

REMARKS ON THE GENUS *ICHNUSELLA* (CRUSTACEA, COPEPODA, HARPACTICOIDA) AND DESCRIPTION OF TWO SPECIES FROM SUBTERRANEAN FRESHWATER HABITATS IN LATIUM AND SARDINIA, ITALY

V. COTTARELLI, M.C. BRUNO, R. BERERA

Dipartimento di Scienze Ambientali, Università degli Studi della Tuscia,
Via San Camillo De Lellis, 01100, Viterbo, Italy
E-mail : Cottarel@unitus.it

HARPACTICOID COPEPODS
ICHNUSELLA
LEPTOPONTIIDAE
PSAMMON
SYSTEMATICS
DISTRIBUTION

ABSTRACT. – This work concerns the description and discussion of two new species of *Ichnusella* collected in subterranean freshwater, near the rivermouth of some Italian rivers and streams. *I. tertia* n. sp. has been collected in the interstitial habitat of the Fiora River and from the small Valfragida Stream (Viterbo Province, Latium); this species has been studied using scanning electron microscopy. *I. longifurca* n. sp. is described from a similar phreatic habitat of De li Saldi Stream (Sassari Province, Sardinia). In addition new morphological features for *I. pasquini* (Cottarelli, 1969) and *I. eione* Cottarelli, 1971 are described. An amended generic diagnosis and a key to the four known species are presented. Some remarks on the ecology and distribution of these species are also given.

COPÉPODES HARPACTICOÏDES
ICHNUSELLA
LEPTOPONTIIDAE
PSAMMON
SYSTÉMATIQUE
DISTRIBUTION

RÉSUMÉ. – La présente étude concerne la description de deux espèces d'*Ichnusella* des eaux souterraines douces, proches de l'embouchure de quelques cours d'eau italiens. *I. tertia* n. sp. provient de l'interstitiel du fleuve Fiora, et de la petite rivière Valfragida (province de Viterbo, Latium); cette espèce est étudiée en outre par microscopie électronique à balayage. *I. longifurca* n. sp., provient des eaux phréatiques proches du fleuve De li Saldi (province de Sassari, Sardaigne). Quelques compléments morphologiques concernant *I. pasquini* et *I. eione*, sont apportés. Ces nouvelles acquisitions permettent la révision de la diagnose du genre *Ichnusella* et une clef dichotomique des espèces est établie. Quelques observations et considérations sur l'écologie et la distribution sont également présentées.

INTRODUCTION

The genus *Ichnusella* Cottarelli, 1971 was originally assigned to the subfamily Psammopsyllinae Krishnaswamy, 1956 in the family Cylandropsyllidae Sars, 1909. At that time and until recently this family included, besides the Psammopsyllinae, three other subfamilies: Cylandropsyllinae, Leptastaciinae and Leptopontiinae (Lang, 1948).

The polyphyletic status of the Cylandropsyllidae was first recognized by Huys (1992) who raised the Leptastaciinae to family rank. Subsequently, Martinez Arbizu & Moura (1994) discussed the position of the remaining subfamilial taxa of the Cylandropsyllidae and altered their taxonomic concepts profoundly. These authors allocated the subfamily Cylandropsyllinae Sars, 1909 to the family Canthocamptidae Sars, 1906

in which they included the genera *Cylindropsyllus* Brady, 1880, *Evansula* T. Scott, 1906, *Stenocaris* Sars, 1909, *Cylinula* Coull, 1971, *Boreopontia* Willems, 1981, *Stenocaropsis* Apostolov, 1982, and an as yet unpublished genus *Selenopsyllus* Pottek. They further proposed the new family Leptopontiidae consisting of the subfamilies Leptopontiinae Lang, 1948 (with the only the genus *Leptopontia* T. Scott, 1902), Psammopsyllinae (with *Psammopsyllus* Nicholls, 1945, *Sewellina* Krishnaswamy, 1956, *Ichnusella*, *Notopontia* Bodiou, 1977, *Syrlicola* Willems & Claeys, 1982, *Parasewellina* Cottarelli, Saporito & Puccetti, 1986, and *Prosewellina* Mielke, 1987) and a new subfamily Arenopontiinae (with the only genus *Arenopontia* Kunz, 1937).

Huys *et al.* (1996) recognized the Leptopontiidae as a valid family, including in it the Psammopsyllinae, but assigning the genera *Notopontia* and *Syrlicola* to the Leptopontiinae (Huys &

Ohtsuka 1993, Huys & Conroy-Dalton 1996). Moreover, these authors raised the third subfamily, Arenopontiinae, to the rank of family, the Arenopontiidae, retaining in this family the genus *Pararenopontia* Bodiou & Colomines, 1986. Finally, they did not follow Martinez-Arbizu & Moura's (1994) placement of the *Cylindropsyllinae* in the *Canthocamptidae* and instead regarded the taxon as a valid family accommodating the genera *Cylindropsyllus*, *Evansula*, *Stenocaris*, *Boreopontia*, *Stenocaropsis*, *Willemsia* Huys and Conroy-Dalton, 1993 and *Navalonia* Huys and Conroy-Dalton, 1993.

It is clear that the high level systematics of these taxa is currently in a state of flux. Even though we accept the *Psammopsyllinae* as defined by Huys *et al.* (1996) as a natural group, its present position as a subfamily within the *Leptopontiidae* should be regarded as provisional (Huys, pers. comm.). Its status and that of the *Arenopontiinae* should be reviewed in the light of forthcoming in-depth studies of various genera. There are several indications that the *Psammopsyllinae* deserves upgrading to full family status, however, its recognition as a separate lineage will require a comprehensive phylogenetic analysis, discussing the potential relationships with other interstitial taxa such as the *Parastenocarididae* Chappuis, 1933 (Bruno *et al.* 1998).

Two species of *Ichnusella* are known thus far: *I. pasquinii* (Cottarelli, 1969), from the phreatic habitat of a small stream on a beach near Sperlonga Village (Latina Province, Latium), and *I. eione* Cottarelli, 1971, from a similar habitat in the rivermouth of Cedrino River (Nuoro Province, Sardinia). Recent research on the interstitial fauna of rivermouth ecotones, boundary lines between two communities (Cottarelli *et al.* 1995, Cottarelli & Bruno 1996) has resulted in the discovery of two new species of *Ichnusella* to be described and discussed below.

I. tertia n. sp. has been collected in the rivermouth of two rivers (Fiora River and Valfragida Stream), which enter the Tyrrhenian Sea at about 6.3 km distance between each other. In Sardinia (Sassari Province) we have collected *I. longifurca* n. sp. in the rivermouth of De li Saldi Stream and many specimens of *I. eione* in the rivermouth of the Coghinis River, thus widening the distribution area of the latter species. New morphological features have been observed both for *I. eione* and *I. pasquinii* using the new material and the old specimens retained in our collection. Comparison of these data with those referring to the two new species, has been used to propose a more precise diagnosis of the genus. The phylogenetical implications are discussed and new information concerning the ecology and the distribution of these very characteristic members

of the interstitial rivermouth taxocenosis, are presented.

MATERIALS AND METHODS

Specimens were collected using the Karaman-Chappuis method (Delamare Deboutteville 1960), fixed in 5 % buffered formalin and mounted on permanent slides with Faure's medium. They were drawn at 1250 X, with an oil immersion lens, using a drawing tube mounted on a Zeiss Axioskop® phase contrast microscope. All scale bars represented in the figures correspond to 0.025 mm.

For scanning electron microscopy, some specimens fixed for 24 hours in 10 % formalin were washed twice in cacodylate buffer (pH 7.2), post-fixed in 1 % osmium tetroxide 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 1200 JEOL JEM® EX II scanning electron microscope.

The following abbreviations are used, when required, throughout the text and figures: A1 = antennule; A2 = antenna; Bs = basis; Enp = endopod; Exp = exopod; Fu = caudal rami; Ga = genital field; Gsg = genital somite; Md = mandible; Mxl = maxillule; Mx = maxilla; Mxp = maxilliped; Op = anal operculum; P1-P5 = thoracic appendages; R = rostrum.

The nomenclature and descriptive terminology follow Huys *et al.* (1996).

The holotype and one female paratype will be deposited in the collection of the Museo Civico di Storia Naturale G. Doria, Genova (Italy); the remaining material of the type series is deposited at the "Dipartimento di Scienze Ambientali, Università della Tuscia", Viterbo (collection of the senior author).

Latitude and longitude were determined using a Garmin GPS (Global Position System) 38™.

SYSTEMATICS

Family Leptopontiidae Lang, 1948

Subfamily Psammopsyllinae Krishnaswamy, 1956

Ichnusella tertia n. sp.

Material – Holotype: 1 male, L. Ciampicali coll., 23-11-1993, rivermouth of Fiora River (Viterbo Province, Latium; 42° 19' 34'' N, 11° 34' 57'' E), 2 m from the right river side, 55 m from the sea; dissected and mounted on a slide labelled: Fiora River-*Ichnusella tertia* ht. Paratypes: 1 male and 1 female, L. Ciampicali coll., same date and place; 1 male and 2 females, L. Ciampicali leg, 15-03-1997, rivermouth of Valfragida Stream (Viterbo Province, Latium; 42° 16' 914'' N,

11°39' 75'' E), 40 m from the sea, mounted on slides labelled: Valfragida Stream – *Ichnusella tertia* pt.

Description of the holotype

Body cylindrical and elongate, pitted, unpigmented, eyeless; length from rostrum to distal apex of caudal rami: 0.30 mm. Cephalic shield and anal somite with a dorsal (Fig. 1) and a ventral (Fig. 3) hyaline integumental window, respectively.

Anal operculum (Fig. 3): distal margin smooth and straight, medially drawn out into paired curved, spinous processes bilaterally.

Caudal rami (Fig. 3): slightly longer than anal somite, of approximately conical shape; length to width ratio: 3.75. Armature consisting, according to Huys *et al.* (1996), of: a long anterolateral seta, a posterolateral seta, an outer terminal seta, a long and strong inner terminal seta, a short terminal accessory seta near the inner terminal corner, a dorsal composite seta with a small spine close to it; produced dorsally into a long curved apical tip.

Rostrum and antennule (Fig. 1): rostrum as figured. A1: 7-segmented. First segment with 2 rows of transverse spinules; second segment with 6 distal setae. Third segment with 4 setae, fourth segment enlarged, with 1 distal seta and a strong apical tubercle bearing 2 long setae and an aesthetasc. Fifth segment bare, with enlarged proximal part. Sixth segment without armature, last segment ending in a tip, with 6 setae.

Antenna (Fig. 5): 1-segmented exopod with an apical seta, allobasis bare. Endopod bearing distally 3 geniculate, 2 normal, 1 pinnate and 1 transformed setae.

Mandible (Fig. 6): 2-segmented palp, basis with 1 seta and 1 spinule on the distal margin, endopod with 2 apical setae of same length and 3 subapical ones of different length.

Maxillule (Fig. 8): arthrite of the praecoxa with four slightly curved apical spines, and a curved seta inserted at about half length of arthrite. Coxal endite with 2 distal setae; basis with 3 apical setae, a small tubercle bearing 1 seta (exopod?).

Maxilla (Fig. 9): syncoxa with 1 endite bearing 2 setae of different length. Basis prolonged in an apical curved tip; small endopod with 1 seta and 1 short spine.

Maxilliped (Fig. 10): slim and elongate; syncoxa and basis unarmed, endopod represented by a long, pinnate apical claw.

P1 (Fig. 11): basis with a pore and a seta near the exopod insertion and an inner seta near the endopod insertion. Exopod 1-segmented, with an apical seta. Endopod 2-segmented, enp-1 about

four times as long as the enp-2, with a long proximal inner seta. Enp-2 bearing 2 apical geniculate setae of different length and 1 vestigial inner subapical seta and 1 subapical spinule.

P2 (Fig. 13): basis with an outer seta and a pore. Exopod 3-segmented, armature shown in figure. Endopod 1-segmented, shorter than exp-1, with 2 apical normal and 1 transformed subapical setae.

P3 (Fig. 14): squat, basis with a long seta, 1 pore and 2 groups of distal spinules. Exopod 3-segmented, exp-1 very enlarged with 1 distal outer pinnate seta and some spinules near its insertion; exp-2 with 1 outer pinnate seta and the distal inner corner prolonged in a tip. Last segment very small, with 1 apical long and transformed seta, flanked by 2 thin setae. Endopod represented by a short segment with an apical spinule and shorter subapical ones.

P4 (Fig. 16): basis with outer seta and a pore close to it. Exopod 3-segmented, last segment with a subapical transformed seta and 2 apical normal ones of different length. Endopod as long as half of exp-1, with 2 apical setae of different length.

P5 (Fig. 17): an almost rectangular plate, with 2 distal lobes. A short curved spine at the distal inner corner, and another curved spine at the outer corner of the innermost lobe; on the outermost lobe, a strong curved spine at the inner corner, 1 seta and 1 spine on the distal margin, a long seta at the distal outer corner.

P6 (Fig. 19): 2 asymmetrical rectangular plates, both of them having a small tip and a short spinule on the distal margin.

Description of the female paratype

Length: 0.30 mm. R (Fig. 2), A2, Mx1, Mx, Mxp, P1 (Fig. 12), P2, P4, Op (Fig. 4), cephalic (Fig. 2) and abdominal (Fig. 4) window as in the male.

Caudal rami (Fig. 4): similar to those of the male but a little shorter, length to width ratio: 3.45.

Antennule (Fig. 2): 6-segmented, first segment with 2 transverse rows of spinules, second segment with 6 distal setae. Third segment with 2 setae; fourth segment with a tubercle bearing 2 setae and 1 aesthetasc. Fifth segment with a distal seta, last segment with 9 setae.

Mandibular palp (Fig. 7): with a seta and 2 spinules on the distal margin of basis; endopod longer than in the male, with 2 apical setae and 3 subapical ones of different length.

P3 (Fig. 15): basis with outer seta and pore. Exopod 3-segmented, exp-3 with 2 apical setae

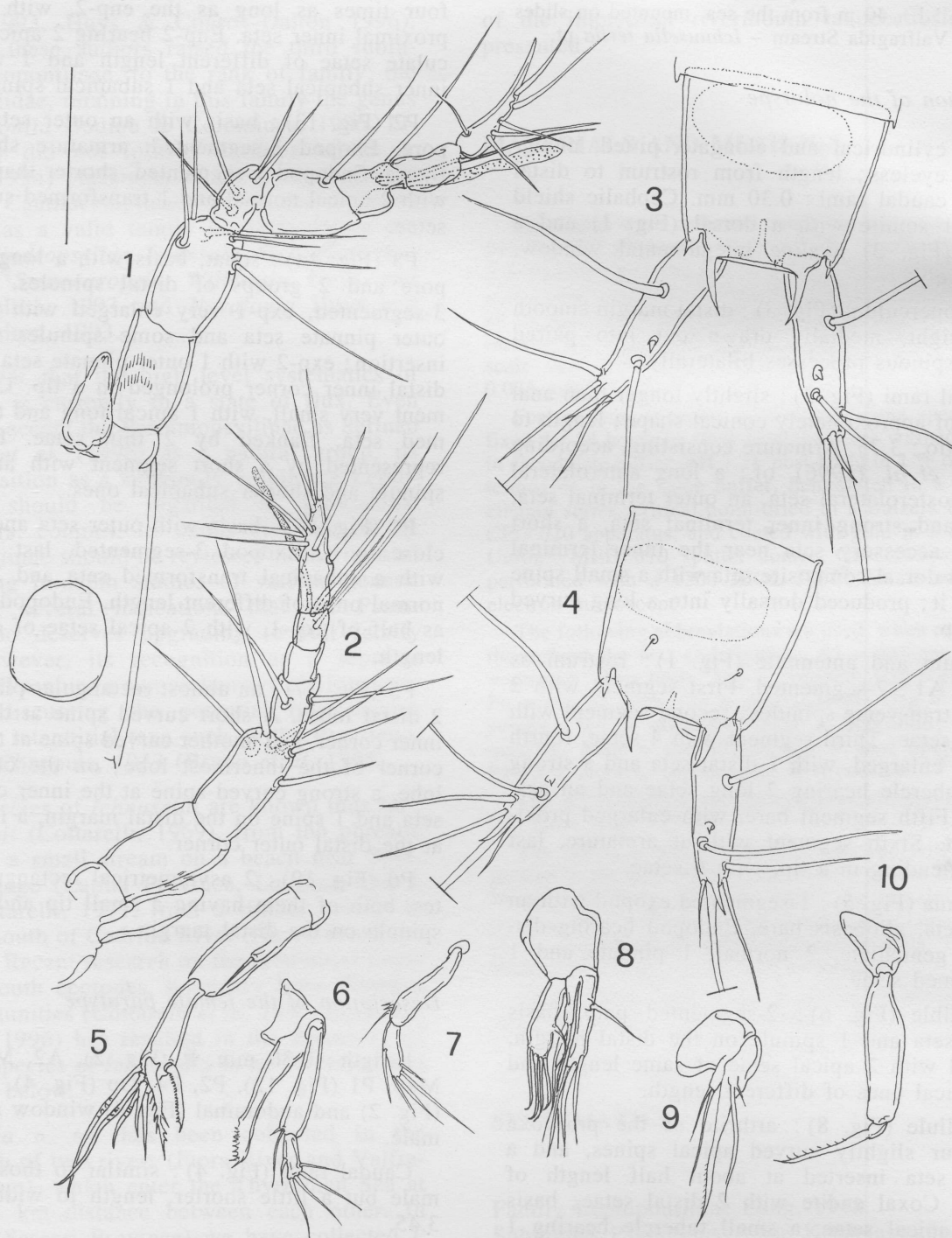


Fig. 1-10. — *Ichnusella tertia* n. sp. Holotype, male. 1, antennule, rostrum and cephalic integumental window; 3, anal operculum, caudal rami and integumental window, dorsal view; 5, antenna; 6, mandible; 8, maxillule; 9, maxilla; 10, maxilliped. Paratype, female. 2, antennule, rostrum and cephalic integumental window; 4, anal operculum, caudal rami and integumental window, dorsal view; 7, mandibular palp.

and 1 subapical transformed one; remaining ornamentation as in figure. Endopod represented by a cylindrical segment, shorter than exp-1, with strong apical seta.

P5 (Fig. 18): 2 plates of almost triangular shape bearing, along the distal margin from the inner to the outer corner: a short spine, a very strong and long spine, a seta, a short spine, a very long seta.

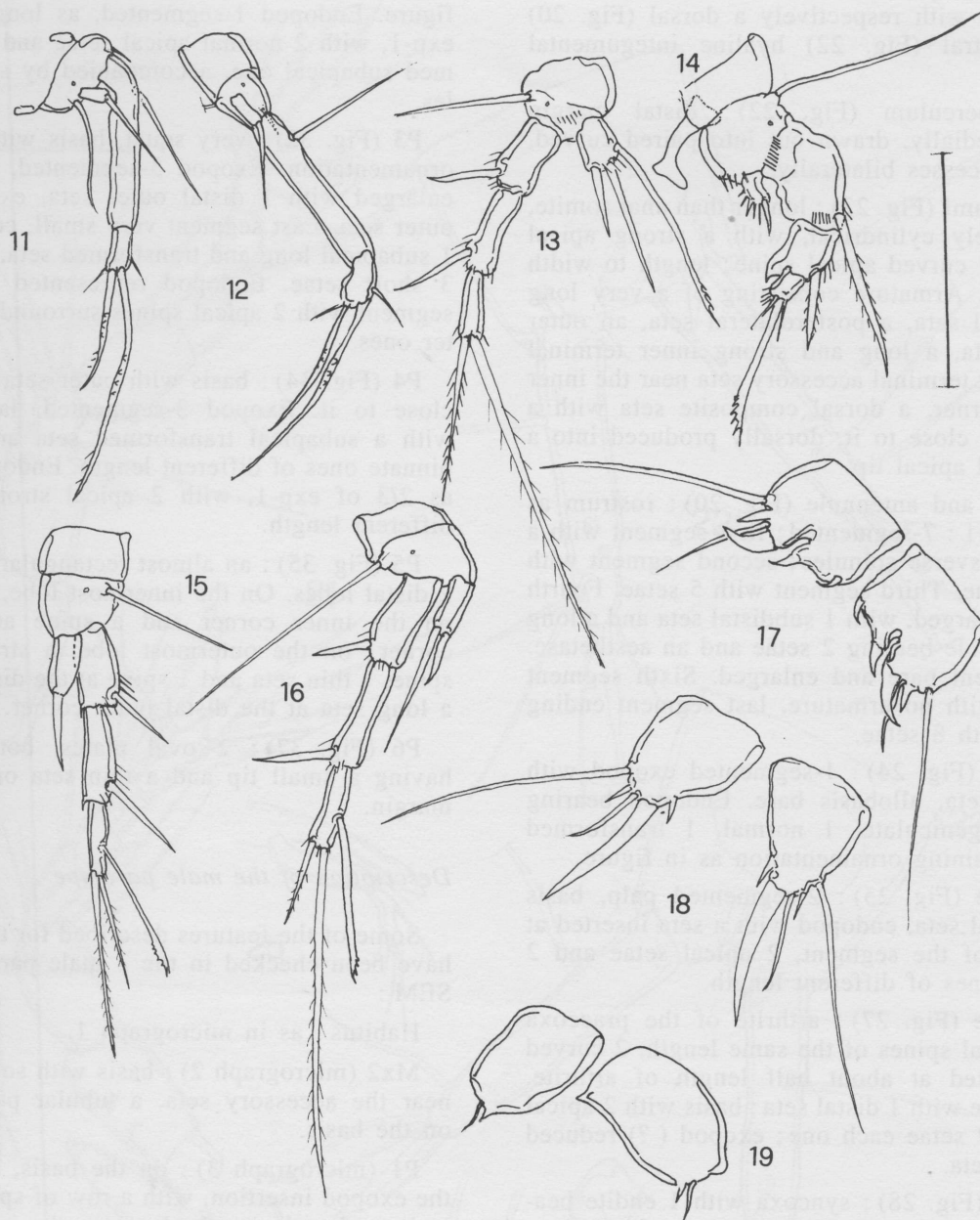


Fig. 11-19. – *Ichnusella tertia* n. sp. Holotype, male. 11, P1; 13, P2; 14, P3; 16, P4; 17, P5; 19, P6. Paratype, female. 12, P1; 15, P3; 18, P5.

Derivatio nominis

The species name is derived from the Latin adjective “*tertia*”, meaning “the third”. This species is in fact the third one ever collected for the genus.

Ichnusella longifurca n. sp.

Material – Holotype : 1 male, V. Cottarelli and M.C. Bruno coll., 25-04-1997, rivermouth of De li Saldi Stream (Sassari Province, Sardinia; 41° 7' 831'' N, 9° 5' 295'' E), 200 cm from the left stream side, 20 m from the sea, dissected and mounted on a slide labelled :

De li Saldi Stream – *Ichnusella longifurca* ht. Paratypes : 4 males and 8 females, V. Cottarelli and M.C. Bruno coll., same date and place, mounted on slides labelled : De li Saldi Stream – *I. longifurca* pt. 3 males and 2 females, prepared for scanning electron microscopy, on a stub labelled : De li Saldi Stream – *I. longifurca* pt.

Description of the holotype

Body cylindrical and elongate, pitted, unpigmented, eyeless; length from rostrum to distal apex of caudal rami : 0.35 mm. Cephalothorax and

anal somite with respectively a dorsal (Fig. 20) and a ventral (Fig. 22) hyaline integumental window.

Anal operculum (Fig. 22): distal margin straight, medially, drawn out into paired curved, spinous processes bilaterally.

Caudal rami (Fig. 22): longer than anal somite, approximately cylindrical, with a strong apical and slightly curved apical spine; length to width ratio: 4.28. Armature consisting of a very long anterolateral seta, a posterolateral seta, an outer terminal seta, a long and strong inner terminal seta, a short terminal accessory seta near the inner terminal corner, a dorsal composite seta with a small spine close to it, dorsally produced into a long curved apical tip.

Rostrum and antennule (Fig. 20): rostrum as in figure. A1: 7-segmented; first segment with a row of transverse spinules; second segment with 6 distal setae. Third segment with 5 setae. Fourth segment enlarged, with 1 subdistal seta and a long apical tubercle bearing 2 setae and an aesthetasc. Fifth segment bare and enlarged. Sixth segment enlarged, with no armature, last segment ending in a tip, with 8 setae.

Antenna (Fig. 24): 1-segmented exopod with an apical seta, allobasis bare. Endopod bearing distally 3 geniculate, 1 normal, 1 transformed setae. Remaining ornamentation as in figure.

Mandible (Fig. 25): 2-segmented palp, basis with a distal seta, endopod with a seta inserted at about 1/2 of the segment, 2 apical setae and 2 subapical ones of different length.

Maxillule (Fig. 27): arthrite of the praecoxa with 4 apical spines of the same length, 2 curved setae inserted at about half length of arthrite. Coxal endite with 1 distal seta; basis with 2 apical groups of 2 setae each one; exopod (?) reduced to a long seta.

Maxilla (Fig. 28): syncoxa with 1 endite bearing 1 normal and 1 pinnate setae of different length. Basis prolonged in an apical curved and pinnate claw; accessory ornamentation consisting of a seta.

Maxilliped (Fig. 29): slim and elongate; syncoxa and basis unarmed, endopod represented by a long, curved, pinnate apical claw.

P1 (Fig. 30): basis with 2 pores, a short seta near the exopod insertion and another short 1 on the distal inner margin, near the endopod insertion. Exopod 1-segmented, with an apical seta. Endopod 2-segmented, enp-1 about 4 times as long as the enp-2, with a long proximal inner seta; enp-2 bearing 2 apical geniculate setae of different length and 1 vestigial inner subapical seta and 1 subapical spinule.

P2 (Fig. 31): basis with a short outer seta and a pore. Exopod 3-segmented, armature shown in

figure. Endopod 1-segmented, as long as 1/2 of exp-1, with 2 normal apical setae and 1 transformed subapical one, accompanied by some spinules.

P3 (Fig. 32): very squat, basis with the usual ornamentation. Exopod 3-segmented, exp-1 very enlarged with 1 distal outer seta, exp-2 with 1 outer seta. Last segment very small, conical, with 1 subapical long and transformed seta, flanked by 3 short setae. Endopod represented by a short segment with 2 apical spines surrounded by shorter ones.

P4 (Fig. 34): basis with outer seta and a pore close to it. Exopod 3-segmented, last segment with a subapical transformed seta and 2 apical pinnate ones of different length. Endopod as long as 2/3 of exp-1, with 2 apical strong setae of different length.

P5 (Fig. 35): an almost rectangular plate, with 2 distal lobes. On the innermost lobe, a sinuosity on the inner corner and a spine at the outer corner; on the outermost lobe, a strong curved spine, 1 thin seta and 1 spine at the distal margin, a long seta at the distal outer corner.

P6 (Fig. 37): 2 oval plates, both of them having a small tip and a thin seta on the distal margin.

Description of the male paratype

Some of the features described for the holotype have been checked in the 3 male paratypes with SEM:

Habitus: as in micrograph 1.

Mx2 (micrograph 2): basis with some spinules near the accessory seta, a tubular pore dorsally on the basis.

P1 (micrograph 3): on the basis, a pore near the exopod insertion, with a row of spinules close to it, and a short spinules near the inner seta.

P3 (micrograph 4): 2 pores on the inner and 2 pores on the outer surface of the basis. Endopod as in micrograph 4.

P5 and P6: as in micrograph 5.

Description of the female paratype

Length: 0.34 mm. R (Fig. 21), A2, Mx, Mx, Mxp, P1, P2, P4, Op (Fig. 23), cephalic (Fig. 21) and abdominal (Fig. 23) windows as in the male.

Caudal rami (Fig. 23): similar to those of the male but a little thicker, length to width ratio: 5.45.

Antennule (Fig. 21): 6-segmented; second segment with 5 setae; third segment with 2 setae; fourth segment bearing a tubercle with 3 setae

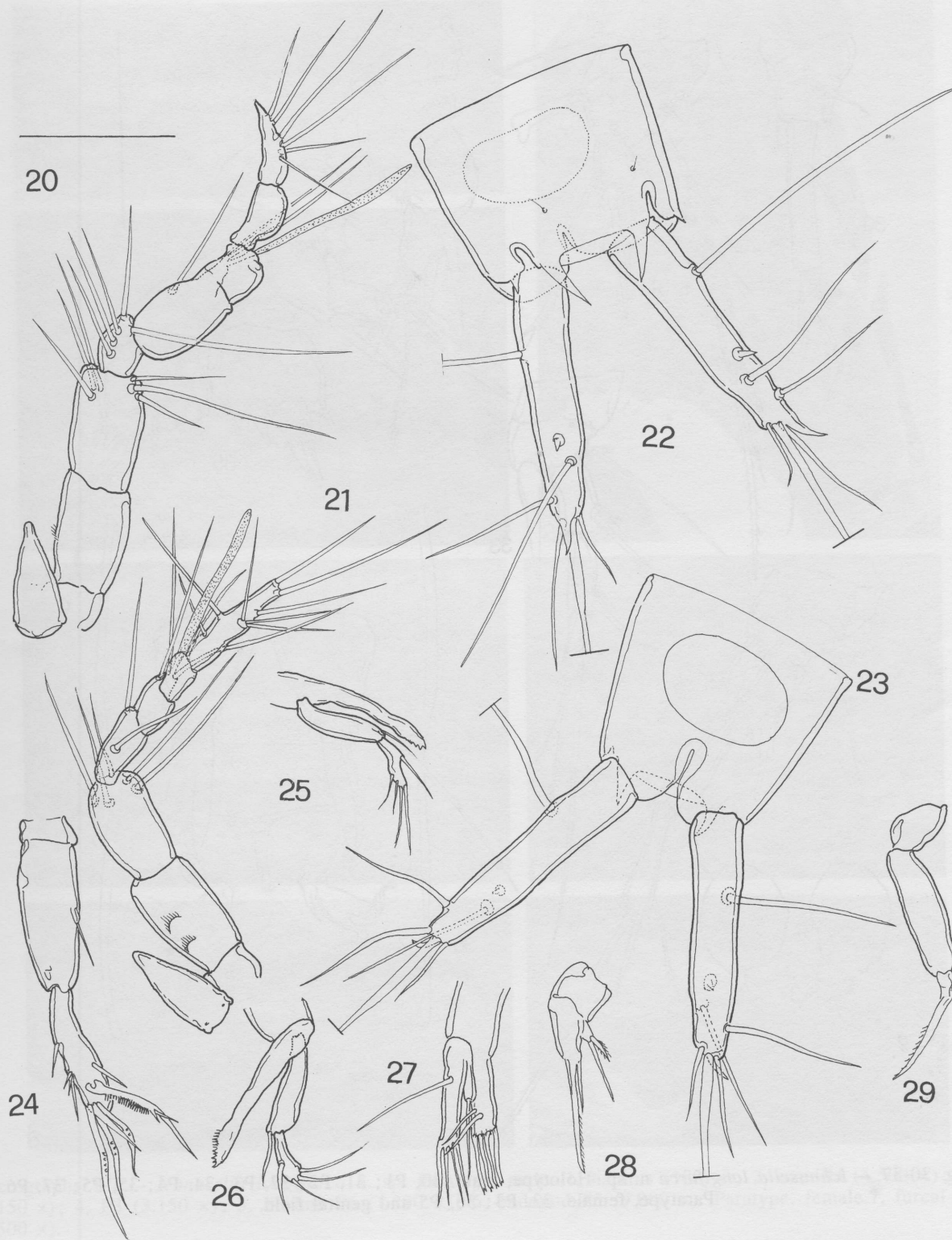


Fig. 20-29. — *Ichnusella longifurca* n. sp. Holotype, male. 20, antennule, rostrum and cephalic integumental window; 22, anal operculum, caudal rami and integumental window, dorsal view. 24, antenna; 25, mandible; 27, maxillule; 28 maxilla; 29, maxilliped. Paratype, female. 21, antennule, rostrum and cephalic integumental window; 23, anal operculum, caudal rami and integumental window, ventral view; 26, mandible.

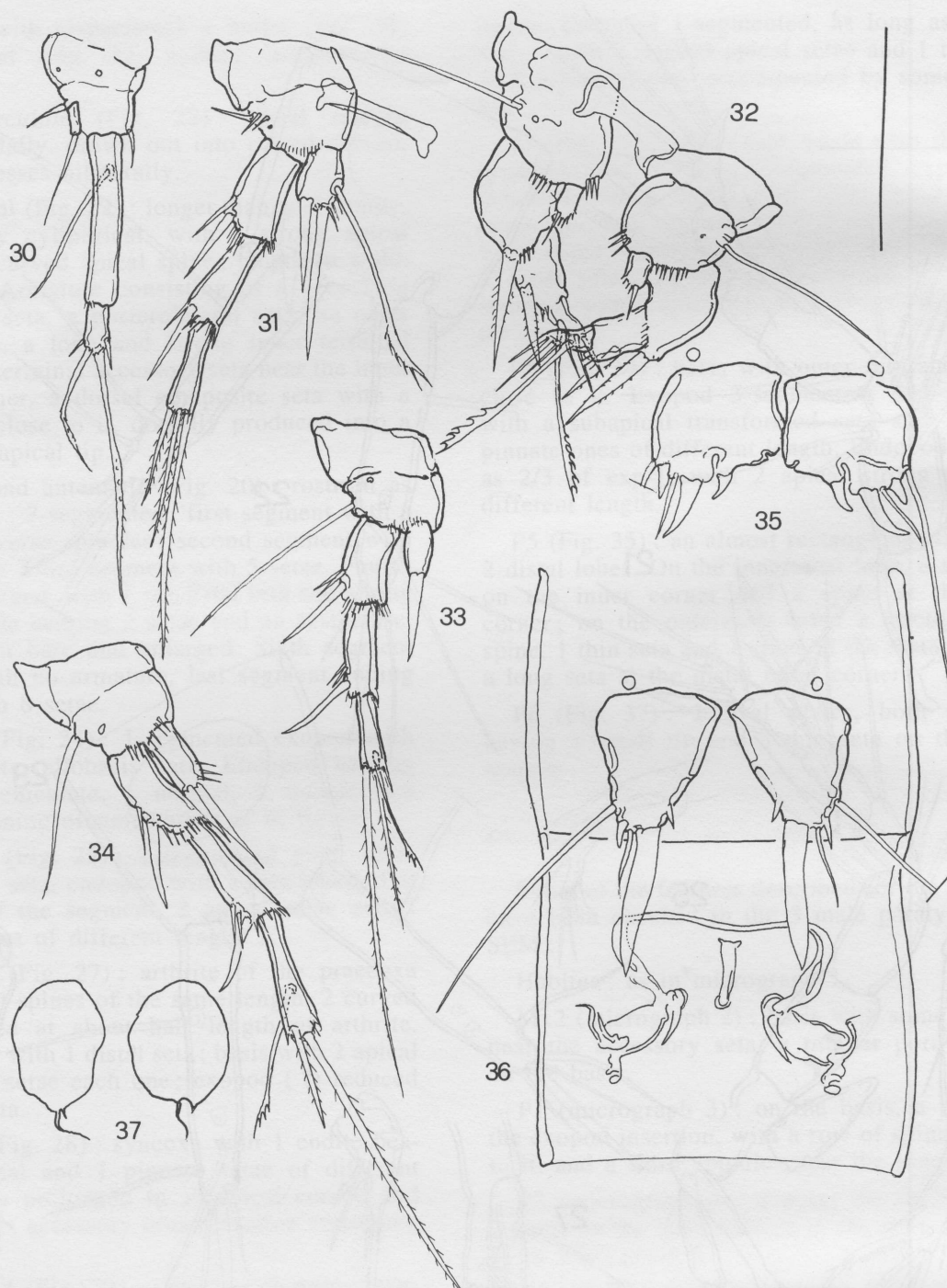


Fig. 30-37. — *Ichnusella longifurca* n. sp. Holotype, male. 30, P1; 31, P2; 32, P3; 34, P4; 35, P5; 37, P6. Paratype, female. 33, P3; 36, P5 and genital field.

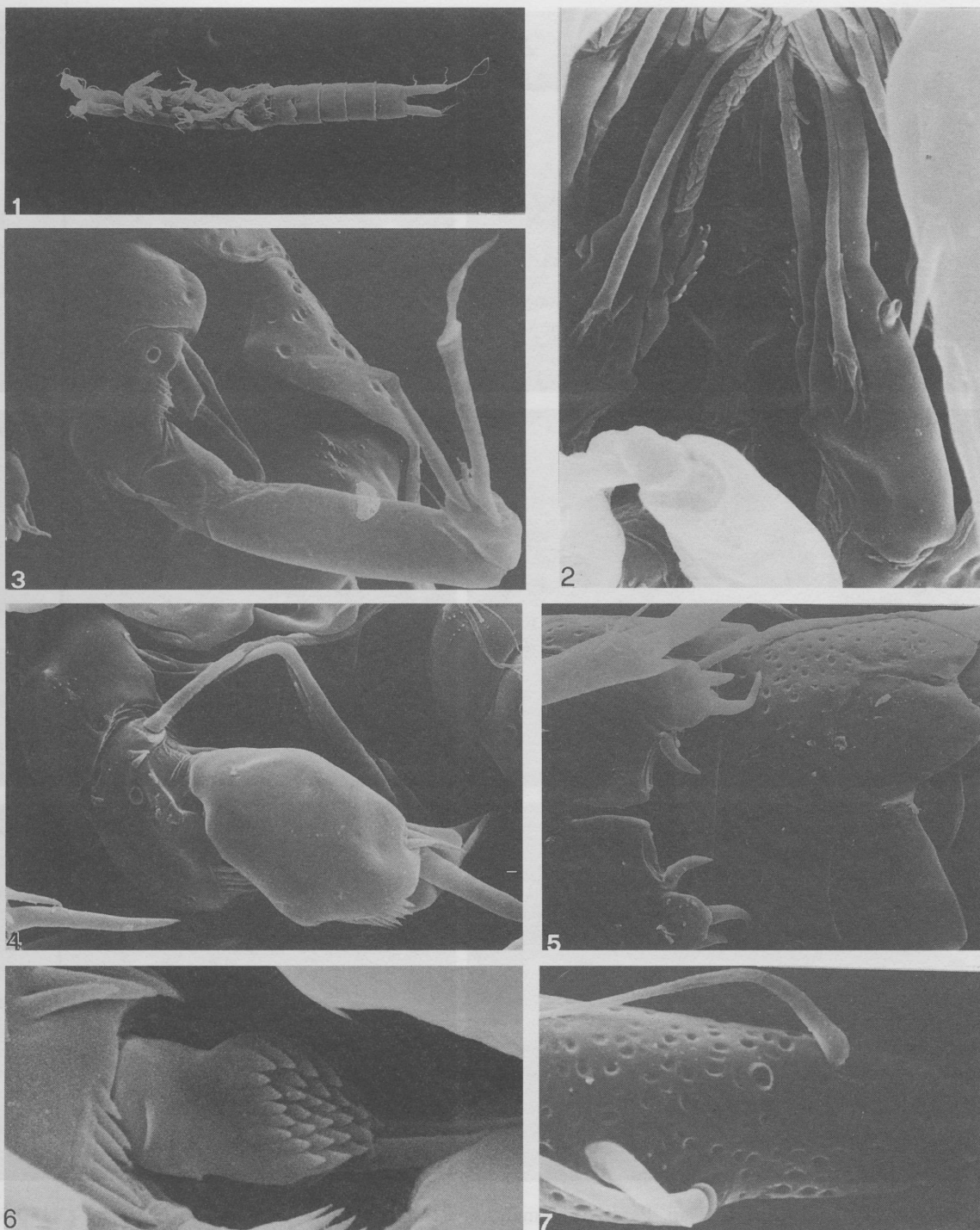
and an aesthetasc. Fifth segment with a distal seta, last segment with 8 setae.

Mandibular palp (Fig. 26): with a seta on the distal margin of the basis, endopod with 2 apical setae and 2 subapical ones of different length.

P3 (Fig. 33): basis with an outer seta and a pore. Exopod 3-segmented, ornamentation as in

figure. Endopod 1-segmented, almost as long as $1/2$ of exp-1, with an apical strong spiniform seta.

P5 (Fig. 36): 2 plates of almost triangular shape with, along the distal margin from the inner to the outer corner: a short spine, a very strong and long spiniform seta, a thin setae, a short spine, a very long seta.



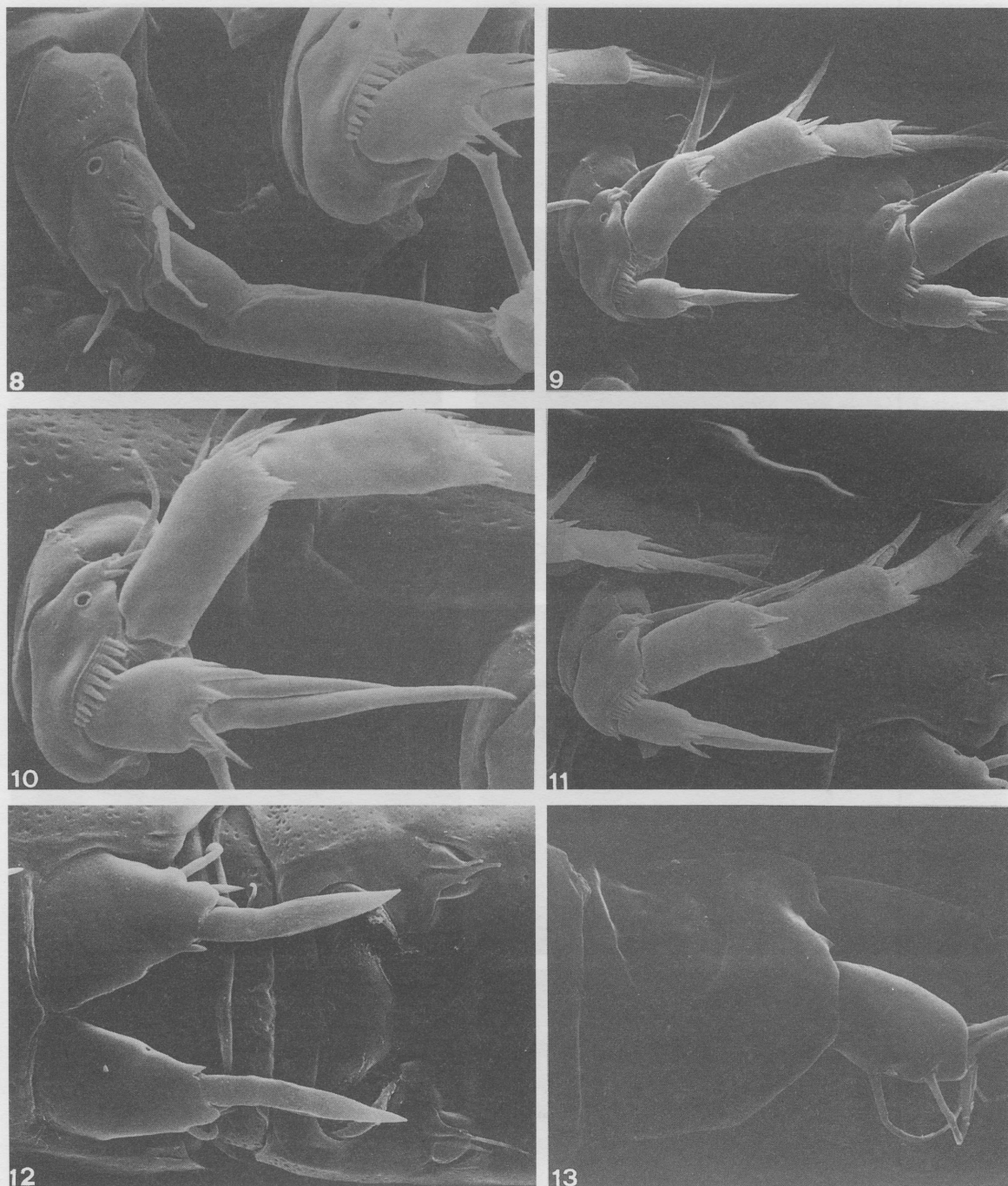
Micrographs 1-7. — *Ichnusella longifurca* n. sp. Paratype, male. 1, habitus (180 ×); 2, maxilla (4.500 ×); 3, P1 (3.150 ×); 4, P3 (3.150 ×); 5, P5 and P6 (1.800 ×); 6, endopod P3 (9.000 ×). Paratype, female. 7, furcal ramus (4.500 ×).

Genital field as in figure 36.

Some of the features described for the paratype have been checked in the 2 female specimens with SEM :

Genital field as in micrograph 12.

Caudal rami (micrograph 7) : with a subapical pore near the posterolateral seta. P1 (micrograph 8) : ornamentation as in the male paratype, but the inner seta is shorter. P2 (micrograph 10) : basis with a pore near the exopod and a comb of spinules near the endopod insertion. P3 (micro-



Micrographs 8-13. — *I. longifurca* n. sp. Paratype, female. 8, P1 (3.150 \times); 9, P3 (1.800 \times); 10, P2 (3.150 \times); 11, P4 (1.800 \times); 12, P5 and genital field (1.800 \times). *Psammopsyllus maricae*. Male. 13, anal somite and integumental window (1.800 \times).

graph 8): basis with a pore near the exopod and a comb of spinules near the endopod insertion. Some spinules at the origin of the apical seta of the endopod. P4 (micrograph 11): basis with a pore and some spinules near the endopod insertion. P5 (micrograph 12) with a pore on the inner margin. P6 (micrograph 12): vestigial, represented by 2 setae of different length.

Derivatio nominis

The species name refers to the caudal rami, which are the longest ones for the known species of the genus.

For the two following *Ichnusella* already described, we present some morphological features that had not been considered before, or that had not been described precisely.

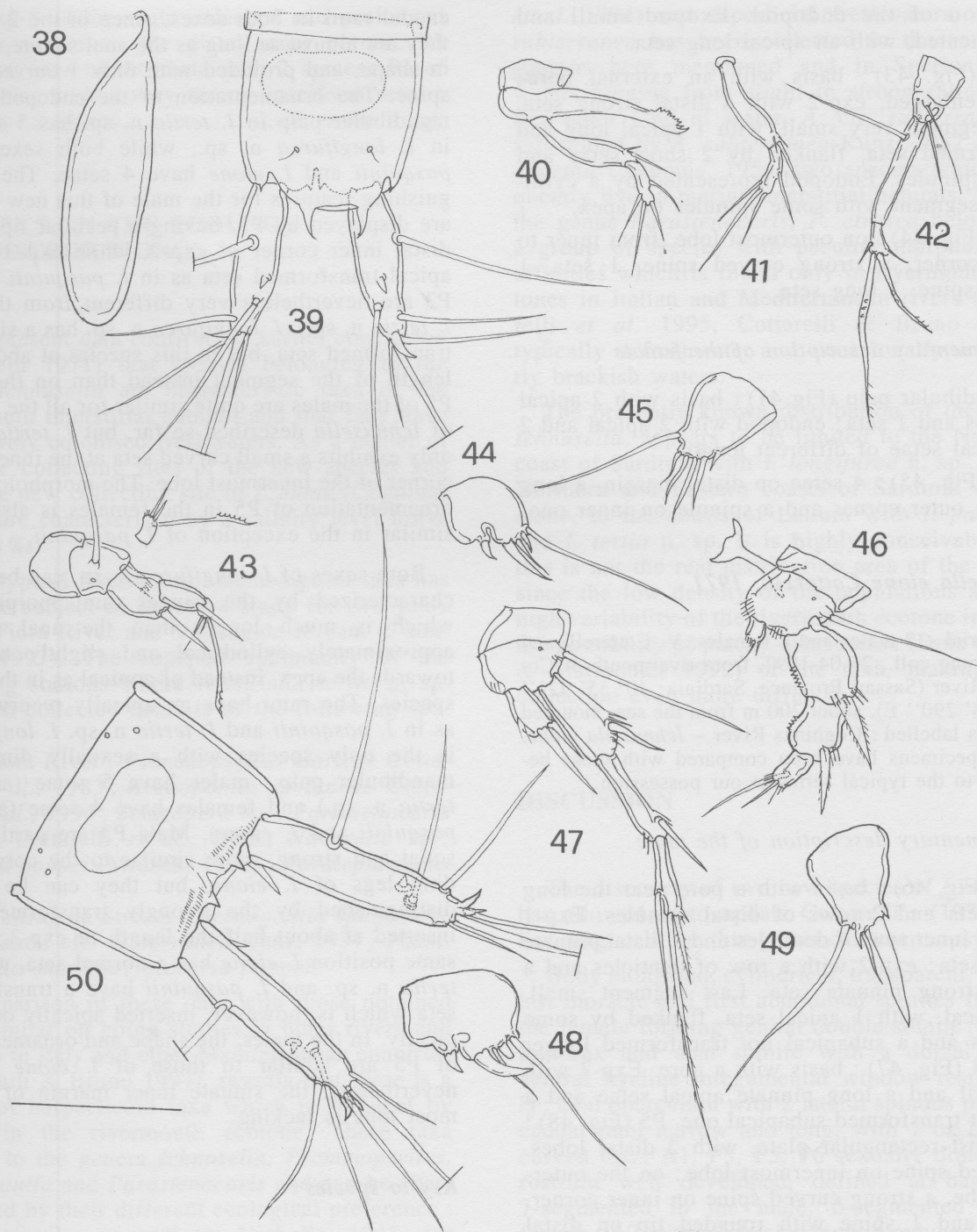


Fig. 38-50. — *Ichnusella pasquinii*. Male. 38, spermatophore; 39, integumental window, dorsal view; 40, mandible; 41, mandibular palp; 42, P1; 43, P3; 44, P5. Female. 45, P5. *Ichnusella eione*. Male. 46, P3; 47, P4; 48, P5. Female. 49, P5; 50, anal operculum, caudal rami and integumental window, ventral view.

***Ichnusella pasquinii* (Cottarelli, 1969)**

Supplementary description of the male

Cephalothorax and anal somite (Fig. 39): with respectively a dorsal and a ventral hyaline integumental window. Spermatophore as in fig. 38.

Mandible (Fig. 40): 2-segmented palp, basis distally enlarged, with 1 distal seta and 1 distal spinule. Endopod with 2 apical setae and 2 subapical ones of different length.

P1 (Fig. 42): basis with 1 pore and a seta near the insertion of the exopod, and 1 seta near the

insertion of the endopod. Exopod small and 1-segmented, with an apical long seta.

P3 (Fig. 43): basis with an external pore; exp-1 enlarged, exp-2 with a distal strong seta. Last segment very small, with 1 apical long and transformed seta, flanked by 2 short setae and some spinules. Endopod represented by a cylindrical segment with some spinules on apex.

P5 (Fig. 44): on outermost lobe, from inner to outer corner, a strong curved spine, 1 seta, 1 strong spine, 1 long seta.

Supplementary description of the female

Mandibular palp (Fig 41): basis with 2 apical spinules and 1 seta; endopod with 2 apical and 2 subapical setae of different length.

P5 (Fig. 45): 4 setae on distal margin, a long seta on outer corner and a spinule on inner one.

Ichnusella eione Cottarelli, 1971

Material – 2 males and 2 females, V. Cottarelli and M.C. Bruno coll., 26-04-1997, from rivermouth of Coghinias River (Sassari Province, Sardinia; 40° 55' 921'' N, 8° 48' 290'' E), about 200 m from the sea, mounted on slides labelled: Coghinias River – *Ichnusella eione*. These specimens have been compared with those belonging to the typical series in our possession.

Supplementary description of the male

P3 (Fig. 46): basis with a pore near the long outer seta and 2 rows of distal spinules. Exp-1 with an inner row of denticles and a distal pinnate strong seta; exp-2 with a row of denticles and a distal strong pinnate seta. Last segment small, cylindrical, with 1 apical seta, flanked by some spinules and a subapical not transformed longer seta. P4 (Fig. 47): basis with a pore. Exp-3 with a normal and a long pinnate apical setae and a long not transformed subapical one. P5 (Fig. 48): an almost rectangular plate, with 2 distal lobes. A curved spine on innermost lobe; on the outermost lobe, a strong curved spine on inner corner, 1 seta and 1 spine with rounded tip on distal margin, a long seta on distal outer corner.

Supplementary description of the female

Caudal rami and anal somite as in fig. 50. P5 (Fig. 49): with a tip on the inner corner instead of a spinule.

REMARKS ON THE SYSTEMATICS

I. tertia n. sp. is reminiscent of *I. pasquinii* in the morphology and the ornamentation of the

caudal rami in both sexes, since in the 2 species they are almost as long as the anal somite, conical in shape, and provided with only 1 curved distal spine. The ornamentation of the endopod of the mandibular palp in *I. tertia* n. sp. has 5 setae as in *I. longifurca* n. sp., while both sexes of *I. pasquinii* and *I. eione* have 4 setae. The distinguishing features for the male of this new species are displayed by P3, having a peculiar tip on the distal inner corner of exp-2, while exp-3 has an apical transformed seta as in *I. pasquinii* (whose P3 are nevertheless very different from those of *I. tertia* n. sp.). *I. longifurca* n. sp. has a similarly transformed seta, but in this species at about half length of the segment instead than on the apex. P5 of the males are quite similar for all the species of *Ichnusella* described so far, but *I. tertia* n. sp. only exhibits a small curved seta at the inner distal corner of the innermost lobe. The morphology and ornamentation of P5 in the females is also quite similar in the exception of *I. pasquinii*.

Both sexes of *I. longifurca* n. sp. can be easily characterized by the caudal rami morphology, which is much longer than the anal somite, approximately cylindrical and slightly tapering towards the apex, instead of conical as in the other species. The rami have an apically recurved tip, as in *I. pasquinii* and *I. tertia* n. sp. *I. longifurca* is the only species with a sexually dimorphic mandibular palp: males have 5 setae (as in *I. tertia* n. sp.) and females have 4 setae (as in *I. pasquinii* and *I. eione*). Male P3 are particularly squat and strong, more similar to the corresponding legs of *I. eione*, but they can be easily distinguished by the strongly transformed seta inserted at about half the length of exp-3. In the same position *I. eione* has a normal seta, while *I. tertia* n. sp. and *I. pasquinii* have a transformed seta which is, however, inserted apically or subapically. In the males, the shape and ornamentation of P5 are similar to those of *I. eione* where, nevertheless, the sinuate inner margin of innermost lobe is lacking.

Key to species:

- 1 – Caudal rami of both sexes distinctly longer than anal somite..... 2
- Caudal rami of both sexes shorter than or as long as anal somite..... 3
- 2 – Apex of caudal rami in both sexes with 2 curved spines..... *I. eione*
- Apex of caudal rami in both sexes with 1 curved spine..... *I. longifurca* n. sp.
- 3 – P3 exp-2 of the male not very enlarged; P5 innermost lobe of the male with only 1 curved spine; P5 distal margin of the female with 4 setae of different length..... *I. pasquinii*

– P3 exp-2 of the male enlarged and prolonged in a strong tip on the distal inner corner; P5 innermost lobe of the male with 2 spines; P5 of the females with a very strong spiniform seta, a long seta, 2 spines..... *I. tertia* n. sp.

REMARKS ON THE ECOLOGY AND DISTRIBUTION

The present data confirm the earlier conclusion (Cottarelli 1971) that species belonging to the genus *Ichnusella* are characteristic taxa of the rivermouth interstitial habitat, and that they have exclusively colonised freshwaters. As a matter of fact, the collecting sites of the new species and even the new collecting site of *I. eione* (Coghinas River) are characterised by a salinity level lower than 20 ‰.

The typical rivermouth assemblage of taxa has been the subject of intensive study for two years in the Fiora River and Valfragida Stream (Ciamicali 1997). The copepod community of the sampling stations where *Ichnusella tertia* n. sp. has been collected consists of the following species:

1) Fiora River: *Psammopsyllus maricae* Cottarelli *et al.*, 1983; *Arenopontia speluncae* Cottarelli *et al.*, 1994; *Schizopera* sp.; *Parastenocaris etrusca* Cottarelli *et al.*, 1995; *Nitocrella* sp.; *Moraria poppei* (Mrázek, 1893); *Paraleptastacus* sp.

2) Valfragida Stream: *Arenopontia speluncae*; *Arenopontia* cfr. *subterranea* Kunz, 1937; *Psammopsyllus maricae*; *Moraria poppei*.

Comparison of these data with those obtained from similar on going studies in other rivers and streams in Italy and other Mediterranean countries (Cottarelli & Bruno 1997), reveals that there is a group of harpacticoid taxa which is frequent by found in the rivermouth ecotone. These taxa belong to the genera *Ichnusella*, *Psammopsyllus*, *Arenopontia* and *Parastenocaris* and can be characterised by their different ecological preference: *Psammopsyllus operculatus* Nicholls, 1945, for example, has been collected not only in marine, but also in brackish interstitial waters of varying salinity level (Cottarelli *et al.* 1983, Cottarelli *et al.* 1984), while *P. maricae* (collected in the stations previously mentioned and in many more rivermouths of peninsular Italy and Sardinia) is the only species of the genus living up the river, where the salinity level is typically that of slightly brackish or freshwaters. All the species of *Ichnusella* are exclusively freshwater inhabiting. Within the genus *Arenopontia*, *A. speluncae* seems to share the same ecological niche with *P. maricae*, *A. phreatica* lives both in phreatic (Cottarelli

et al. 1994) and superficial freshwaters, *A. cfr. subterranea* has been collected in the sampling stations here mentioned and in Sperlonga, in waters ranging from slight to strong salinity. In this latter kind of water, *A. cfr. subterranea* is substituted by *A. subterranea* Kunz, 1937 and *A. acantha* Chappuis, 1954, two species which frequently live in marine interstitial habitats. Within the genus *Parastenocaris*, *P. etrusca* belongs to a group of species with peculiar morphological affinities which is found only in rivermouth ecotones in Italian and Mediterranean rivers (Cottarelli *et al.* 1995, Cottarelli & Bruno 1997), typically in freshwater and, occasionally, in slightly brackish waters.

The presently known distribution of the genus *Ichnusella*, appears to be limited to the Northern coast of Sardinia with *I. longifurca* n. sp., to the Northern and Eastern coasts of Sardinia with *I. eione*, to the coasts of Latium with *I. pasquinii* and *I. tertia* n. sp. It is highly conceivable that this is not the real distribution area of the genus, since the low density of the populations and the high variability of the rivermouth ecotone increase the possibility of patchy distribution (Dole Olivier & Marmonier 1992) of the taxa, making their collection even more difficult.

DISCUSSION

The discovery of two new species of *Ichnusella* has allowed us to update Cottarelli's (1971) earlier diagnosis for this genus of Psammopsyllinae.

Body elongated, cylindrical, transparent, pitted; last thoracic and first abdominal somites fused in the female forming genital double-somite; cephalothorax and anal somite with a dorsal and a ventral hyaline integumental window respectively; anal operculum with 2 lateral spinous process; caudal rami narrow and long, with 1 or 2 apical curved tips; A1, P3, P5, P6 sexually dimorphic; rostrum well developed, defined at base; A1 7-segmented in the male, 6-segmented in the female; A2 with allobasis, exopod reduced, 1-segmented, with apical seta; two-segmented mandibular palp; Mx2 with only 1 endite with 2 setae; legs P1-P4 with at least 1 pore on the basis near the outer seta; P1 basis with inner seta; exopod 1-segmented and very reduced with only 1 apical spine, endopod 2-segmented, prehensile, enp-1 much longer than enp-2, with a very long proximal seta on the inner margin; legs P2-P4 with 3-segmented exopod and 1-segmented endopod; endopod P2 with a long subapical transformed seta; P3 transformed into a copulatory organ in the male; P5 of male with trace of division in 2 lobes, reduced to a triangular plate in the females.

As already stated by Cottarelli (1971), the genus *Ichnusella* shows closest affinities with the genus *Psammopsyllus* Nicholls, 1945, and particularly with the *arenarius* species group (Cottarelli *et al.* 1984). The close relationship between these taxa is illustrated by the long seta on P1 enp-1. Moreover, in the genus *Psammopsyllus*, the morphology and ornamentation of P2-P4 endopods (small and with reduced chaetotaxy) and of P5 of both sexes (but mainly of the females), are reminiscent of the corresponding legs of *Ichnusella*. Finally, at least for a new species of this group (*P. maricae*) currently under study, remarkable similarities with *Ichnusella* exist in the morphology and ornamentation of Mx (micrograph 2), and in the presence of a hyaline integumental window on the anal somite (micrograph 13).

The collection of the two new species has also allowed to confirm the peculiar transformation of P3 exopod in the males as a generic diagnostic. This structure is more or less enlarged and curved in the different species, and is most likely involved in mating. A similar trend is typical of the family Parastenocarididae Chappuis, 1933, where this leg is much more transformed (exp-1 and exp-2 merged, exp-3 reduced and creating, together with the prolonged exp-2, a pair of "pliers"; endopod very reduced or missing). In any case, it can still be considered that the transformation of P3 exopod of *Ichnusella* is due to convergence (Cottarelli 1971), even if, as previously stated, research in progress indicates a certain phylogenetical relationship between *Psammopsyllinae* and *Parastenocarididae* (Bruno *et al.* 1998).

ACKNOWLEDGEMENTS. – This work was supported by grants from the Italian Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST, funds 40 % and 60 %). We want to thank Dr. Rony Huys, Crustacea Research Group, Natural History Museum, London, for the important exchange of opinions and informations regarding this work, the referees for the comments that improved the manuscript, and the staff of the "Centro Interdipartimentale di Microscopia Elettronica" of the Università della Tuscia, who helped us in the Scanning Electron Microscopy. We also thank Dr. Lara Ciampicali, who collected the specimens of *I. tertia* n. sp.

REFERENCES

- Bruno MC, Cottarelli V, Berera R 1998. Preliminary remarks on the cladistic systematics in some taxa of Leptopontiidae and Parastenocarididae (Copepoda, Harpacticoida). *Boll Mus Civ St nat Verona* 13 (2): 69-79.
- Ciampicali L 1997. Le taxocenosi di Arpacticoidi interstiziali della foce del Fiume Fiora – osservazioni sulla biologia ed ecologia. Tesi sperim. Laurea Sci. Biol. Univ. Tuscia, Unpublished. 121 p.
- Coineau N, Boutin C 1993. Biological processes in space and time. Colonization, evolution and speciation in interstitial stygobionts. In *The natural history of biospeleology*. A.I. Camacho ed. Monographias 7: 423-451.
- Cottarelli V 1969. Un nuovo Crostaceo di acque interstiziali italiane: *Psammopsyllus pasquinii* n. sp. (Harpacticoida, Cylindropsyllidae). *Ist Lomb Acc Sc Lett* 103 (B): 8-21.
- Cottarelli V 1971. *Ichnusella eione* n. gen. n. sp. (Copepoda, Harpacticoida), nuovo crostaceo di acque interstiziali italiane. *Ist Lomb Acc Sc Lett* 105 (B): 57-70.
- Cottarelli V, Saporito PE, Puccetti AC 1983. Una nuova specie di *Psammopsyllus* (Crustacea, Copepoda, Harpacticoida) di acque interstiziali di foce: *Psammopsyllus maricae* n. sp. *Fragm Entomol* 17 (1): 11-18.
- Cottarelli V, Puccetti AC, Saporito PE 1984. Osservazioni sul genere *Psammopsyllus* (Copepoda, Harpacticoida, Cylindropsyllidae) e descrizione di tre nuove specie. *Boll Mus Civ St nat Verona* 11: 1-29.
- Cottarelli V, Bruno MC 1994. *Parastenocaris rivi* n. sp. di acque iporreiche dell'isola di S. Pietro (Sardagna). *Ann Mus Civ Stor nat Giacomo Doria* 90: 509-519.
- Cottarelli V, Bruno MC, Venanzetti V 1994. First record in phreatic freshwater of Harpacticoids belonging to the genus *Arenopontia* (Crustacea, Copepoda) and description of two new species. *Ann Mus Civ Stor nat Giacomo Doria* 90: 471-484.
- Cottarelli V, Bruno MC, Venanzetti F 1995. Two new species of *Parastenocaris* from the interstitial waters of rivermouths in Latium and Sardinia (Crustacea, Copepoda, Harpacticoida). *Fragm Entomol* 26 (2): 229-247.
- Cottarelli V, Bruno MC 1997 (1996). First record of Parastenocarididae (Crustacea, Copepoda: Harpacticoida) from subterranean waters of insular Greece and description of two new species. *Int J Speleol* 25 (1-2): 43-57.
- Delamare Deboutteville C 1960. Biologie des eaux souterraines littorales et continentales. *Vie Milieu suppl.* 9, 740 p.
- Dole Olivier MJ, Marmonier P 1992. Patch distribution of interstitial communities: prevailing factors. *Fresh Biol* 27: 177-191.
- Dussart B, Defaye D 1990. Répertoire mondial des Crustacés Copépodes des eaux intérieures. III. Harpacticoides. *Crustaceana Suppl* 16: 1-364.
- Dussart R, Defaye D 1995. Introduction to the Copepoda. Guide to the identification of the Microinvertebrates of the Continental Waters of the World. SPB Academic Publishing, 277 p.
- Huys R 1992. The amphiatlantic distribution of *Leptastacus macronyx* (T. Scott, 1892) (Copepoda, Harpacticoida): a paradigm of taxonomic confusion; and a cladistic approach to the classification of the Leptastacidae Lang, 1948. *Meded K Acad Wet Lett sch Kunst Belg* 54: 21-196.
- Huys R, Ohtsuka S 1993. A new species of *Syrticola* Willems & Claeys, 1982 (Copepoda: Harpacticoida) from Japan with notes on the type species. *Bull Br Mus nat Hist Zool* 59 (1): 83-94.

- Huys R, Conroy-Dalton S 1996. A revision of *Leptopontia* T. Scott (Copepoda : Harpacticoida) with description of six new species. *Zool J Linn Soc* 118 : 197-239.
- Huys R, Gee JM, Moore CG, Hamond R 1996. Marine and brackish water Harpacticoid Copepods. Part I. RSK Barnes and JH Crothers eds 352 p.
- Lang K 1948. Monographie der Harpacticiden. Nordiska Bøkhäandeln, Stockholm, 2 vol. 1682 p.
- Martinez Arbizu P, Moura G 1994. The phylogenetic position of the Cyindropsyllinae Sars (Copepoda, Harpacticoida) and the systematic status of the Leptopontiinae Lang. *Zool Beitr NF* 35 (1) : 55-77.

Reçu le 18 mars 1998; received March 18, 1998
 Accepté le 25 mai 1998; accepted May 25, 1998