



# *Microcharon novariensis*, a new microparasellid isopod from groundwater in Italy (Crustacea, Isopoda, Janiroidea)

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## ABSTRACT

A new species of *Microcharon* from hyporheic waters of Piedmont, northern Italy, described herein as *Microcharon novariensis* sp. n., shows some affinities with the eastern Mediterranean group of *Microcharon*; its enigmatic origin and phylogenetic relationships are discussed. *Microcharon marinus* is for the first time recorded from the island of Elba.

**KEY WORDS:** Isopoda - *Microcharon* - Stygobiont - Biogeography - Phylogeny - Italy.

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## INTRODUCTION

The genus *Microcharon* Karaman, 1934 is widely distributed in the Mediterranean area. Among microparasellid isopods, this genus shows the highest radiation into inland groundwater with some 85 species and subspecies (Coineau *et al.*, 1994) known world-wide, most of which distributed in the Mediterranean area. Until now, only five species are known from Italy, namely *M. marinus* Chappuis & Delamare, 1954, from brackish coastal habitats of Campania (southern Italy), Sardinia, Sicily and the island of Montecristo (Argano & Pesce, 1975; Argano, 1979; Pesce & Galassi, 1988a), *M. arganoi* Pesce & Tété, 1978, from phreatic waters of Apulia (southern Italy), *M. angelicae* Pesce & Galassi, 1988, from phreatic waters of Latium (central Italy), *M. nuragicus* Pesce & Galassi, 1988 and *M. silverii* Pesce & Galassi, 1988, both from brackish, phreatic waters of Sardinia. Additionally, *Microcharon* sp. was also reported by Pesce & Galassi (1988a) from a freshwater well in Sardinia.

A small sample of interstitial isopods entrusted to one of us (F. S.) by the kindness of Andrea Bertola (Novara) revealed the presence of a new species of *Microcharon*, described herein as *M. novariensis* sp. n. Moreover, recent stygobiological investigations resulted in the discovery of *M. marinus* from phreatic waters on Elba, confirming the presence of this species along the Tyrrhenian coasts of Italy. A brief discussion of the taxonomic status of the specimens is reported and compared with the previous descriptions and figures given by Chappuis & Delamare (1954) and Coineau (1962).

## MATERIALS AND METHODS

Qualitative samples were taken from interstitial habitats using a Bou-Rouch pump (Bou & Rouch, 1967) and filtered through a 100- $\mu$ m mesh net; samples from wells were obtained using a modified Cvetkov net (Vigna Taglianti *et al.*, 1969). Specimens were stored in 70% ethanol with 10% glycerin added. Selected specimens were dissected in glycerin, and permanently mounted on slides in Faure's medium. Drawings and measurements were made using a camera lucida on a Leitz Laborlux contrast microscope. The terminology used by Wilson & Wägele (1994) is adopted in the description.

## TAXONOMIC ACCOUNT

Family MICROPARASELLIDAE S. Karaman, 1934

Genus *Microcharon* S. Karaman, 1934

*Microcharon marinus* Chappuis & Delamare, 1954

### *Material examined*

One juvenile, phreatic waters of Bagnai, island of Elba, Italy; house of Cecchini, well in the courtyard, 10 m a.s.l.; depth: 7 m; water level: 4 m; temperature: 18.4° C;

pH: 8.0; electrical conductivity: 1100  $\mu\text{S}/\text{cm}$ ; redox potential: 202 mV;  $\text{Na}^+$ : 0.12 g/l; 30 March 1994, F. Stoch coll. One male completely dissected and mounted in Faure medium, house of Cecchini, well near Valle brook, 11 m a.s.l.; depth: 9 m; water level: 5 m; temperature: 16.5° C; pH 7.8; electrical conductivity: 750  $\mu\text{S}/\text{cm}$ ; redox potential: 202 mV;  $\text{Na}^+$ : 0.09 g/l; 30 March 1994, F. Stoch coll.

#### Remarks

The only adult male (Fig. 1a-f) collected differs from the original description (Chappuis & Delamare, 1954), and from the following one given by Coineau (1962), in the presence of a pectinate seta on the inner endite of the second maxilla (Fig. 1c) and rows of tiny spinules on male first pleopod (Fig. 1d). According to Coineau (1986, 1994), Dole & Coineau (1987), Pesce & Galassi (1988b), Galassi (1991), Galassi *et al.* (1995a, b), Boulanour *et al.* (1995) and Yacoubi-Khebiza *et al.* (1997), differences on these microcharacters may have a high significance in taxonomic distinction. Interestingly, the same differences with respect to the available descriptions of *M. marinus* were noticed also by Pesce & Galassi (1988b) for several Spanish populations, only dubitatively assigned to the nominate species. These morphological details may have been overlooked in the original description, and, for this reason, our material is assigned to *M. marinus*.

*Microcharon marinus* is an interstitial coastal element, widely distributed along the western Mediterranean coasts in brackish habitats, as a thalassobiont element. The record from brackish wells on Elba confirms the presence of the species along the Tyrrhenian coasts of Italy. This species has been also quoted by Coineau (1986) for the eastern Mediterranean Sea (Greece); however, this record requires confirmation since it was reported without locality data (Galassi *et al.*, 1995b).

### *Microcharon novariensis* sp. n.

#### Material examined

Male holotype, 1 female paratype, completely dissected and mounted in Faure medium, deposited in the Museo Civico di Storia Naturale (Verona, Italy). Other material: paratypes, four males, nine females, preserved in 70% ethanol with 10% glycerin added, in the Stoch collection, Trieste; one male, one female in the Galassi collection, Dipartimento di Scienze Ambientali, University of l'Aquila.

#### Type locality

Several sampling stations along the Agogna stream, between Novara and Borgomanero, downstream from Agogna spring, Piedmont, northern Italy; interstitial habitat; 4 March 1996, A. Bertola coll.

#### Description

Body slender, colourless and eyeless. Length, excluding antennae and uropods, 1.3 mm (male, holotype); 1.2 mm (female, paratype).

Cephalon broader anteriorly than posteriorly; pereopods inserted dorsolaterally. Pleotelson longer than broad (length/width ratio: 1.3), longer than any pereonite, nearly rectangular in dorsal view, with well developed caudal and pleural setae. Chaetotaxy as in Figure 1h.

Antennula short (Fig. 2a), 6-segmented, not sexually dimorphic in the armature; segment 1 with two naked setae; segment 2 bearing two sensorial and one naked lateral setae, one subapical naked seta, and one distal plumose seta, overreaching tip of distal segment; segment 3 naked; segment 4 with two distal setae, segment 5 with one distal seta and one aesthetasc; segment 6 bearing one aesthetasc and two setae apically, one subapical seta, one hyaline sensorial and one lateral setae.

Antenna (Fig. 2c): segment 3 longer than segments 1, 2 and 4, with exopodal scale, segment 6 armed as in the detail of Figure 2c; flagellum consisting of eight segments.

Upper lip (Fig. 2h) rounded, with thin setules along distal margin. Lower lip consisting of two large elongate lobes, armed as in Figure 2i.

Left mandible (Fig. 2f): lacinia mobilis with three subconical teeth; pars incisiva with four strong teeth; pars molaris with three setae, one of which unipinnate, the remaining ones plumose; between lacinia mobilis and pars molaris there are two crenulate spines, two plumose setae and some thin setules. Right mandible (Fig. 2e) lacking lacinia mobilis; pars incisiva with five subconical teeth, pars molaris with three setae, one of which unipinnate, and the remaining ones plumose; between pars incisiva and pars molaris there are two unipinnate setae, three crenulate spines and some thin setules. Mandibular palp (Fig. 2g) consisting of three segments, distal one bearing three claw-like fringed spines and one row of setules; remaining armature as in Figure 2g.

First maxilla (Fig. 2d) with two endites, the outer one with 11 distal denticulate or naked spines, and numerous setules along outer margin; inner endite with one distal and one subdistal spinule and some thin setules along both inner and outer margins distally.

Second maxilla (Fig. 2b) with three endites, of about the same length; inner endite wider than the other two, bearing eight setae: one pectinate, three with rounded apex, two slender naked setae inserted apically and two setae along the inner margin; minor armature as in Figure 2b; outer and medial endites with four and five setae, respectively, some of which denticulate along inner margins.

Maxilliped (Fig. 3a): epipodite slender; palp consisting of five segments, segments 2 and 3 expanded, segment 4 and 5 slender; segment 2 with one seta, segment 3 with three inner and one outer setae, segment 4 with four setae, segment 5 with seven setae; the basipo-

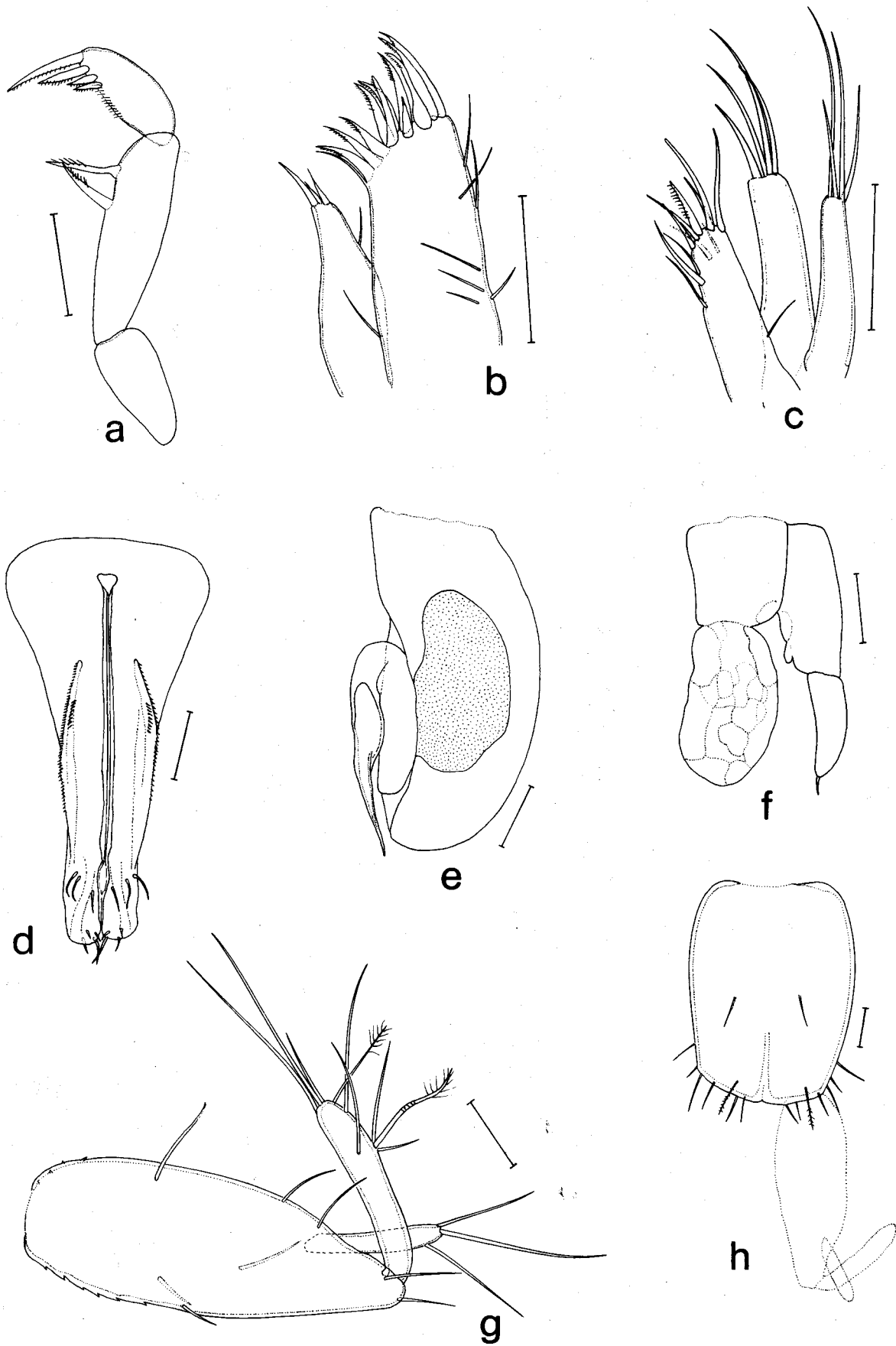


Fig. 1 - *Microcharon marinus*, male from Elba island: a, mandibular palp; b, first maxilla; c, second maxilla; d, first pleopod; e, second pleopod; f, third pleopod. *Microcharon novariensis* sp. n., paratype male: g, right uropod; h, pleotelson. Scale bars, 25  $\mu$ m

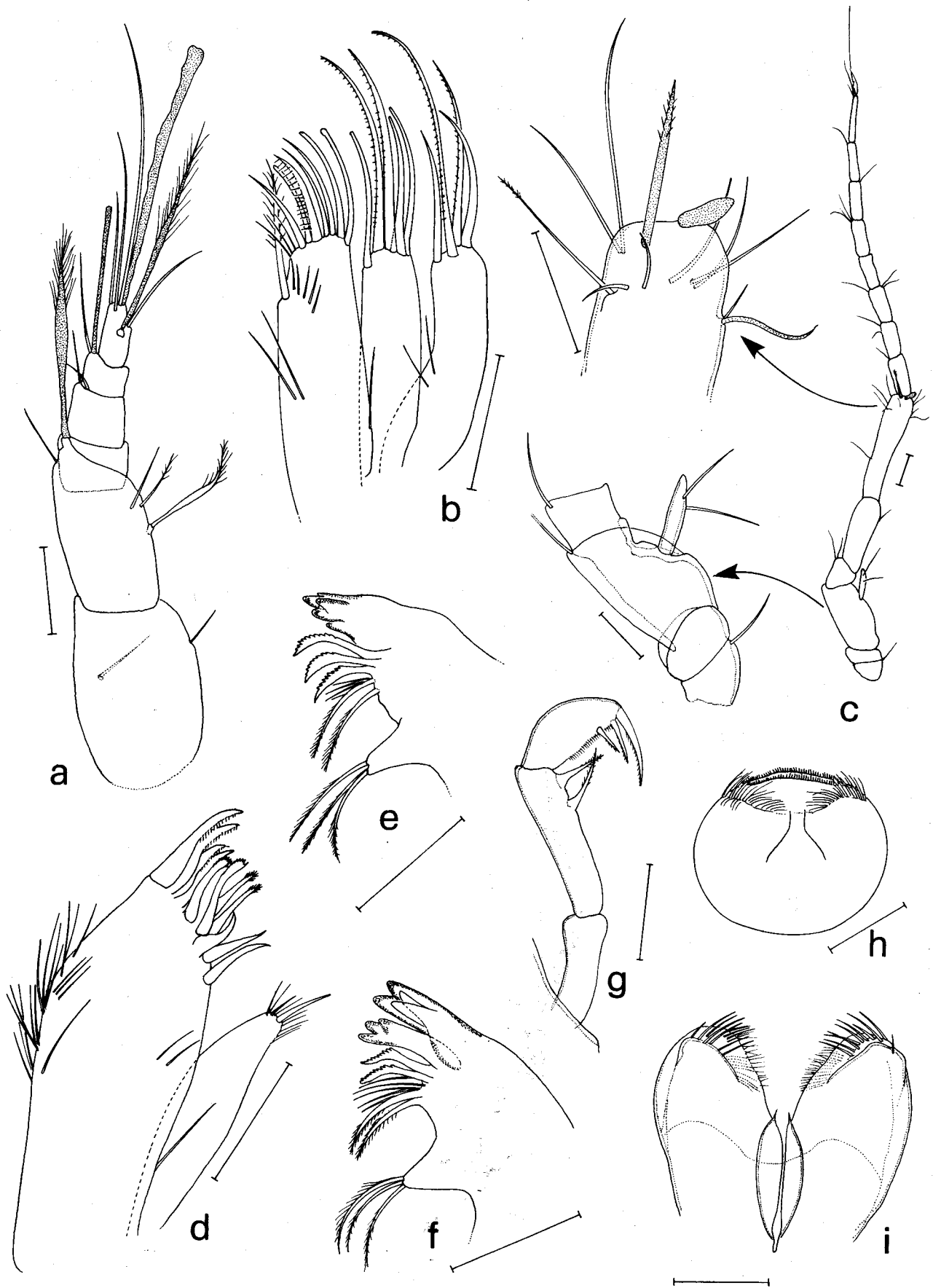


Fig. 2 - *Microcharon novariensis* sp. n., holotype male: a, antennula; b, second maxilla; c, antenna, with details of segments 1-4 and 6; d, first maxilla; e, right mandible; f, left mandible; g, left mandibular palp; h, upper lip; i, lower lip. Scale bars, 25  $\mu$ m.

dal endite bears apical and subapical spines of different length, one of which plumose; inner margin with thin spinules, remaining armature as in Figure 3a.

Pereopods rather similar in shape (Fig. 3b-e), with small differences in the armature: one bifid spine on P1 propodus vs two bifid spines on P2-P7, and six setae on P1 dactylus vs five on P2-P7. Moreover, a spur has been observed on the distal part of P1 propodus, while it is absent in P2-P7; unguli on dactyls relatively short (Fig. 3c, e).

First male pleopod (Fig. 3f) consisting of two halves, both distally enlarged; hyaline edge not sticking out beyond apex of pleopod, folding the laterodistal third of the same pleopod. Basal part of pleopod slightly enlarged, about 2/3 of the total length, reaching a surface ridge on the distal third of pleopod, which represents the coalescent line between the basal and the apical parts of endopod. Armature as follows: two subapical surface setae inserted near the free distal margin of the pleopod, one apical inner seta, one short lateral surface seta, three surface subdistal setae, the innermost being the longest.

Second male pleopod (Fig. 3g): sympod elongate, suboval in shape, pointed at mediiodistal corner (some degree of variation of this character has been observed, most probably artefacts); endopod curved, distal part represented by a stylet, with truncated apex, slightly overreaching tip of sympod.

Second female pleopod operculum (Fig. 3j) slightly longer than wide (length/width ratio: 1.07), distally rounded, with two setae.

Third pleopod (Fig. 3h) as usual in the other freshwater species of the genus.

Fourth pleopod (Fig. 3i) rudimentary.

Uropods (Fig. 1g) longer than pleotelson; endopod short, about half as long as the sympod, armed with three apical simple setae, two plumose lateral setae, and four naked setae; exopod short with two apical and one subapical setae.

#### Etymology

The specific name *novariensis* refers to the Latin *Novaria*, the ancient name of Novara, the town in the neighbourhood of which the new species was collected.

#### Remarks and affinities

*Microcharon novariensis* sp. n., as most freshwater species of the genus, is characterized by a 6-segmented antennula and by a third pleopod with naked endopod. The new species is easily distinguishable from any other species of *Microcharon* by the peculiar morphology of the male first pleopod and by the unique combination of characters such as the armature of the distal segment of mandibular palp with only three spines, the short pereopodal unguli, the pointed sympod of second male pleopod, and the second female pleopod armed with two setules.

According to the evolutionary trend of characters proposed by Dole & Coineau (1987) and Coineau (1994)

within the genus *Microcharon*, the new species shows the following characters in plesiomorphic state: long sensorial seta on segment 2 of antennula, numerous pinnate setae on the outer endite of first maxilla and on the endites of second maxilla, relatively short pereopodal unguli, pointed sympod of the second male pleopod and female operculum armed with two setules. *Microcharon novariensis* sp. n. shares some affinities with the Balkanic group of *Microcharon*, as defined by Galassi *et al.* (1995a, b) on the basis of the following characters: second segment of the antennula with a long plumose seta, mandibular palp armed with four claw-like fringed distal spines, inner endite of the second maxilla with one or more pectinate setae, second female pleopod armed with two (rarely four) setules. Nevertheless, the new species shows only three spines on the distal segment of mandibular palp (apomorphic character state with respect to the condition of four spines on the same segment) and does not fit the diagnosis of the two monophyletic groups (the *major* and the *profundalis* groups) established by Galassi *et al.* (1995a, b). With respect to the eastern Mediterranean group of *Microcharon* as defined by Coineau (1994), *M. novariensis* sp. n. shows only weak affinities with *M. apolloniacus* Cvetkov, 1964 (*latus* group *sensu* Coineau, 1994) known from spring waters of Sozopol (Bulgaria) in the gross morphology of the first male pleopod, short in the distal third and with short hyaline edge not protruding beyond the distal part of the pleopod, in the presence of pectinate or denticulate setae on both maxillae, and in the relatively short pereopodal unguli. On the other hand, the new species differs from *M. apolloniacus* in the different morphology of the second male pleopod (sympod elongate, endopod with pointed truncated tip in *M. novariensis* sp. n. vs sympod suboval, endopod with pointed tip in *M. apolloniacus*), and in the different armature of the mandibular palp (three claw-like spines in *M. novariensis* sp. n. vs four in *M. apolloniacus*). The new species is also only distantly related to *M. latus* Karaman, 1934 and *M. prespensis* Karaman, 1954 in the general construction of the first male pleopod, and to *M. prespensis* also in the morphology of the second male pleopod. The most relevant difference with respect to the eastern group of species regards the number of spines on the distal segment of the mandibular palp (three in *M. novariensis* sp. n. vs four in the eastern congeners), a character that could have a relevant phylogenetic significance for assessing natural groups within the genus as a whole (Galassi *et al.*, 1995a, b). Among the species having a mandibular palp with only three claw-like spines, mostly belonging to the western Mediterranean group of species, *M. novariensis* sp. n. shows weak affinities with some derived Italian congeners, like *M. angelicae* Pesce & Galassi, 1988 (in the general morphology of the first male pleopod, with slightly enlarged distal third and short hyaline edge) and the Sardinian *M. nuragicus* Pesce & Galassi, 1988 (in the gross morphology of the

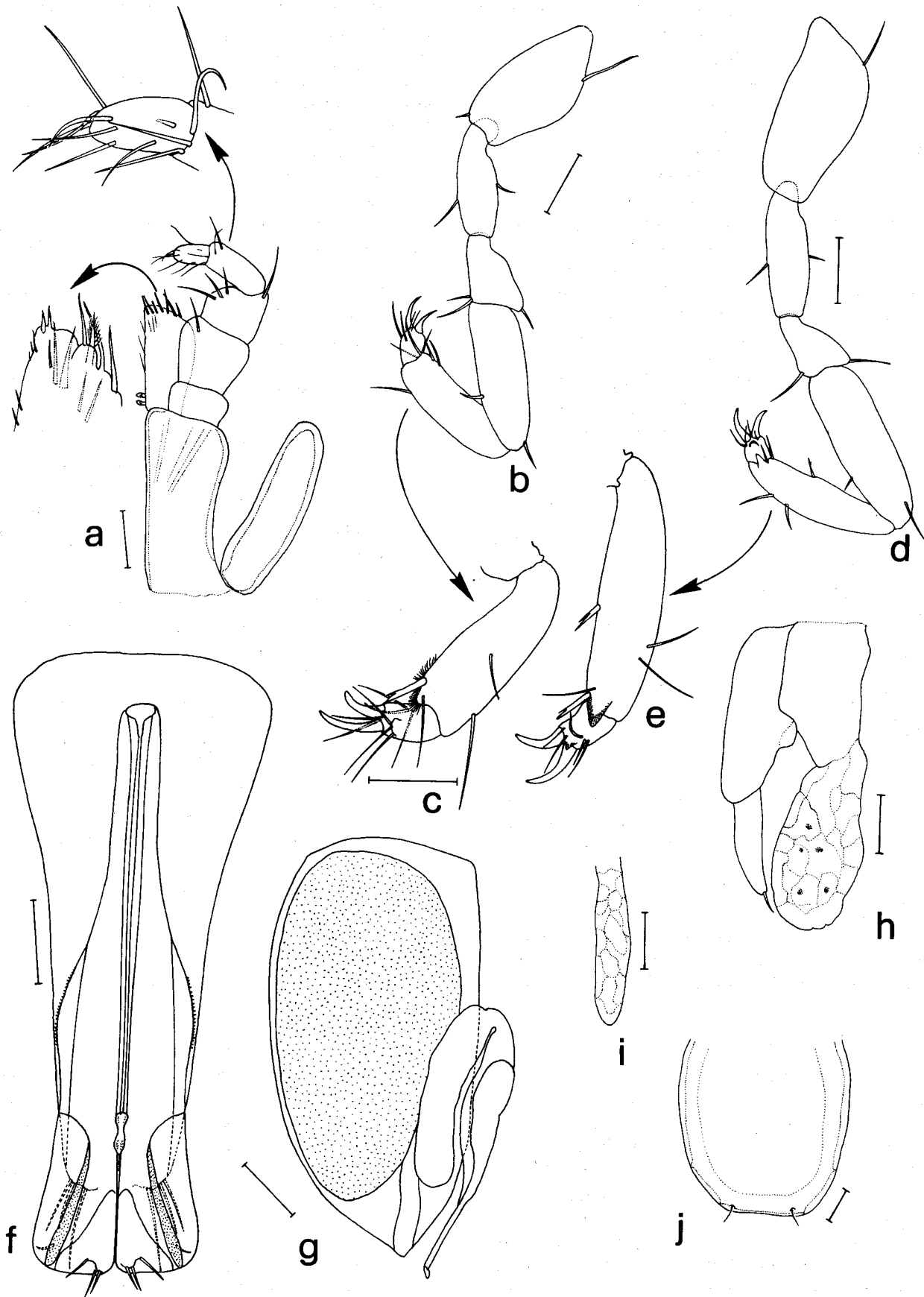


Fig. 3 - *Microcharon novariensis* sp. n., holotype male: a, maxilliped, with details of the distal part of basipodal endite and palp; b, c, pereopod 1; d, e, pereopod 7; f, first pleopod; g, second pleopod; h, third pleopod; i, fourth pleopod. Paratype female: j, second pleopod. Scale bars, 25 µm.

first male pleopod). Nevertheless, from both species, as well as from all members of the western Mediterranean group of *Microcharon*, the new species differs in the long sensorial seta on the second segment of the antennula, the relatively short pereopodal unguli and the presence of two setules on the second female pleopod. In the new species, all these characters are present in a plesiomorphic state versus their apomorphic state in the western Mediterranean species, all with short sensorial seta on segment 2 of the antennula, long pereopodal unguli, and second female pleopod naked. For this reason, the new species occupies an enigmatic position in the phylogeny of the genus hypothesised by Coineau (1994), most probably because of a 'mosaic evolution' of characters.

#### DISCUSSION

According to Coineau (1986, 1994), Dole & Coineau (1987), and Galassi (1991), the isopod genus *Microcharon* has a marine origin, and secondarily colonized inland groundwater during marine regressions. The dynamic events, which led to the colonization and speciation in inland groundwater, have been widely discussed by several Authors for some crustacean taxa (Stock, 1980; Rouch & Danielopol, 1987; Boutin & Coineau, 1990; Notenboom, 1991; Coineau & Boutin, 1992; Holsinger, 1994; Stoch, 1995). As regards the genus *Microcharon*, a good correlation between patterns of relationship among the species and sequence of epicontinental sea regressions in the Mediterranean basin was suggested by Coineau (1994), who postulated a colonization of groundwater from marine ancestors and a subsequent speciation by vicariance, according to the "modèle biphasé" (Boutin & Coineau, 1990). The new species quite fits this scenario, although its origin is possibly somewhat distant from the evolutionary history depicted by Coineau (1992) for the remaining Italian species of the genus. The more relevant phylogenetic affinities with the Balkanic group of *Microcharon*, as well as the number of characters that *M. novariensis* sp. n. shows in a plesiomorphic state, suggest an ancient origin of this species from a marine ancestor living along the paleocoastlines of the Tethys Sea when it was still connected to the Mediterranean Proper through a northern Adriatic corridor (Adriatic-Pannonian connection), until late Burdigalian (Hsü, 1978; Steininger & Rögl, 1979; Dercourt *et al.*, 1993). The subsequent closure of the Paratethys marked the independent evolution of the Paratethyan biota during the Middle Miocene ("endemic facies" of Paratethys, according to Steininger & Rögl, 1979) and possibly resulted in the isolation of the ancestor's population of *M. novariensis* sp. n. from the Balkanic lineages. The chronology of the speciation event which led to *M. novariensis* sp. n. is difficult to assess. The sites from which the new species has been collected were submerged by the sea until Late

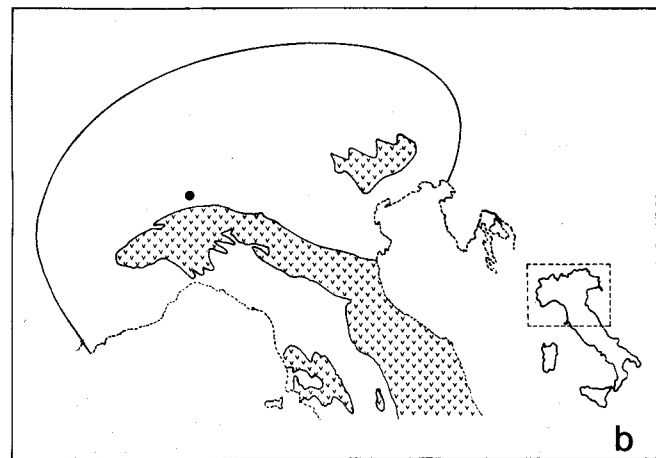
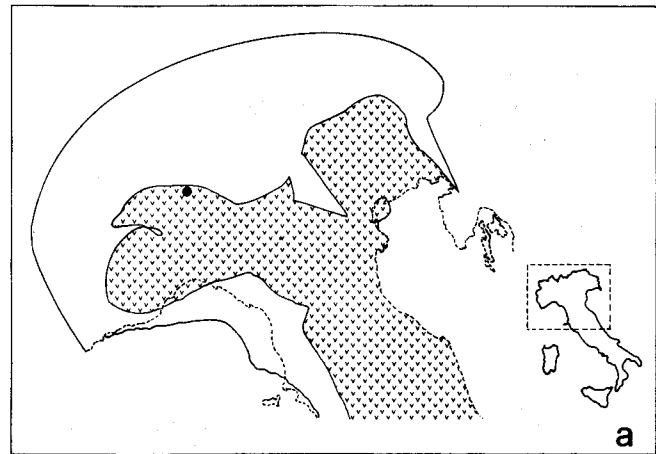


Fig. 4 - Paleogeographical maps showing the distribution of emerged and submerged areas in northern Italy: **a**, Tortonian; **b**, Messinian (after Boccaletti *et al.*, 1990, redrawn). Distribution of *Microcharon novariensis* sp. n. (•); submerged areas (▨); emerged areas (□).

Miocene. The Messinian (5.5 Ma B.P.) is well represented in the Padanian Region with a large (non-marine) lake fed by rivers from the southern Alps and northern Apennines. After this period, the northern limit of the Piedmont basin assumed a continental facies (Steininger *et al.*, 1985; Boccaletti *et al.*, 1990; Cita & Corselli, 1993), although a marine influence is quoted for the same area during Pliocene. The most parsimonious hypothesis may be that isolation and speciation may have taken place during the last regressive phase dated to the Messinian. This hypothesis was already suggested by Caccone *et al.* (1982) to explain the distribution of the cave-dwelling isopod genus *Monolistra* from the Italian Prealps. Moreover, Wägele (1983) suggested that the isopod *Microcerberus ruffoi* Chappuis, 1953, reported from the alluvial sediments of the river Adige (Venetian Prealps), must be regarded as a 'stranded relict' of the Oligocene Tethyan coast, as this species has a 'typical marine morphology'. All these records suggest the main role of the Tertiary marine regressions in shaping the groundwater biodiversity of this area. These species

may be considered distributional and phylogenetic relicts of ancient Tethyan lines now disappeared from the Mediterranean Sea, whose disjunct closest relatives are traceable only in the eastern Mediterranean continental groundwater.

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