FEB 27 1996

Int. J. Speleol., 23, 3-4 (1994): 133-155. 1995

TALLAHASSEE, FLORIDA

**Some remarks on the genus *Microcharon* Karaman
in Greece, and description of *M. agripensis* n. sp.
(Crustacea, Isopoda, Microparasellidae)**

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SUMMARY

Several samples of microparasellid isopods of the genus *Microcharon* Karaman were obtained in groundwater habitats of Greece.

Four species are identified, and taxonomical and zoogeographical remarks on some rare or poorly known taxa are made. One species, herein described as *Microcharon agripensis* n. sp., is new to Science. *M. latus prespensis* Karaman, 1954, on account of the different morphology of the first and second male pleopods, and its partially overlapping distribution, in respect to *M. latus* Karaman, 1934, is definitively raised at specific rank. Supplementary descriptions and illustrations are reported for incompletely described species such as *M. latus*, *M. prespensis* stat. nov., *M. major* Karaman, 1954 and *M. othrys* Argano & Pesce, 1979. For some species, such as *M. latus*, *M. othrys* and *M. antonellae* Galassi, 1991, SEM preparations of the mouthparts, not well detailed with the optical microscopy, were carried out. According to data from the present study, a paleogeographical scenario of the Balkan Peninsula is briefly depicted in order to sketch the most significative events which led to the colonization and speciation of the *Microcharon* species in this area.

INTRODUCTION

The genus *Microcharon* is widespread in the Balkan Peninsula, with 20 named species and subspecies, most of which are present in Greece.

Only the species *M. latus* and *M. prespensis* show a wider distribution, in contrast with the high degree of endemism shared by the other *Microcharon* species.

Our recent collections of microparasellid isopods from different groundwater substrates of Greece led us to carefully examine some incompletely described species such as *M. latus* Karaman, 1934, *M. prespensis* Karaman, 1954, *M. othrys* Argano & Pesce, 1979.

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Description

Body length 1.04-1.49 mm (♀ ♀); 1.36-1.60 (♂ ♂), male holotype: 1.55 mm. Cephalosome slightly longer than broad; pleotelson about as long as broad; chetotaxy as in Fig. 17.

First antenna (Fig. 2), 6-segmented, not sexually dimorphic in the armature; segment 1 bearing 3 setae; segment 2 with 2 lateral sensorial plumose setae and 1 distal and 2 subdistal setae; a long plumose seta is inserted on the distal protrusion of the same segment; segment 3 naked, segment 4 with a little aesthete and a transformed plumose seta; segment 5 bearing an aesthete, segment 6 with 1 aesthete, 1 long plumose, 1 distal and 1 subdistal setae. Second antenna: exopodite with 2 setae, flagellum of 9 segments.

Upper lip (Fig. 11) rounded, with thin setules along laterodistal margins. Lower lip consisting of 2 elongates lobes, as in Fig. 7.

Left mandible (Fig. 8): lacinia mobilis with 4 subconical teeth; pars incisiva with 7 teeth, not equally developed; pars molaris with 3 setae; between lacinia mobilis and pars molaris 2 denticulated spines and 5 setae are inserted.

Right mandible (Fig. 3), lacking lacinia mobilis; pars incisiva with 5 subconical teeth; pars molaris with 3 setae; between pars incisiva and pars molaris there are 3 denticulated spines, 4 setules and 2 plumose setae.

Mandibular palp built as usual in the genus, consisting of 3 segments, distal one bearing 4 claw-like fringed spines and 2 rows of thin cuticular spines; other armature as in Fig. 5.

First maxilla with 2 endites, the outer one (Fig. 10) with 10 distal denticulated spines and 3 thin setules; inner endite (Fig. 6) with 1 apical spine and some distal and subdistal setules.

Second maxilla (Fig. 9) with 3 endites, about of the same length, inner endite the wider; outer and central endites with 4 denticulated setae; inner endite with 5 apical setae and 1 strong pectinate spine; 2 spines and 3 setules are inserted along the inner margin.

Maxilliped: epipodite slender; palp of 5 segments; segments 2 and 3 expanded, segments 4 and 5 slender, segment 4 with 4 setae, segment 5 with 7 setae. Basipodal endite armed as in Fig. 4.

Pereopods (Figs. 13, 20) rather similar in shape, with small differences in the armature, especially of the dactylus (5 setae in P1 vs 4 in P2-P7).

First male pleopod (Fig. 18): with simple apex, more or less

Figs. 2-11 — *M. agripensis* n. sp., females paratypes: 2. first antenna; 3. right mandible; 4. maxilliped; 5. mandibular palp; 6. first maxilla, inner endite; 7. lower lip; 8. left mandible; 9. second maxilla; 10. first maxilla, outer endite; 11. upper lip.

rounded; hyaline edge slightly sticking out the apex of the same pleopod. Exopodite relatively long; basal part slightly enlarged. Distal armature consisting of 7 setae: 1 subdistal and 2 lateral setae, 1 short subapical and 3 apical setae.

Second male pleopod (Fig. 12): sympodite elongate, subovalar and subrounded at mediodistal corner; endopodite recurved, filiform, reaching tip of exopodite.

Second female pleopod (Fig. 16) about as long as broad (mean length/width ratio: 1.01; $n=3$); distal margin with 2 setae and a more or less pronounced, medial notch.

Third pleopod (Fig. 19) as usual in other freshwater species of the genus.

Fourth pleopod rudimentary (Fig. 15).

Uropods (Fig. 14) much longer than pleotelson; endopodite short, more than half as long as the sympodite; exopodite as long as the sympodite.

Etymology

Specific epithet after «Agripo», the ancient name of Euboea island.

Remarks

M. agripensis n. sp. belongs to a morphological group (*profundalis* group sensu Galassi & al., in press) of species, characterized by a hyaline edge sticking out the apex of the first male pleopod. At present, the *profundalis* group includes, besides the new species, the following ones: *M. profundalis* Karaman, 1940, from phreatic and hyporheic waters of former Yugoslav Macedonia; *M. hercegovinensis* Karaman, 1959, from phreatic and hyporheic waters of Hercegovina; *M. phlegethonis* Cvetkov, 1967, from spring waters near Devnja and phreatic waters near Varna, Sevlievo and Rouse (Bulgaria) and *M. bureschi* Cvetkov, 1976, from phreatic waters of Sofia. Within the group, *M. agripensis* n. sp. most closely resembles *M. hercegovinensis*, on account of the construction of the first male pleopod and the morphology and ornamentation of the second female pleopod. The diagnostical differences regard especially the different bend of the apex of the first male pleopod (inner corner drawn down in *M. agripensis* vs the same, straight in *M. hercegovinensis*), the setation of the same pleopod (7 setae in *M.*

Figs. 12-20 — *M. agripensis* n. sp., male holotype (12, 18), females paratypes (13-17, 19, 20): 12. second pleopod; 13. first pereopod, dactylus; 14. uropod; 15. fourth pleopod; 16. second pleopod; 17. telson; 18. first pleopod; 19. third pleopod; 20. seventh pereopod, dactylus.

agripensis vs 6 in *M. hercegovinensis*) and the morphology of the inner corner of the sympodite of the second male pleopod (subrounded in *M. agripensis* vs pointed in *M. hercegovinensis*).

From a zoogeographical point of view, the speciation within *profundalis* group could be related to the eastern Paratethys evolution (Rögl & Steininger, 1984; Steininger & al., 1985; Cita & Corselli, 1993). On this regard, the new species shows more similarities with *M. hercegovinensis* and *M. profundalis* than with *M. phlegethonis* and *M. bureschi*. It seems to be plausible that the origin and the age of *M. agripensis* could be related with the speciation of *M. hercegovinensis* and *M. profundalis*, during the more recent (Mio-Pliocene age) fragmentation and drainage of the central-eastern Paratethys (plausible Dacian-Pannonian correlations; Steininger, in litt.), along sud-Dinaric Adriatic and Morava-Vardar Aegeic «channels». Particularly, *M. agripensis* could be originated during the late Miocene - lower Pliocene involution of the Aegean «lago-mare» (Hsü, 1978; Hsü et al., 1973), which partially covered the Euboea island, determining the invasion of »Paratethyan relicts« into the Mediterranean, as mentioned by many authors (Danielopol, 1980; Bianco, 1990; Banarescu, 1991).

Microcharon major Karaman, 1954 (Figs. 21-37)

Material

3 ♂♂, 2 ♀♀; hyporheic habitat, little stream of the Evinos river basin, on the road to Platanos (Etholie); 300 m a.s.l.; water temperature: 21°C, E.c.: 0.240 mS/cm, pH 7.8, salinity: 0.5‰, (Karaman-Chappuis sampling method); 22 June 1990; coll. A. Tiberio & D. Galassi.

Supplementary description

Body length about 2.03 mm (range: 1.86-2.17 mm) (♂♂), 1.73 mm (range: 1.67-1.80 mm) (♀♀). Pleotelson much longer than broad, with well developed caudal and pleural setae, as in *M. antonellae*; chetotaxy as in Fig. 35.

First antenna (Fig. 21), 6-segmented, not sexually dimorphic in the armature; segment 1 naked, segment 2 with 2 lateral plumose and 2 subdistal setae; a long plumose seta is inserted on the distal protrusion of the same segment; segment 3 naked, segment 4 with

Figs. 21-30 — *M. major* Karaman, 1954. 21. First antenna; 22. first maxilla, inner endite; 23. mandibular palp; 24. maxilliped; 25. second maxilla; 26. lower lip; 27. first maxilla, outer endite; 28. left mandible; 29. upper lip; 30. right mandible.

a little lateral seta, segment 5 with 1 aesthete and 1 seta, segment 6 with 2 setae, 1 aesthete, 2 plumose setae of different length. Second antenna missing in all specimens.

Upper lip (Fig. 29) rounded, with thin cuticular spines along the distal margin.

Lower lip (Fig. 26) consisting of 2 elongated lobes.

Left mandible (Fig. 28): lacinia mobilis with 5 subconical teeth; pars incisiva with 4 teeth; pars molaris with 3 setae; between lacinia mobilis and pars molaris 2 denticulated spines, 5 setules and 2 plumose setae are inserted.

Right mandible (Fig. 30), lacking lacinia mobilis; pars incisiva with 5 subconical teeth; pars molaris with 3 setae; between pars incisiva and pars molaris there are 3 denticulated spines, 3 setules and 2 plumose setae.

Mandibular palp built as in other *Microcharon* species, consisting of 3 elements, distal one bearing 4 claw-like fringed spines and 2 rows of cuticular spines; remaining armature as in Fig. 23.

First maxilla with 2 endites, the outer one (Fig. 27) with 10 distal denticulated spines and 3 thin setules; inner endite (Fig. 22) with 1 apical spine and numerous distal and subdistal setules.

Second maxilla (Fig. 25) with 3 endites; inner endite the wider; outer and central endites with 4 setae, most of which denticulated; inner endite with 5 apical setae and 1 strong pectinate spine; 1 seta and some thin setules are inserted along the inner margin.

Maxilliped: epipodite slender; palp of 5 segments; segments 2 and 3 slender, segment 4 and 5 with 4 and 5 setae respectively. Basipodal endite as in Fig. 24.

Pereopods (Figs. 32, 34) with small differences in the armature, especially of the dactylus (5 setae in P1 vs 4 in P2-P7); unguli relatively short, close resembling those of *M. antonellae*.

First male pleopod (Fig. 31) consisting of 2 halves, distally protruding in a marked protuberance, bearing 2 setules; remaining armature of the distal part as follows: 3 distal, outer setae and 2 lateral more developed setae. As pointed out by Galassi (1991) the species was originally (Karaman, 1954) described with 1-2 apical setae; successively, with 1 apical setula by Cvetkov (1967, 1968). Well developed hyaline membrane.

Second male pleopod (Fig. 33): sympodite elongated, subrectangular and pointed at mediobasal corner; endopodite recurved, not filiform, weakly overreaching tip of exopodite.

Figs. 31-37 — *M. major*. 31. First male pleopod; 32. seventh pereopod, dactylus; 33. second male pleopod; 34. first pereopod, dactylus; 35. telson; 36. third pleopod; 37. second female pleopod.

Second female pleopod (Fig. 37) slightly longer than wide; distal margin with a medial notch, armed with 4 setae.

Third pleopod (Fig. 36) as usual in other freshwater species of the genus.

Uropods missing in all the specimens.

Remarks

M. major was originally described by Karaman (1954) from hyporheic habitats of Pcinja river (Skoplje) and from Bregalnica stream and some, not more detailed, hyporheic habitats near Prilep (Macedonia). Successively, Cvetkov (1967) recorded the species from hyporheic of Strouma river (Blagoevgrad, Bulgaria). Our finding in Etholie greatly enlarged southward the range of the species, suggesting that *M. major* could be more widely distributed than previously thought.

M. major, owing to the morphology of the first and second male pleopods and the armature of the second female pleopod, quite fits the *major* group of species as established by Galassi (1991). The phylogenetic analysis yielded by Coineau (1994) clearly recognized the monophyletic value of the group, although its sister relationships are still not clear. The «intuitive» zoogeographical model, as proposed by Galassi & al. (in press) placed the main speciation event within the group at the Messinian.

Microcharon latus Karaman, 1934 (Figs. 39-52; 58a)

Material

1 ♂, 1 ♀; phreatic waters (well), along the main-road Arta-Agrinion, near cross-road to Stanos, Etholie; 6 May 1977, coll. G.L. Pesce, D. Maggi & M. Miranda. 13 ♀♀, 8 ♂♂, hyporheic habitat, little stream of the Evinos river basin; for locality data see *M. major*. Numerous ♀♀ and ♂♂, freshwater well of Kephallinia (Sami, Asosos, Argostolion), Corfu (Kassiopi, Ipsos) (for more details on locality data, see Argano & Pesce, 1979).

Supplementary description

Body lenght 1.61 - 1.76 mm (♀♀), 1.23 - 1.43 (♂♂). Pleotelson much longer than broad, with well developed caudal and pleural setae; chetotaxy as in Fig. 47.

Fig. 38 — Updated distribution of *M. latus* Karaman, 1934 and *M. prespensis* Karaman, 1954 (● *M. latus*, * *M. prespensis*). The arrows indicate the hypothetical dispersion way of the species during the Quaternary continental freshwater flooding.

First antenna (Fig. 39), 6-segmented, not sexually dimorphic in the armature; segment 1 with 3 setae, one of which plumose, segment 2 with 2 lateral plumose and 3 subapical setae; distal protrusion of the same segment bearing a long plumose seta, reaching tip of the antenna, segment 3 naked, segment 4 with 3 setae, segment 5 with 1 aesthete, segment 6 with 1 plumose seta, 1 aesthete and 4 setae of different length. Second antenna lacking in all specimens.

Left mandible (Fig. 42): lacinia mobilis with 3 teeth, pars incisiva with 4 teeth; pars molaris with 3 setae; between lacinia mobilis and pars molaris 2 denticulated spines, 3 setules and 2 plumose setae are inserted.

Right mandible (Fig. 45): pars incisiva with 3 subconical teeth; pars molaris with 3 setae; between pars incisiva and pars molaris there are 3 denticulated spines, 4 thin setules and 2 plumose setae.

Mandibular palp consisting of 3 elements, distal one bearing 4 claw-like fringed spines; remaining armature as in Fig. 40.

First maxilla with 2 endites, the outer one (Fig. 43) with 12 distal spines, most of which denticulated, the inner endite (Fig. 46) bears an apical spine and some thin setules.

Second maxilla (Figs. 44, 58a) consisting of 3 endites, the inner the broader. Outer and central endites with 4 setae, some of which denticulated; inner endite with 1 strong pectinate spine and 4 apical setae. One plumose seta and some thin setules are inserted along the inner margin.

Maxilliped: palp and basipodal endite as in Fig. 41.

Pereopods rather similar in shape, armature of the dactylus as in Fig. 51, except P1 with 5 setae. Unguli relatively long.

First male pleopod (Fig. 48) with simple apex, more or less rounded, inner corner drawn in; distal part bearing well developed setae; armature as follows: 3 apical setae, 1 short subapical one; 3 setae are inserted at the distal third of the same pleopod. Exopodite relatively long, basal part slightly enlarged. Hyaline membrane not distally sticking out the apex of the pleopod.

Second male pleopod (Fig. 49): sympodite elongated, subovalar and subrounded at mediiodistal corner; endopodite recurved, filiform.

Second female pleopod (Fig. 50) about as long as broad, with 2 distal setules; distal margin with a faint notch.

Third pleopod as in Fig. 52.

Remarks

M. latus s. l. includes, besides the nominal subspecies, *M. latus prespensis*, described by Karaman (1954) as an endemism of Prespa lake.

According to more recent data, *M. latus* s.str. is known from some localities of former Yugoslav Macedonia and of Crna-Gora (Montenegro), Etholie and Ionic islands of Greece. On the other hand, *M. latus prespensis* is recorded from former Yugoslav Macedonia, Epirus, Peloponnesus and some Aegean islands of Greece (Fig. 38).

As suggested by Argano & Pesce (1979), their partially overlapping distribution as well as their well defined diagnostical differences,

Figs. 39-46 — *M. latus* Karaman, 1934. 39. First antenna; 40. mandibular palp; 41. maxilliped; 42. left mandible; 43. first maxilla, outer endite; 44. second maxilla; 45. right mandible; 46. first maxilla, inner endite.

of the same weight if compared to congeners in the Balkan Peninsula, constrained ourself to raise the above taxa to higher specific level. It seems to be plausible that further investigations in this region could enlarge the patchy distribution of these species.

M. latus and *M. prespensis* appear to be good dispersers, widely distributed in the Balkan area, in different subterranean biotopes. However, an active migration mechanism, enlarging the original species' range from the «origin center» is an hypothesis statistically weak, due to the feeble dispersion ability shared by the other *Microcharon* species and by many interstitial stygobionts (Boutin & Coineau, 1990; Dole & Coineau, 1987; Coineau, 1994; Coineau & Boutin, 1992; Holsinger, 1992; Notenboom, 1991). On the other hand, an ancient, continuous range, more or less fragmented since Neogene times, including both islands and mainland populations, appear to be an unlikely explanation for the actual distributional patterns of *M. latus* and *M. prespensis*. In fact, two objections could be raised: 1) marked morphological divergences should be expected between islands and mainland populations; 2) stygobiont *Microcharon* populations, stranded in freshwater habitats should not have survived during the following marine transgressions which partly covered both the coastal mainland and the islands, during the basal Pliocene transgression and the Quaternary interglacial periods.

Therefore, a possible explanation for the distributional pattern of both species is offered by a dispersal scenario due to a passive colonization mechanism, supported by the more recent paleoecological and paleogeographical history of the Balkan area. On this regard, a freshwater flooding from the continental freshwaters (probable «origin center» of the species) to the Ionian sea and especially to the Aegean sea (Dermitzakis, 1990; Kotsakis, 1990; Sondaar et al., 1986), during Quaternary humid (often interglacials) periods (Bethoux, 1984; Thunneil & Williams, 1983; Chamley, 1983; Cramp & Collins, 1988; Cramp et al., 1988) is demonstrated by lithological and biostratigraphical reconstruction of sediments. According to the above data, a relatively recent invasion from mainland to islands for both *M. latus* and *M. prespensis* could be reasonably dated in the Würm interglacial; consequently, their presence in insular coastal habitats could represent a secondary colonization from some continental, more or less ancient, populations. According to Botosaneanu & Holsinger (1991), «dispersal processes of stream

Figs. 47-52 — *M. latus*. 47. Telson; 48. first male pleopod; 49. second male pleopod; 50. second female pleopod; 51. seventh pereopod, dactylus; 52. third pleopod.

meiofauna can be explained mainly by a passive, hydrodinamical model; recruitment in new areas downstream occurs mostly by dispersal through the water; water-column movements, not infaunal migrations, appears to be the most important dispersal mode in streams».

Microcharon prespensis Karaman, 1954 **stat. nov.**

Material

2 ♂♂, 1 ♀; freshwater well, Arta, Epirus. 2 April 1978; coll. G.L. Pesce, D. Maggi & M. Miranda. Numerous ♂♂, ♀♀; freshwater wells, Peloponnesus (Kokkinorraki, Amikle, Egie and Kalamata); 12 May 1977; coll. G.L. Pesce, D. Maggi & M. Miranda. 2 ♀♀, 1 ♂; freshwater well, along the main-road Kimi-Paralia, near Kimi (Euboea), 30 June 1984; coll. G.L. Pesce. 3 ♂♂, freshwater well, near Mistegna, Skala (Lesbos); 30 July 1982; coll. G.L. Pesce, D. Maggi & G. Silverii. 4 ♂♂, 3 ♀♀; hyporheic habitat, Lakos Marion stream, along the road to Maries, Thasos. 50 m a.s.l.. Water temperature: 20°C, E.c.: 0.240 mS/cm, salinity: 0.5‰; pH 6.5. 28 June 1990; coll. A. Tiberio & D. Galassi.

The rather brief description, given below, deals with the characters overlooked in the original description as well as with the diagnostical morphological features, within the genus, in respect to *M. latus*.

The main differences regard the morphology of the first and second male pleopods. Particularly, the distal part of the first male pleopod is rounded in *M. prespensis* and with a symmetrical circular bend in each ramus versus a distal part asymmetrical, being the inner corner drawn inward in *M. latus*. Moreover, the second male pleopod shows a sympodite with pointed mediodistal corner (although we observed a little variation of this character, which could be related with a different orientation on slide), when in *M. latus* the same appears to be subrounded. No marked differences were observed in the other morphological characters.

Microcharon othrys Argano & Pesce, 1979 (Figs. 53-57, 58b)

Material

1 ♂, 1 ♀; freshwater well, along the road Thebes-Lamia, near Scamnos; 10 May 1977, coll. G.L. Pesce, D. Maggi & M. Miranda.

Figs. 53-57 — *M. othrys* Argano & Pesce, 1979. 53. Second maxilla, outer and central endites; 54. mandibular palp; 55. first maxilla, inner endite; 56. first maxilla, outer endite; 57. second maxilla, inner endite.

M. othrys was originally described by Argano & Pesce (1979), without particular attention to mouthparts and to the first male pleopod (Galassi, 1991). The availability of type-material let us to improve some not well detailed characters.

First maxilla consisting of 2 endites, the outer one bearing 10 spines, most of which denticulated (Fig. 56), inner endite (Fig. 55) with 1 apical spine and numerous distal and subdistal thin setules.

Second maxilla with 3 endites; outer and central endites (Fig. 53) bearing 4 denticulated setae, inner endite (Figs. 57, 58b) with 5 apical setae, one of which plumose and 1 strong pectinate spine; 1 plumose seta and some setules are inserted along the inner margin.

Mandibular palp consisting of 3 segments, the distal one bearing 4 claw-like fringed spines; remaining armature as in Fig. 54.

CONCLUSION

The paleogeographical and paleoecological reconstruction of the Hellenic area is the major topic for outline an evolutionary scenario for the stygobiont *Microcharon* in Greece and in the Balkan Peninsula at all (Banarescu, 1991; Sket, 1994).

The ancient origin of the region as well as the numerous, not well recognizable, paleogeographical events (viz. Neogene marine transgressions, Paratethys involution, Quaternary glacial-interglacial alternance) which followed one another, made the paleohistory of the Hellenic area difficult to assess. Any biogeographical reconstruction feels the effects of such a vagueness. The little ecological and taxonomical knowledge of the *Microcharon* species does not aid to solve the question.

Nevertheless, the Balkanic *Microcharon* occur, sometimes in syntopy, in different subterranean biotopes, such as springs, phreatic and hyporheic habitats and limnostygial, showing an interesting niches diversification and high variation of morphological planes. It seems to be plausible that different events in time and space led to numerous colonization's waves and consequently to the present-day distribution and diversification of the genus in this area.

0.01 mm

0.01 mm

0.01 mm

Fig. 58 — SEM preparations of the second maxilla in: a. *M. latus*; b. *M. othrys*; c. *M. antonellae*.

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