella pescei sp. n.

Material examined

1 ♀, holotype, and 8 ♂♂, 15 ♀♀ paratypes, completely dissected and mounted in polyvinyl lactophenol; small lateral hollows of the Presciano spring system (Capestrano, L'Aquila, central Italy); coordinates: 42°16'05"N 13°46'56"E; altitude: 330 m a.s.l.; interstitial biotope, 150 cm below the bottom; temperature: 11.0° C, electrical conductivity (25° C): 483 µS/cm, pH 7.40, dissolved oxygen:

7.1 mg/l, sediment composed by a small amount of gravel and coarse sand on the carbonate bedrock; 9 July 1996; coll. A. Marchegiani, P. De Laurentiis & D. M. P. Galassi. 1 ♀, dissected and mounted as above; upwelling zone, 20 m downstream from the main spring hollows; 70 cm below the bottom; temperature: 13.3° C, electrical conductivity (25° C): 480 µS/cm; pH 7.8; dissolved oxygen: 6.9 mg/l, sediment composed by sand and a small amount of clay; same data and collectors. 13 ♂♂ paratypes, completely dissected and mounted as above; 1♂ and 1♀ paratypes mounted without dissection; upwelling zone in the same locality, 150 cm below the bottom; temperature: 10.57° C, electrical conductivity (25° C): 510 µS/cm, pH 7.50, dissolved oxygen: 6.97 mg/l, alluvial sediment composed by sand and a small amount of gravel; 18 September 1996; coll. A. Marchegiani & P. De Laurentiis. 3 ♀♀ dissected and mounted as above; a large hollow on the left side of the same karstic spring system; 120 cm below the bottom; temperature: 10.4° C, electrical conductivity (25° C): 250 µS/cm, pH 7.4, dissolved oxygen: 8.6 mg/l, sediment composed by medium-sized sand on fissured carbonate rocks; 22 July 1996; coll. A. Marchegiani & P. De Laurentiis. No dissected specimens from numerous sampling sites preserved in 70° alcohol.

Types

Holotype and one male paratype in the Natural History Museum, London; remaining material in Galassi's collection at the Dipartimento di Scienze Ambientali, University of L'Aquila (Italy).

Description

Female

Length, excluding caudal setae, from 431 to 490 µm (440 µm, holotype). Body cylindrical, colourless. Rostrum with subrounded tip, with 2 setules at basis. Free thoracic somites and ventral margins of all abdominal somites with weakly developed hyaline frill, not incised. Posterior dorsal margins of abdominal somites with weakly incised hyaline frill. Genital and first abdominal somites unfused both on dorsal and ventral surfaces (Fig. 1A); ventral surface of genital somite with sparse hair-like elements; numerous rows of tiny spinules and a continuous row of stronger spinules on the distal margin of all abdominal somites on ventral side. Anal somite ventrally with 8 transverse spinulose rows and tiny setules sparsely. Dorsal surface of the genital segment and abdominal somites with tiny spinules sparsely. Anal somite dorsally with 9 transverse rows of spinules (Fig. 1B). Anal operculum slightly convex, armed with numerous spinules on dorsal surface and hair-like elements on free distal margin.

Caudal rami (Fig. 1B) slightly divergent, longer than wide (length/width ratio, measured at proximal part of the caudal ramus: about 1.17), subrectangular in shape. Armature as follows: seta I minute, setae II and III slender and bare, of about the same length, setae IV and V well developed, seta VI bare, seta VII long, inserted on a small tubercle; a row of spinules near its insertion on caudal ramus.

Antennule (Fig. 1C) of moderate length, 8-segmented; segment 4 with an aesthetasc reaching the tip of antennule. Armature formula: 1-[1], 2-[9], 3-[5], 4-[3+(1+ae)], 5-[2], 6-[2], 7-[3], 8-[7].

Antenna (Fig. 1D): basis unarmed, with some spinules along inner margin; exopod 1-segmented, bearing 3 distal setae, one of which bipinnate; endopod 2-segmented, segment 1 with 2 rows of spinules along inner margin, segment 2 with 3 bare setae, 5 apical geniculate setae and 1 outer bipinnate seta, inserted near the basis of the outermost geniculate seta.

Mandible (Fig. 1E): coxal gnathobase elongate, cutting edge with 4 teeth and a crenulate chitinous lamella; 1 seta at dorsal corner. Mandibular palp 2-segmented; segment 1 armed with a strong bipinnate seta, segment 2 with 5 slender and bare setae.

Maxillule (Fig. 1F): praecoxal arthrite well developed, with 2 strong and curved spines and 1 distally crenulate spine, 1 slender seta and 2 stout curved spines in a subdistal position; 2 slender setae on anterior surface. Coxal endite with 3 slender setae and 1 geniculate unipinnate spine. Basal endite with 5 setae; exopod represented by a rudimentary tubercle bearing 1 bipinnate seta. Endopod not recognizable by setation.

Maxille (Fig. 1G): syncoxa with 2 endites; proximal endite well developed, with rounded margins, bearing 2 bipinnate setae, not defined at base; distal endite cylindrical, with 3 apical slender setae and 1 strong seta, crenulate distally. Allobasis with strong unipinnate claw, not defined at base; accessory armature consisting of 1 lateral seta, with enlarged and crenulate apex. Endopod reduced to a little tubercle bearing 2 bipinnate setae.

Maxilliped (Fig. 1H) prehensile. Syncoxa with 1 plumose seta. Basis unarmed. Endopod as a strong, curved unipinnate claw.

P1 with both exopod and endopod 3-segmented. P2-P4 with 3-segmented exopod and 2-segmented endopod. Intercoxal sclerites without ornamentation.

P1 (Fig. 2A): coxa with 1 marginal and 2 surface rows of spinules. Basis with outer spiniform seta and inner strong unipinnate spine; two rows of spinules, each inserted, respectively, near the insertion of the outer seta and the inner spine, and a distal row of spinules between exopod and endopod. Exopod slightly longer than endopod; segments 1 and 2 each with 1 outer spine, a row of spinules along outer margins and long setules on inner margins; segment 2 with also 1 inner seta; segment 3 with 2 outer spines and 2 apical geniculate setae. Endopod: outer margins of segments with a row of spinules, inner margins with some long setules; segment 1 slightly longer than the first exopodal segment, unarmed; segment 2 unarmed, segment 3 with 2 apical geniculate setae and inner minute and bare seta.

P2-P4 (Fig. 2B-D): basis with a slender and bare outer seta and 2 rows of spinules. Exopod: all segments provided with outer rows of spinules and some hair-like elements along inner margins. Segment 1 with 1 outer spine, segment 2 with 1 outer spine and 1 inner short, unipinnate seta; segment 3 with 2 outer spines and 2 long plumose setae. Endopod P2-P3 longer than endopod P4; outer margins of segments with continuous rows of spinules, inner margins with some long se-

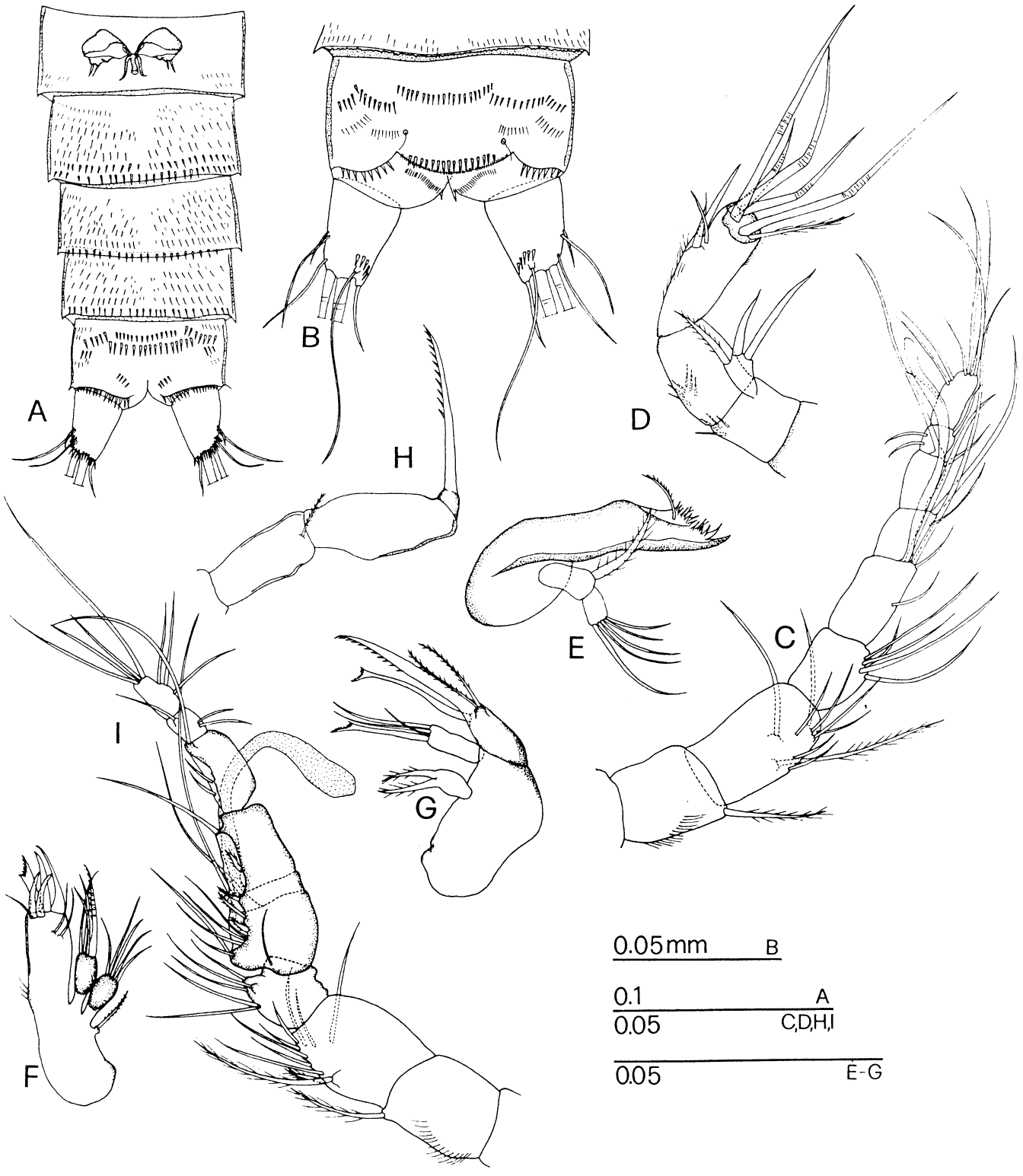

Fig. 1 - *Nitocrella pescei* sp. n. A, Abdomen and caudal rami (♀, paratype). B, Anal somite and caudal rami (♀, paratype). C, Antennule (♀, paratype). D, Antenna (♀, holotype). E, Mandible (♀, holotype). F, Maxillule (♀, holotype). G, Maxille (♀, holotype). H, Maxilliped (♀, holotype). I, Antennule (♂, paratype).

tules; segment 1 unarmed, segment 2 with 1 spine and 1 long plumose seta. Endopod P4 strongly reduced in size, shorter than the first exopodal segment; minor armature as in P2-P3; segment 1 unarmed, segment 2 with 1 long apical plumose seta.

P5 (Fig. 2E-F): baseoendopod not prominent, armed

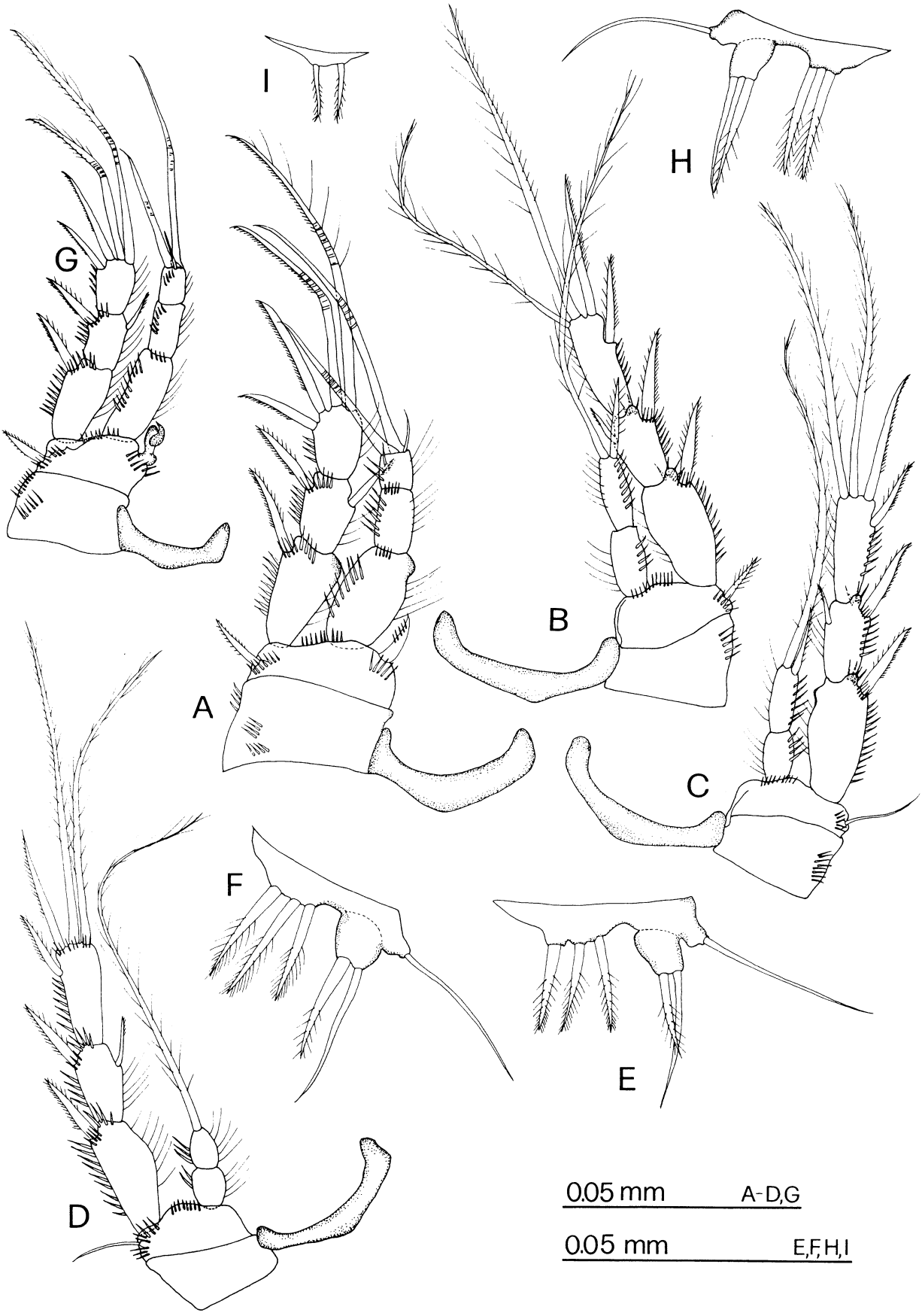

Fig. 2 - *Nitocrella pescei* sp. n. A, P1 (♀, holotype). B, P2 (♀, holotype). C, P3 (♀, holotype). D, P4 (♀, holotype). E, P5 (♀, holotype). F, P5 (♀, paratype). G, P1 (♂, paratype). H, P5 (♂, paratype). I, P6 (♂, paratype).

with 3 bipinnate setae, outer basal seta slender and bare; exopod as long as wide, not well defined at base, armed with 1 bipinnate seta and a longer bare seta.

P6 (Fig. 1A) represented by a little rudimentary plate, bearing 2 short setae.

Male.

Body length, excluding caudal setae, from 376 to 438 µm. Hyaline frills and armature of both body somites and caudal rami as in female.

Antennule (Fig. 1I) 9-segmented, haplocer; geniculation between segments 6 and 7; a long aesthetasc on segment 4; soft sclerotization of segments 4, 5 and 6.

P1 (Fig. 2G) quite similar to that of female, the only difference regarding the transformed inner spine on basis.

P5 (Fig. 2H): baseoendopod armed with 2 bipinnate setae; basal outer seta slender and bare; exopod not well defined at base, bearing 2 setae, the outer bare, the inner bipinnate, of the same length.

P6 (Fig. 2I) as a chitinous lamellar plate, with 2 bipinnate setae, of the same length.

Variability

A little variation was observed in body size, depending on different sampling times. A few variations perceived in the degree of fusion between P5 baseoendopod and exopod in both sexes.

Etymology

Specific name after Prof. Giuseppe L. Pesce (University of L'Aquila, Italy), who greatly promoted groundwater investigations in Italy.

Affinities

Nitocrella pescei sp. n. fits the *hirta*-group of *Nitocrella*, according to Petkovski (1976), owing to the armature of the distal segment of P4 exopod with 3-4 elements. Within this group, the new species is close to *Nitocrella juturna* Cottarelli, 1975, from phreatic waters of Abruzzo (central Italy) and to *N. fedelitae* Pesce, 1985, from phreatic waters of Molise (southern Italy), due to the identical armature of P1-P4 exopods, P2-P4 endopods and the general morphology and armature of caudal rami. The new species is easily distinguished from the above taxa (Table I) by the exclusive combination of the following characters: P1 without inner seta on endopodal segment 2, P5 (♀) with 2 setae on exopod and 3 inner setae on baseoendopod. Moreover, in both sexes, the exopod is more or less fused to baseoendopod: a few perceived insertion line of P5 exopod *are* noticeable only on anterior side; but, in all specimens, both exopod and baseoendopod are confluent on posterior side.

With regard to the armature of P1 endopod, which has a high taxonomic significance within the genus, *Nitocrella fedelitae* has been described and figured with naked endopodal segments 1 and 2 (Pesce, 1985). The re-examination of the type-material revealed the presence of an inner seta on the second segment of P1 endopod and a complete armature of caudal rami (seta I minute and bare present, seta VII about 3 times longer than seta VI).

Nitocrella kunzi sp. n.

Material examined

1♀, holotype, and 1♂, 1♀ paratypes, completely dissected and mounted in polyvinyl lactophenol; Presciano spring system (Abate spring, Capestrano, L'Aquila, central Italy); coordinates: 42°16'04"N 13°47'37"E; altitude: 330 m a.s.l.; interstitial biotope, 150 cm below the bottom; temperature: 10.6° C, electrical conductivity (25° C): 300 µS/cm, pH 7.16, dissolved oxygen: 7.58 mg/l; sediment composed by fine sand; 27 June 1996; coll. A. Marchegiani, P. De Laurentiis. 3♂♂ paratypes completely dissected and mounted as above; upwelling zone in the same locality, interstitial biotope, 150 cm below the bottom; temperature: 10.45° C, electrical conductivity (25° C): 508 µS/cm, pH 7.50, dissolved oxygen: 6.97 mg/l; alluvial sediment composed by sand and a small amount of gravel; 18 September 1996; coll. A. Marchegiani & D. M. P. Galassi.

Types

Holotype in the Natural History Museum, London; other material in Galassi's collection at the Dipartimento di Scienze Ambientali, University of L'Aquila (Italy).

Description

Female

Body length, excluding caudal setae, by sum of all somites, from 319 µm to 384 (319 µm, holotype). Body cylindrical, colourless.

Table I - *Setation formula of P1-P5 in the closely related* Nitocrella *species:* N. juturna *Cottarelli,* N. fedelitae *Pesce,* N. pescei *sp. n.*

Nitocrella	P1		P2		P3		P4		P5 ♀	
species	exp	enp	exp	enp	exp	enp	exp	enp	exp	benp
N. juturna	0.1.022	0.1.120	0.1.022	0.020	0.1.022	0.020	0.1.022	0.010	3	3
N. fedelitae	0.1.022	0.1.120	0.1.022	0.020	0.1.022	0.020	0.1.022	0.010	3	3
N. pescei	0.1.022	0.0.120	0.1.022	0.020	0.1.022	0.020	0.1.022	0.010	2	3

Free thoracic and abdominal somites with weakly developed hyaline frill, not incised, both on ventral and dorsal surfaces. Genital and first abdominal somites unfused on dorsal surface and partially fused on ventral surface (Fig. 3A); first abdominal somite with two rows of spinules on the latero-distal third of the ventral surface and a continuous row of spinules along the posterior ventral margin. Remaining abdominal somites armed as in Figure 3 A, B. Dorsal surface of the genital segment and of all abdominal somites with sparse rows of tiny spinules; a continuous row of tiny spinules is present on the distal margin of each abdominal somite. Anal operculum slightly convex, armed with numerous hair-like elements.

Caudal rami (Fig. 3B) divergent, stout and short, wider than long (length/width ratio: about 0.52). Hyaline frill of anal somite fully-incised, subulate, ventrally. Armature as follows: seta I minute, setae II and III bare and minute, of about the same length, setae IV and V well developed, setae VI and VII bare.

Antennule (Fig. 3C) of moderate length, 8-segmented; segment 4 with aesthetasc, not reaching the tip of the antennule. Armature formula: 1-[1], 2-[8], 3-[6], 4-[3+(1+ae)], 5-[2], 6-[2], 7-[4], 8-[7].

Antenna (Fig. 3D): basis unarmed, with tiny hairs along the inner margin; exopod 1-segmented, with 3 distal setae, one of which bipinnate; endopod 2-segmented, first segment naked, second segment with 2 inner unipinnate setae, 1 inner slender and bare seta, 5 apical geniculate setae and 1 outer short plumose seta inserted near the basis of the outermost geniculate seta.

Mandible (Fig. 3E): coxal gnathobase elongate, cutting edge with 6 teeth and a crenulate chitinous lamella; 1 seta at dorsal corner. Mandibular palp 2-segmented; first segment unarmed, second segment with 5 apical setae.

Maxillule (Fig. 3F): well developed praecoxal arthrite, represented by a rectangular lobe with 6 apical elements, 1 lateral seta and 2 setae on anterior surface. Coxal endite with 3 apical setae; basal endite with 4 setae; exopod represented by a rudimentary tubercle, bearing 2 strong and plumose setae; endopod not recognizable by setation.

Maxille (Fig. 3G): syncoxa with 2 endites; proximal endite rudimentary, as a little tubercle, not defined at base, bearing 2 apical stout spines; distal endite cylindrical, with 3 apical plumose setae, the proximal the largest. Allobasis with a strong bipinnate claw, not defined at base; accessory armature consisting of 1 lateral strong plumose seta. Endopod reduced to a tubercle bearing 2 bipinnate setae.

Maxilliped (Fig. 3H): syncoxa with 1 plumose seta. Basis unarmed. Endopod as a strong, curved unipinnate claw.

P1 with both exopod and endopod 3-segmented. P2-P4 with 3-segmented exopod and 2-segmented endopod. Intercoxal sclerites without ornamentation.

P1 (Fig. 4A): coxa with a row of spinules on anterior surface; basis with inner bipinnate spine and outer unipinnate seta. Exopod shorter than endopod; segment 1 and 2 with 1 outer spine and a row of thin spinules along the outer margins; segment 3 with 2 outer spines, and 2 long apical geniculate setae. Endopod: outer margins of segments with a row of thin spinules, inner margin with some long setules; segment 1 slightly shorter than exopodal segments 1 and 2 together, and armed with an inner plumose seta; segment 2 unarmed; segment 3 with 2 apical geniculate setae and 1 inner slender and plumose seta.

P2-P4 (Fig. 4B-D) quite similar in shape and armature: basis with a slender and bare seta on the outer margin and 2-3 rows of hair-like elements. Exopod: all segments provided with outer rows of spinules and some hair-like elements along inner margins. Segment 1 and 2, each with 1 outer spine, segment 3 with 2 outer spines and 2 long apical plumose setae. Endopod: 2-segmented, reaching about the tip of the exopodal segment 2 in P2-P3, shorter in P4. Outer margins of segments with numerous hair-like elements, inner margin with some long setules; segment 1 unarmed, segment 2 with 1 spine and 1 long plumose seta.

P5 (Fig. 4E): baseoendopod not prominent, armed with 2 spiniform setae, outer basal seta plumose; exopod: slightly longer than wide; armature consisting of 2 spiniform setae and 2 bare and slender setae of different length.

P6 (Fig. 3A): rudimentary, with 2 setae of different length.

Male

Body length, excluding caudal setae, from 403 to 411 µm. Hyaline frills and armature of body somites and caudal rami as in female.

Antennule (Fig. 3I,L): 9-segmented, haplocer; geniculation between segments 6 and 7; a swollen and long aesthetasc on segment 4; segment 5 represented by a rudimentary ring; segments 4, 5 and 6 partially enveloped by a chitinous sclerite.

P1 (Fig. 4F) quite similar to that of female, the only difference regarding the transformed inner spine on basis and the relative length of the endopodal segment 1.

P5 (Fig. 4G): minor differences with respect to the female in the relative lengths of the armature elements.

P6 (Fig. 4H) with 2 setae of about the same length.

Etymology

Specific name after Dr. Helmut Kunz, a leading scientist in the systematics of marine and brackish water harpacticoids.

Affinities

According to Petkovski's (1976) revision of the genus *Nitocrella, N. kunzi* sp. n. belongs to the *hirta*-group, but it shows no close affinities with any other species within this group. The absence of the inner seta on P1-P4 exopodal segments 2, the peculiar morphology of caudal rami, and the anal operculum armed with hair-

Fig. 3 - *Nitocrella kunzi* sp. n. A, Abdomen and caudal rami (♀, paratype). B, Anal somite and caudal rami (♀, paratype). C, Antennule (♀, holotype). D, Antenna (♀, holotype). E, Mandible (♀, paratype). F, Maxillule (♀, holotype). G, Maxilla (♀, holotype). H, Maxilliped (♀, holotype). I, Antennule (♂, paratype). L, Antennule (♂, paratype), detail of segments 4, 5 and 6.

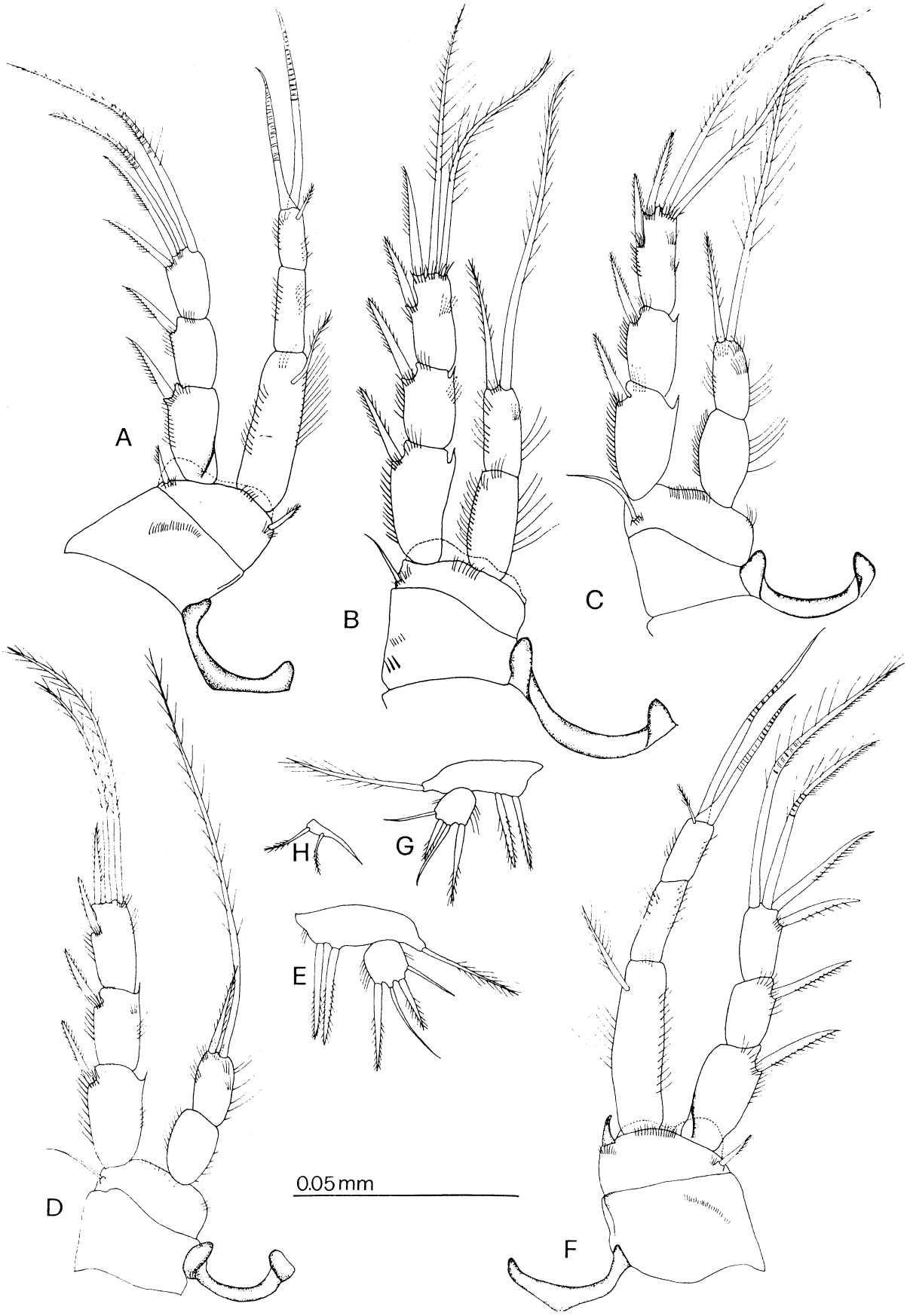

Fig. 4 - *Nitocrella kunzi* sp. n. A, P1 (♀, holotype). B, P2 (♀, holotype). C, P3 (♀, holotype). D, P4 (♀, holotype). E, P5 (♀, paratype). F, P1 (♂, paratype). G, P5 (♂, paratype). H, P6 (♂, paratype).

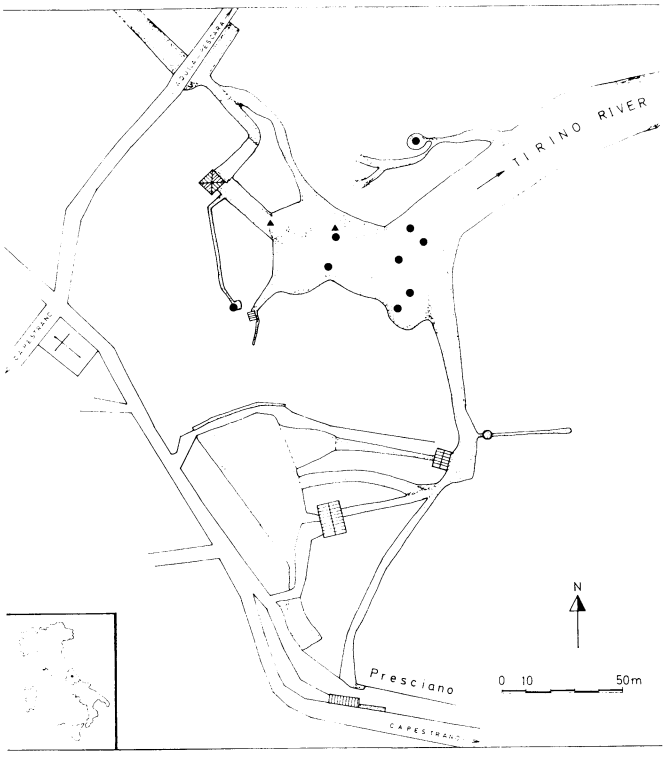

Fig. 5. Fine-scale distribution of both *Nitocrella pescei* sp. n. (●) and *N. kunzi* sp. n. (▲) in the Presciano spring system, Italy.

like elements give this species a relatively isolated position within the *hirta*-group. It shares only weak affinities with *N. slovenica* Petkovski, 1959, from phreatic waters of Slovenia, *N. psammophila* Chappuis, 1954, from both phreatic and hyporheic habitats of Italy and Croatia, *N. skyrensis* Pesce, 1981, from phreatic waters of Skyros island (Greece) and *N. morettii* Pesce, 1984, from phreatic waters of Umbria (central Italy), all showing a P1 endopod 1 with inner seta.

DISCUSSION

Both *Nitocrella pescei* sp. n. and *N. kunzi* sp. n. appear to be sympatric but rarely syntopic in the Presciano spring system, exhibiting different spatial distributional patterns (Fig. 5). In a fine-scale ecological perspective, *N. pescei* is widely distributed in the study area, showing higher occurrence and abundance at 150 cm depth, near the wider openings of the carbonate rock. Moreover, it was found with lower occurrence and abundance in other sites, located in different upwelling areas of the spring, also in alluvial sediments. On the other hand, *N. kunzi* was collected from few sites only (2 out of a total of 50) from the Presciano spring system, all distributed near the left side, in a restricted upwelling area, mainly characterized by abundant alluvial sediments. Neither *N. pescei* nor *N. kunzi* were ever collected from surface-subsurface microhabitats. Consequently, they are to be considered true stygobiont species, sensitive to the hydrological pattern of the spring and linked to deeper groundwaters, showing an interesting spatial segregation in the study area. Although little is known about the autoecology of the stygobiont taxa (Dole-Olivier & Marmonier, 1992; Dole-Olivier *et al.*, 1994; Rogulj *et al.*, 1994; Strayer, 1994), it seems plausible that *N. pescei* has a wider ecological tolerance to sediment characteristics, with marked preferences for a karstic habitat, while *N. kunzi* lives only in an interstitial one.

Moreover, comparisons between the patterns of character changes revealed a great interspecific variation of

TABLE II - *A comparison between mouthparts in* Nitocrella pescei *sp. n. and* N. kunzi *sp. n.*

Mouthparts	*Nitocrella pescei*	*Nitocrella kunzi*
Mandibular palp segment 1	with seta	without seta
Maxillule		
proximal endite	5 setae	4 setae
distal endite	3 plain +1 transformed setae	3 plain setae
praecoxal arthrite	with 8 elements	with 9 elements
exopod	with 1 seta	with 2 setae
Maxille		
proximal endite	developed	rudimentary
distal endite	with 3 plain+1 crenulate setae	with 2 slender+1 strong bipinnate setae
allobasis	with 1 accessory distally crenulate seta	with 1 accessory bipinnate seta
Maxilliped		
syncoxa/basis	length ratio: ca 0.8	length ratio: ca 0.5

the mouthparts, suggesting an important role of trophic specialization in the niche diversification of the stygobiont species of *Nitocrella* (Table II). A similar situation was described by Boxshall & Evstigneeva (1994) within the Baikalian cyclopid flocks of *Acanthocyclops/Diacyclops* species.

From a phylogenetic point of view, *N. pescei* shows a higher number of characters in a plesiomorphic state (segment 1 of mandibular palp with seta, well developed proximal endite of maxille, inner seta on P1-P4 exopodal segment 2 present, genital and first abdominal somites unfused) with respect to *N. kunzi*. Consequently, the co-presence of these two species in the Presciano spring system could be the result of two different colonization events. With regard to this, the more ancient origin of *N. pescei* could further explain the wider fine-scale distribution of this species, compared to that of *N. kunzi*. Further investigations, in both fine- and mesoscale perspectives, might corroborate the above proposition.

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