THE *ACANTHOCYCLOPS KIEFERI* COMPLEX (COPEPODA, CYCLOPOIDA) FROM SOUTH-EASTERN EUROPE, WITH DESCRIPTION OF A NEW SPECIES

BY

SANDA IEPURE[1,3]) and DANIELLE DEFAYE[2,4])

[1]) Institutul de Speologie "Emil Racoviţă", Cluj Department, Clinicilor, 5, Cluj Napoca, RO-400006, Romania

[2]) Muséum national d'Histoire naturelle, Département Milieux et Peuplements Aquatiques, UMR 5178, 61, rue de Buffon, F-75005 Paris, France

ABSTRACT

The *Acanthocyclops kieferi* (Chappuis, 1925) complex (Copepoda, Cyclopoida) includes exclusively stygobitic species, mainly widespread in the cave habitats of south-eastern Europe. A new species for the group, *Acanthocyclops milotai* n. sp., collected from pools supplied by percolation water in a cave in south-western Romania, is described. The new species is distinguished from its congeners by the following characters: the ornamentation pattern of the basis of the antenna with four spinules placed in an arch; the coxa of leg 4 with four spinules on its distal margin; furcal rami divergent, about 2.2 times as long as wide, with the lateralmost, terminal furcal seta 1.14 times as long as the medialmost one, and these both setiform. Inter-populational variability of some characters in the new species such as the shape of the anal operculum and the ornamentation of the coxa of leg P4 are discussed. Furthermore, the characters generally used to differentiate the taxa of the *kieferi* group are re-examined and discussed, and a brief description of the adaptive traits of these species to the groundwater is given. Biogeographical and ecological considerations about the *kieferi* species-complex in Romanian groundwater habitats are also mentioned.

RÉSUMÉ

Le complexe *Acanthocyclops kieferi* (Chappuis, 1925) est composé d'espèces stygobiontes largement répandues dans les habitats des grottes de l'Europe de l'Est. Une nouvelle espèce de ce groupe, *A. milotai* n. sp., trouvée dans des bassins remplis d'eau de percolation d'une grotte du sud-ouest de la Roumanie, est décrite. La nouvelle espèce se distingue de ses congénères par les caractères suivants: l'ornementation de l'antenne avec 3 spinules disposées en arc sur le basipodite et celle du coxopodite de P4, avec 4 spinules sur son extrémité distale; les rames furcales divergentes, 2,2 fois plus longues que larges et la soie furcale terminale la plus latérale 1,14 fois plus longue que celle plus médiane, les deux sétiformes. La variabilité intra-populationnelle de certains caractères

[3]) e-mail: siepure@hasdeu.ubbcluj.ro

[4]) e-mail: ddefaye@mnhn.fr

Crustaceana 81 (5): 611-630
Also available online: www.brill.nl/cr

chez la nouvelle espèce, comme la forme de l'opercule anal et l'ornementation du coxopodite de P4 est brièvement discutée. Les caractères généralement utilisés pour différencier les taxons de ce groupe sont re-examinés et discutés, et une courte description de leurs traits adaptatifs est donnée. Les caractères biogéographiques et écologiques de la nouvelle espèce et du complexe *A. kieferi* dans les eaux souterraines de Roumanie sont brièvement discutés.

INTRODUCTION

The genus *Acanthocyclops* Kiefer, 1927 currently includes about 60 species and subspecies (Dussart & Defaye, 2006); most of them are cosmopolitan or Holarctic, living in surface or subsurface fresh waters. The number of species is considered an approximate indication, due to the genus' close affinities with *Diacyclops* Kiefer, 1927, which sometimes leads to the transfer of species from *Acanthocyclops* to *Diacyclops* or vice versa (Galassi & De Laurentiis, 2004). The genus is divided into four species groups, of which the *kieferi* complex, first introduced by Stoch (1995), includes strictly subterranean species (Pandourski, 1994; Iepure, 2001). They are distributed mainly in south-eastern Europe and the Mediterranean region, with the exception of *Acanthocyclops kieferi* Chappuis, 1925 (sensu stricto), which is widespread throughout various subterranean habitats in Europe (Chappuis, 1925; Pleşa, 1969; Lescher-Moutoué, 1973; Pleşa & Racoviţă, 1973; Pesce & Galassi, 1984; Moldovan et al., 2000).

The *Acanthocyclops kieferi* complex included ten species and subspecies until 1997, when Pandourski (1997) extended the group to 15 taxa. Recently, careful investigations of both female and male specimens of *Acanthocyclops agamus* Kiefer, 1938 from Castelcivita Cave (southern Italy) allow its addition to the *kieferi* group (Galassi & De Laurentiis, 2004). Two new species described from cave habitats in north-western Romania (Iepure, 2001) and the species newly described herein, *A. milotai* n. sp., increase the number of species in the group. Thus, the complex now includes the following 19 taxa: *Acanthocyclops stygius stygius* (Chappuis, 1924); *A. stygius deminutus* (Chappuis, 1925); *A. kieferi* (Chappuis, 1925); *A. reductus* (Chappuis, 1925); *A. hypogeus* Kiefer, 1930; *A. hispanicus* Kiefer, 1937; *A. macedonicus* (Petkovski, 1954); *A. propinquus* (Pleşa, 1957); *A. biarticulatus* Monchenko, 1972; *A. petkovskii* Pesce & Lattinger, 1983; *A. balcanicus* Naidenow & Pandourski, 1992; *A. chappuisi* Naidenow & Pandourski, 1992; *A. iskrecensis* Pandourski, 1992; *A. radevi* Pandourski, 1993; *A. strimonis* (Pandourski, 1994); *A. agamus* Kiefer, 1938; *A. plesai* Iepure, 2001; *A. balcanicus bisaetosus* Iepure, 2001; and *A. milotai* n. sp.

The differentiation of the *Acanthocyclops kieferi* species-group from the other complexes of the genus is mainly based on morphological characters, such as the segmentation pattern of the swimming legs and the 11-segmented antennules, but

also on their ecological preference for groundwater habitats (Pandourski, 1994). Morphologically, the group is highly heterogeneous and is considered by some authors to be polyphyletic or at least paraphyletic (Galassi & De Laurentiis, 2004). Moreover, the species of this group share a combination of characters and micro-characters that are common in several genera of the Cyclopinae such as *Diacyclops* Kiefer, 1927, *Megacyclops* Jurine, 1921, *Rheocyclops* Reid, Strayer, McArthur, Stibbe & Lewis, 1999, *Itocyclops* Reid & Ishida, 2000, and the newly described *Zealandcyclops* Karanovic, 2005.

The earliest records from Romania assigned to the *Acanthocyclops kieferi* species-group were made by Chappuis (1924, 1925), who reported three species from the tap water of the town of Cluj-Napoca: *Acanthocyclops stygius stygius*, *A. stygius deminutus*, and *Acanthocyclops reductus*. The first two taxa, previously included in *Diacyclops*, were transferred by Pandourski (1992, 1997) to *Acanthocyclops*. A new taxon belonging to the complex, *Acanthocyclops reductus propinquus* (now *Acanthocyclops propinquus*) has been described later from cave habitats of the Măgura Cave (north-western Romania) (Pleşa, 1957, 1961). Further, other specimens were reported from several caves in south-western Romania (Banat karstic area) and assigned by Pleşa to *A. propinquus* (cf. Pleşa et al., 1965; Pleşa, 1985). The re-examination of these specimens, deposited in the Copepoda collection of the Speleological Institute "Emil Racovitza", Cluj Department (Romania) allows us to reconsider the complex and to describe a new taxon, *Acanthocyclops milotai* n. sp. New micro-characters have also been added, which are very useful to understand the phylogenetic relationships between the *kieferi* species-group and several genera of the subfamily Cyclopinae.

The species of the *Acanthocyclops kieferi* complex can be differentiated using a set of morphological characters and micro-characters. Several species of the group need complete redescription. The present paper, which describes a new species of this group, continues the study on the taxonomic revision, the ecology, the distribution, and the phylogeny of the *Acanthocyclops kieferi* species-group from groundwater habitats (Iepure, 2001).

MATERIAL AND METHODS

Specimens of the species described here were collected from the Oase Cave (south-western Romania) by filtering the percolation water supplying small pools, with a 200 μm mesh plankton net. No associated fauna has been collected from the pools investigated. The specimens were dissected and mounted in glycerol and deposited in the collection of the Speleological Institute "Emil Racoviţă", Cluj-Napoca.

Thirty-tree ♀♀ and five ♂♂ of *Acanthocyclops propinquus* from the Măgura Cave (Bihor Mountains, north-western Romania) were used for morphological comparison. The specimens have all been dissected, mounted in glycerol on slides, and deposited in the Copepoda collection of the Speleological Institute "Emil Racoviţă", Cluj-Napoca. In the absence of a holotype, i.e., not expressly nominated by Pleşa (1957), one ♀ has been designated as the lectotype and the other specimens have been designated as paralectotypes. One paralectotype ♀ (MNHN-Cp2417) and one paralectotype ♂ (MNHN-Cp2418), both dissected and mounted in glycerol on slides, are deposited at the Muséum national d'Histoire naturelle, Paris.

Drawings and measurements were made using a camera lucida mounted on a Leica DMLB® phase-contrast and a Nikon Eclipse E 200 microscope. Body length was measured from the base of the rostrum to the posterior edge of the furcal rami. The distal segments of the endopodite of leg 4, as well as the furcal rami were measured following the protocol established by Pleşa (1957). The body width is given as the widest part of the cephalothorax. In the spine and seta formula of the swimming legs, Roman numerals indicate spines and Arabic numerals indicate setae. The terminology of Huys & Boxshall (1991) has been adopted.

Abbreviations used in the text and figures are: ae, aesthetascs; exp1-3, first to third segment of exopod; end1-3, first to third segment of the endopod; legs1-6, first to sixth thoracopods.

TAXONOMY

CYCLOPIDAE Rafinesque, 1815
CYCLOPINAE sensu Dana, 1853
Acanthocyclops Kiefer, 1927
Acanthocyclops milotai n. sp. (figs. 2-6)

Material examined. — Holotype: One ♀ dissected and mounted on slides in glycerol, deposited in the Muséum national d'Histoire naturelle, Paris, MNHN-Cp2415; collected on 4.xi.2001, leg. S. Milota; Oase Cave (Aninei Mountains, south-western Romania).

Paratypes: One ♂ dissected and mounted in glycerol, deposited in the Muséum national d'Histoire naturelle, Paris, MNHN-Cp2416, collected on 4.xi.2001, leg. S. Milota, Oase Cave (south-western Romania). Four ♀♀, two ♂♂, pool, 4.xi.2001, leg. S. Milota, Oase Cave; 2 ♀♀, Ponor Uscată Cave, pool, 16.iii.2002, leg. S. Iepure, O. T. Moldovan; one ♀, Ponor Uscată Cave, pool, 29.iv.2003, leg. S. Iepure, O. T. Moldovan; 12 ♀♀, two ♂♂, Buhui Cave, pool, 29.iv.2003 leg. S. Iepure, O. Moldovan, S. Milota, L. Sarcina; one ♀, Padina Popii Cave, pool, 21.ix.1962, leg. C. Pleşa; six ♀♀, Boilor Cave, pool, 6.v.2003, leg. S. Iepure, S. Milota; two ♀♀, Socolovăţ Cave, pool, 21.xi.1961, leg. C. Pleşa; one ♀, Găurile lui Miloi II Cave, pool, 17.viii.1962, leg. C. Pleşa; three ♀♀, one ♂, Rol Cave, pool, 9.vii.1961, leg. C. Pleşa; all specimens dissected and mounted in glycerol on slides, and deposited in the Copepoda collection of the Speleological Institute "Emil Racoviţă", Cluj-Napoca.

All caves are located in the Aninei Mountains, except for Boilor Cave, which is located in the Locvei Mountains (south-western Romania) (fig. 1).

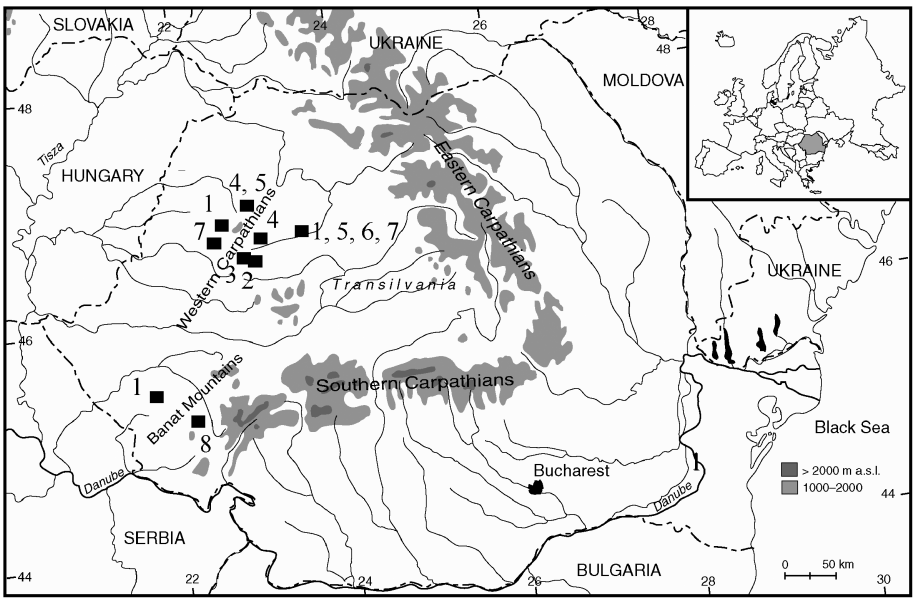

Fig. 1. Distribution of the *Acanthocyclops kieferi* species-complex in groundwater habitats in Romania: 1, *A. propinquus* (Pleşa, 1957); 2, *A. balcanicus bisaetosus* Iepure, 2001; 3, *A. plesai* Iepure, 2001; 4, *A. kieferi* (Chappuis, 1925); 5, *A. reductus reductus* (Chappuis, 1925); 6, *A. stygius stygius* (Chappuis, 1924); 7, *A. stygius deminutus* (Chappuis, 1925); 8, *A. milotai* n. sp. (map modified after Björkman et al., 2002).

Derivatio nominis. — The species is named after the cave diver Ştefan Milota (ProAcva Group, Timişoara, Romania) who explored the Oase Cave and collected the material.

Description of female (unless otherwise stated, data refer to the holotype). — Body length, excluding furcal setae, 0.451 mm (holotype) (paratypes, 0.451-0.560 mm, n = 2); cephalothorax width 0.171 mm (holotype) (paratypes, 0.171-0.190 mm, n = 2). Body shape elongate, without surface ornamentation (fig. 2B).

Genital double somite about 1.4 times as broad as long, seminal receptacle median, with anterior and posterior lobes symmetrical, as in fig. 2F, similar to that of *Acanthocyclops kieferi*.

Posterior margins of all somites smooth. Posterior margin of anal somite ornamented ventrally with small and strong spinules. Anal operculum triangular, with the distal part slightly rounded, ornamented with a row of spinules on its ventral side (fig. 2G). Furcal rami (fig. 2G) divergent, about 2.2 times as long as wide (holotype) (1.75-2.5 times in paratypes, n = 2), without ornamentation except for a row of small spinules ventrally at the base of the medial seta, and four larger spinules at the base of the externalmost terminal furcal seta. Dorsal seta about 1.3

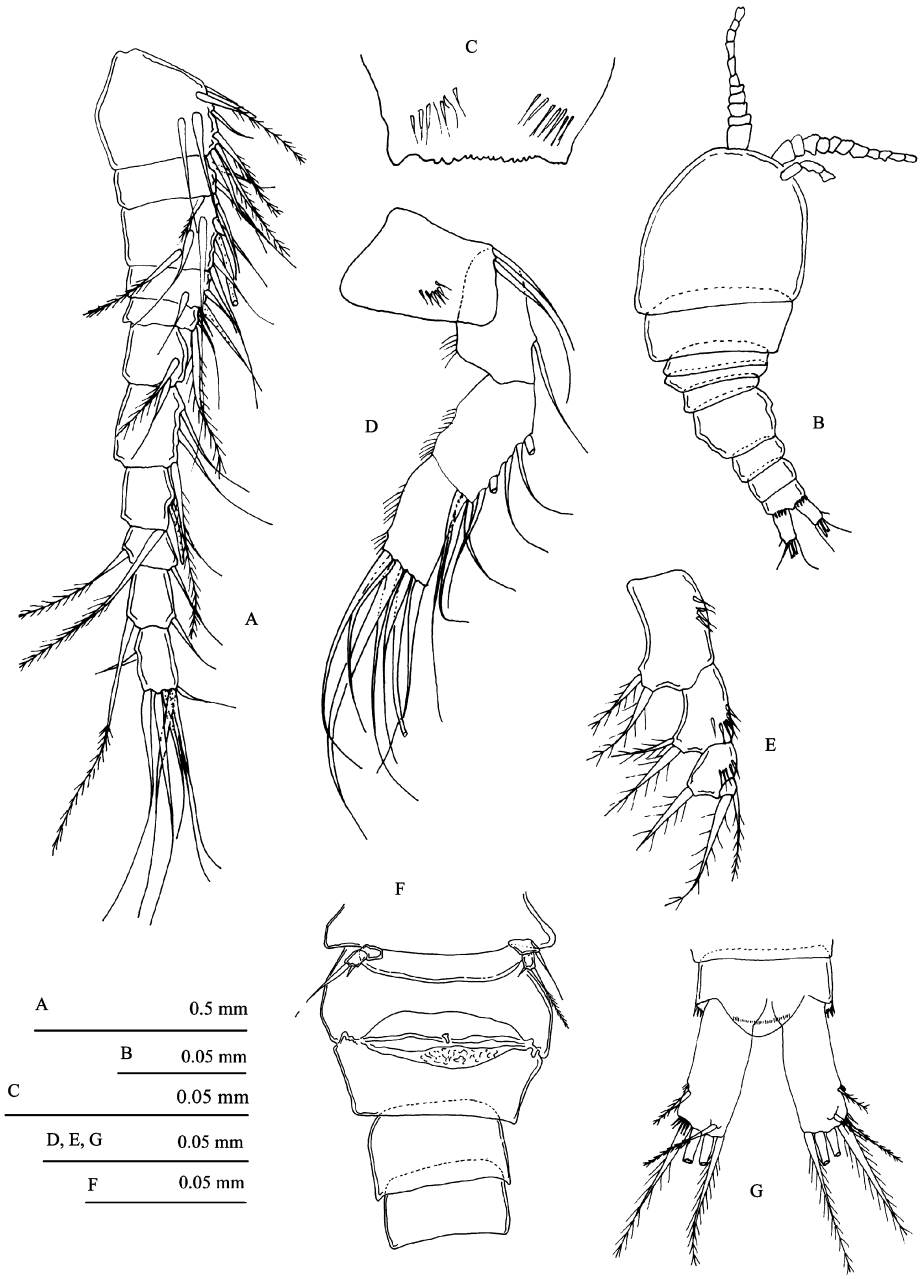

Fig. 2. *Acanthocyclops milotai* n. sp., female: A, antennule; B, habitus dorsal; C, labrum; D, antenna; E, maxilliped; F, pediger 5 and genital somite, ventral; G, anal somite and caudal rami, dorsal.

times the length of the ramus. Lateralmost terminal furcal seta 1.14 times as long as medialmost terminal seta, both setiform.

Antennule (fig. 2A) 11-segmented, surface of segments appearing smooth; setation from proximal to distal segment (number of setae in parentheses): I (8), II (4), III (8), IV (4), V (3), VI (2), VII (3), VIII (2+ae), IX (2), X (3) (one seta broken), XI (8). Aesthetasc on segment 8 reaching distal part of segment 9. Terminal segment without hyaline membrane.

Antenna (fig. 2D) 4-segmented; basis armed with two internal plumose setae inserted antero-distally, and frontal surface ornamented with a group of four spinules placed in an arch on the median outer half of the segment. Exopod absent. Endopod three-segmented, segments 1-3 with 1, 8, and 7 setae, respectively; all segments with a row of setules on the outer margin.

Labrum (fig. 2C) with 15 small and slightly rounded teeth.

Mandible with tiny palp, with the armature hardly visible (two setae observed in one paratype) (not figured).

Maxillule comprising praecoxa with five spines and setae, and a palp of the usual structure for the family, with a total of seven setae (not figured).

Maxilla with praecoxa armed with two plumose setae fixed on a small appendix; distal endite of coxa with two setae; basis with well-developed claw, armed with two setae. Endopod 2-segmented, first segment with two spiniform setae and second with two setiform setae (not figured).

Maxilliped (fig. 2E) proximal to distal segments with 2, 2, 1, and 2 setae, respectively; all segments, except distal one, with a curved row of large spinules.

Legs 1-4 (fig. 3) with three-segmented exopod, 2-segmented endopod on legs 1 and 2, and 3-segmented endopod on legs 3 and 4 (3.2/3.2/3.3/3.3). Armature of legs 1-4 as in table I. Intercoxal sclerites without surface ornamentation.

Leg 1 (fig. 3A) intercoxal sclerite, coxa, and basis with smooth surface. A row of tiny spinules present halfway the external coxa-basis margin. Free distal margin of basis, between the insertion of exopod and endopod, with a row of spinules. Basis bearing one spiniform seta at inner distal corner. Exp1 with one outer spine; exp2 with one outer spine and one inner seta; exp3 with two outer spines and four setae. Enp1 with one inner seta, ornamented with small spinules on its distal part and setules on the outer margin; enp2 with outer indentation marking the original boundary of former segments 2 and 3; armed with one apical spine, ornamented at the basis with small and strong spinules, and three setae.

Leg 2 (fig. 3B) coxa and basis both with smooth surface. Spinule rows present on the free distal margin of the basis between the insertion of exopod and endopod. Basal outer seta short, reaching the distal part of exp1. Exp1 with one outer spine, ornamented with long spinules on distal part; exp2 with one outer spine and one inner seta, and ornamented with long spinules on distal part; exp3 with three outer spines and four setae. All exopodal segments with setules on inner margins. Enp1 with one inner seta and ornamented with small spinules on its distal part; enp2

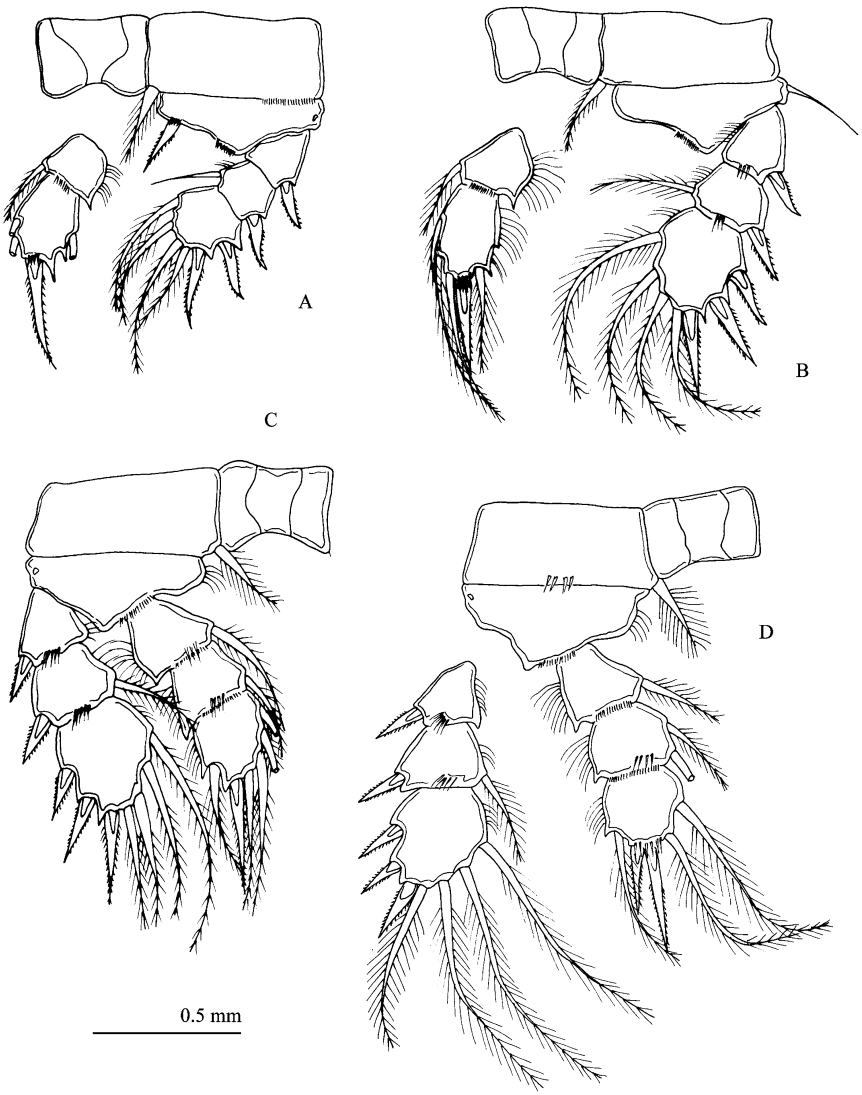

Fig. 3. *Acanthocyclops milotai* n. sp.: A, leg 1 and intercoxal sclerite; B, leg 2 and intercoxal sclerite; C, leg 3 and intercoxal sclerite; D, leg 4 and intercoxal sclerite.

with a slight outer indentation marking the original boundary of former segments 2 and 3, armed with one apical spine and four setae. At the base of the apical spine small, strong spinules are inserted.

Leg 3 (fig. 3C) coxa and basis both with smooth surface. Spinule rows present on the free distal margin of basis, at the base of the endopod. Exp1 with one outer spine, ornamentation of long spinules present on distal part; exp2 with one outer spine, one inner seta, and ornamentation of long spinules on distal part; exp3 with

TABLE I
Armature of legs P1-P4 in *Acanthocylops milotai* n. sp. (female). Spines are in Roman, setae in Arabic numerals

	Coxa	Basis	Exopod	Endopod
Leg P1	0-1	I-1	I-0; I-1; II-1,3	0-1; I-1,2
Leg P2	0-1	0-1	I-0; I-1, III-1,3	0-1; I-1,3
Leg P3	0-1	0-1	I-0; I-1, III-1,3	0-1; 0-1; I-1,4
Leg P4	0-1	0-1	I-0; I-1, III-1,3	0-1; 0-2; II-1,2

three outer spines and four setae. All exopodal segments with setules on inner margins. Enp1 with one inner seta, ornamented with small and long spinules on distal part; enp2 armed with one inner seta, and with the same ornamentation as enp1; enp3 with one apical spine and five setae: four inner and one outer.

Leg 4 (fig. 3D) coxa and basis both with smooth surface. Coxa ornamented with four long spinules inserted near its distal median edge. A small row of spinules present on the free distal margin of the basis at the base of the endopod. Exp1 with one outer spine, ornamented with long spinules on distal margin; exp2 with one outer spine, one inner seta, and ornamented with long spinules on distal margin; exp3 with three outer spines and four setae. All exopodal segments with setules on inner margins. Enp1 with one inner seta and ornamented with small spinules on its distal part; enp2 armed with two inner setae and ornamented with small and long spinules on its distal part; enp3, 1.16 times as wide as long (holotype) (1.11-1.33 times in paratypes, n = 2) armed with two apical spines and three setae, one inserted on outer part and two on inner part of segment. Medio-terminal spine 1.58 times as long as latero-terminal one, and ornamented at the base with a group of spinules. All endopodal segments adorned with setules on the outer margins.

Leg 5 (fig. 2F) 2-segmented, proximal segment bearing a relatively long outer seta; distal segment almost quadrate, about 1.6 times as long as wide, distinctly articulated with the proximal segment, bearing one external terminal seta 6 times as long as the internal spine inserted latero-apically.

Leg 6 in ventro-lateral position, armed with one outer spine and two setae, the inner seta shorter than the medial one.

Description of male. — Body length, excluding furcal setae, 0.376 mm. Habitus elongate, similar to that of female, without ornamentation on body surface (fig. 4A).

Anal somite with a row of spinules on ventral posterior margin, without ornamentation on its dorsal surface. Anal operculum triangular, with distal part slightly rounded, and with ornamentation on ventral face as in female (fig. 4A). Furcal rami slightly shorter than in female, 2.16 times as long as wide (1.50-2.44

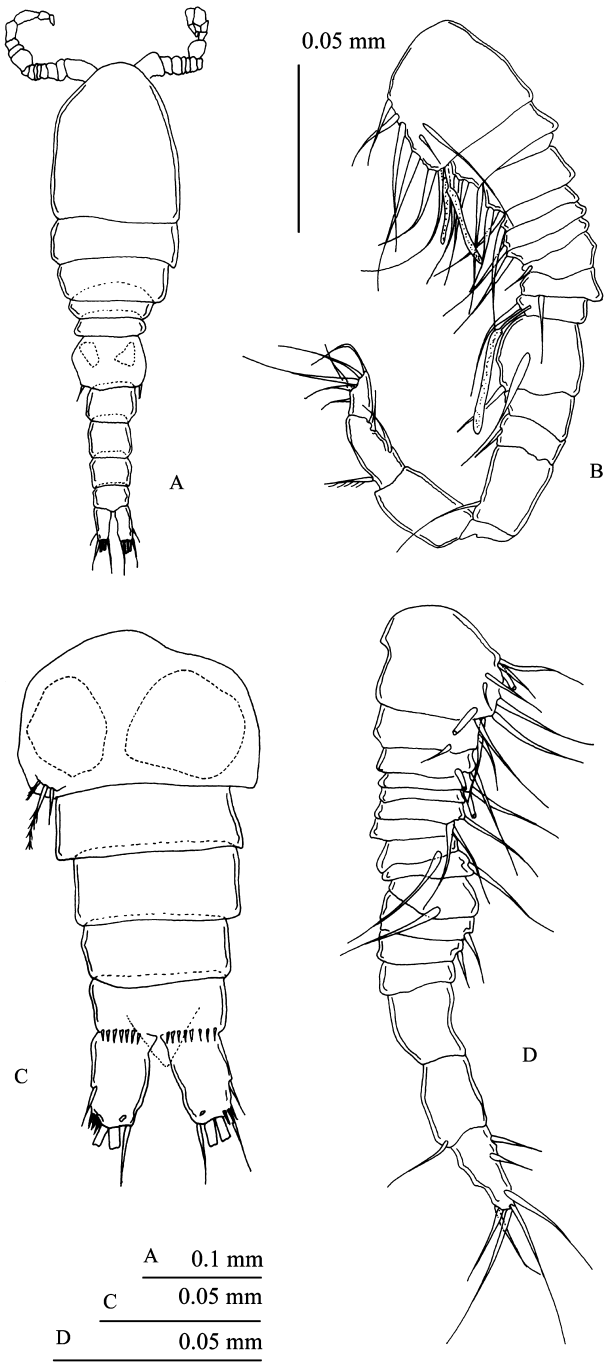

Fig. 4. *Acanthocyclops milotai* n. sp., male: A, habitus, dorsal; B, antennule; *Acanthocyclops propinquus* (Pleşa, 1957), male: C, urosome, ventral; D, antennule.

times in paratypes, n = 6) and less divergent than in female. Ornamentation as in female, with a row of large spinules at the base of the externalmost terminal furcal seta.

Rostrum, antenna, mandible, maxillule, maxilla, and maxilliped as in female. Antenna with the same ornamentation pattern on the basis.

Antennule (fig. 4B) 16-segmented; with surface of articles smooth. Setation from proximal to distal segments (number of setae in parentheses): I (8+2ae), II (3), III (2), IV (2), V (2), VI (2), VII (2), VIII (1), IX (1+1ae), X (2), XI (1), XII (1 sp), XIII (1), XIV (0), XV (3), XVI (6).

No sexual dimorphism observed in segmentation pattern of legs 1-4: 3.2/3.2/3.3/3.3. Spines and setal armature of last segments of exopods similar to that of female: 2.3.3.3 and 4.4.4.4, respectively. Leg 4 coxa with the same spinule ornamentation as in female.

Leg 5, 2-segmented, proximal segment well defined at base, bearing a relatively long outer seta; exopod almost square (about 1.6 times as long as broad), distinctly articulating with the proximal segment, bearing one terminal seta 6 times as long as the apical spine.

Leg 6, inserted in the posterior part of the second urosomal somite, in ventro-lateral position, armed with one outer spine and two setae, the inner seta shorter than the medial one.

DISCUSSION

Taxonomic remarks on *Acanthocyclops milotai* n. sp.

The new species shows close affinities with a few other species of the *kieferi* complex (e.g., *A. propinquus*, *A. radevi*, and *A. iskrecensis*) in the tendency to oligomerization of the swimming legs, but differs in many other characters and micro-characters. We have made, for the first time, a thorough description of the mouthparts, which we consider important in the taxonomy of the complex, although for the other species the mouthparts have been incompletely described and, therefore, no comparison can be made at present.

The species closest to *A. milotai* n. sp. is *A. propinquus*, but that one can clearly be distinguished by the chaetotaxy of the female antennules, lacking the aesthetasc on segment 8 in *A. propinquus*; the ornamentation on the antennary basis, absent in *A. propinquus*; the number of setae on the second endopodal segment of the antenna, with 7 setae (5 in *A. propinquus*); the absence of the ornamentation on the coxa of leg 4; and the triangular shape of the anal operculum, without any ornamentation (fig. 4C). In the males, *A. propinquus* differs by the absence of aesthetascs on the antennules (fig. 4D).

A. milotai n. sp. differs from *A. radevi* by the chaetotaxy of the female antennules, with an aesthetasc present on the 8th segment; the presence of the ornamentation pattern on the antennary basis; the ornamentation of the coxa of leg 4; the ornamentation of the surface of the anal operculum; and finally by the short length of the internalmost furcal setae, 2.2 times as long as the externalmost ones (6 times as long in *A. radevi*) (cf. Pandourski, 1993).

Despite the similar oligomerization of the swimming legs, the shape of the seminal receptacle, with two protuberances on its anterior and posterior part, and the length of the externalmost furcal seta, shorter than the internalmost one, distinctly distinguish *A. iskrecensis* from the new species described here.

Micro-characters appear variable in the new species, such as the spinule ornamentation on the exopods and endopods of P1-P4, the number of spines on the coxa of P4, and the shape of the anal operculum (figs. 5, 6). A brief summary of this variation is given here. Most female and male specimens of *A. milotai* n. sp. have spinulated distal margins on the endopodal and exopodal segments of P1-P4 (fig. 3) but in some cases the ornamentation is lacking. The ornamentation pattern of the P4 coxa, i.e., the number of spinules, varies between populations. Thus, eight spinules have been observed in specimens from Buhui Cave; six spinules in Boilor Cave; four spinules in specimens from Oase Cave; and two spinules in

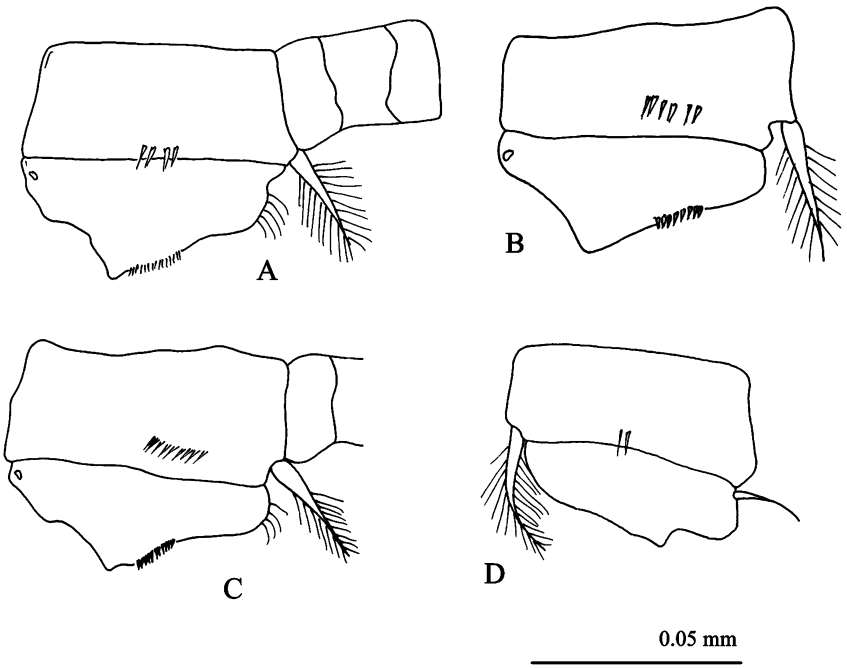

Fig. 5. Variability of the P4 coxa in various populations of *Acanthocyclops milotai* n. sp.: A, Oase Cave; B, Boilor Cave; C, Buhui Cave; D, Padina Popii Cave.

specimens from Padina Popii Cave (fig. 5). No sexual dimorphism has been noted, the male specimens from the Oase Cave showing the same ornamentation pattern as the females.

The micro-characters, such as the spinule pattern on the antennary basis, or the ornamentation of the intercoxal sclerites, the coxa, and the basis of the swimming legs, have not been examined with sufficient precision throughout the stygobitic species of *Acanthocyclops*. Moreover, accurate descriptions and illustrations of the mouthparts of the species of the genus are often missing. These characters have proven their taxonomic interest in the case of *A. milotai* n. sp. They constitute valuable specific characters and need to be investigated in all the species of the group. Fiers & Van de Velde (1984) pointed out that the distinct patterns of armature on the basis of numerous species in the Cyclopinae are fundamental characters for species identification. They stated that the primitive condition of these appendages is probably a remnant feature of the ancestor, which would have had the whole body covered with spines or teeth. Many epigean *Acanthocyclops* species show different spinulation patterns on the antennal basis as well as on the intercoxal sclerites, and the coxa and basis of the swimming legs (e.g., *Acanthocyclops robustus*, *A. vernalis*) (cf. Mirabdullayev & Defaye, 2004). Therefore, this suggests that at least *A. milotai* n. sp. has an ancestor that was highly spinulated, and which had subsequently lost the ornamentation of its appendages and body in its adaptation to groundwater habitats.

The shape of the anal operculum, considered a species-specific character, should be treated with caution, because it appears to be variable in *A. milotai* n. sp. This shape varies from rounded, to triangular and protruding. It has been found to be constant within a population, but variable between the populations investigated. Thus, a rounded shape adorned with a ventral row of small spinules was observed in *A. milotai* n. sp. from Oase Cave, Buhui Cave, Socolovăţ Cave, and Găurile lui Miloi II Cave; crenulated with a row of spines in the specimens from Boilor Cave; and triangular with ornamentation in specimens from Padina Popii Cave (fig. 6). We must mention that large numbers of female and male specimens need further examination in order to confirm the variability of this character at the inter-populational level. No sexual dimorphism has been observed in the ornamentation of the anal operculum of *A. milotai* n. sp. from Oase Cave, the male specimens investigated showing a pattern similar to that of the females.

The differences observed in the ornamentation pattern of the segments of P1-P4, in the ornamentation of the P4 coxa, and in the shape of the anal operculum, express different degrees of morphological diversification in isolated populations of *A. milotai* n. sp. It is very probable that these local populations recorded from caves, situated in the same karst area but in different hydrographical basins, and separated by gaps of tens or even hundred kilometres, have evolved

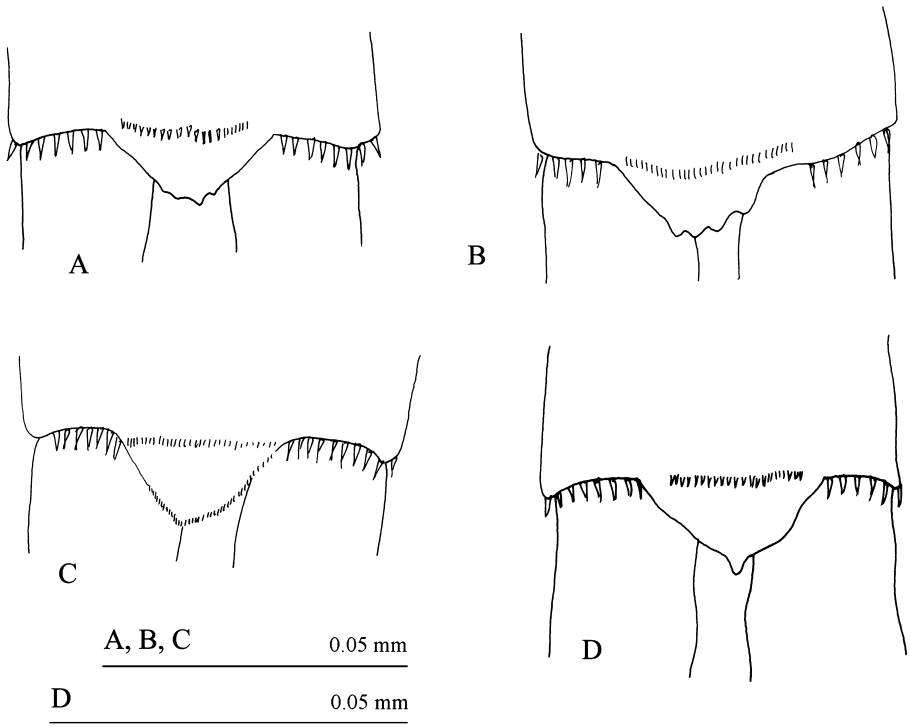

Fig. 6. Variability of the anal operculum shape in various populations of *Acanthocyclops milotai* n. sp.: A, Oase Cave; B, Boilor Cave; C, Buhui Cave; D, Padina Popii Cave.

independently as a result of isolation. The differences observed could increase in time and, together with other characters and micro-characters, enable these forms to genetically diverge and eventually evolve into separate species.

The new species, *Acanthocyclops milotai* n. sp. is assigned to the species-group *kieferi* according to the group characters given by Pandourski (1997): antennules 11-segmented, segmentation pattern of the swimming legs 3.2/3.2/3.3/3.3; spine and setal formula of exopods of P1-P4: 2.3.3.3 and 4.4.4.4; inner surface of furcal rami smooth. The other specific characters are the absence of the exopod on the antenna; the absence of ornamentation on the intercoxal sclerites, and the ornamentation pattern on the coxa of leg 4. In addition to the characters already established to define the *kieferi*-group, three other characters would need to be precised: the presence of an aesthetasc on segment 8 of the female antennule; the mandibular palp with 2 or 3 setae; and the shape of the seminal receptacle. The aesthetasc on segment 8 of the female antennule is present in *A. kieferi*, *A. petkovski*, *A. balcanicus*, *A. biarticulatus* (cf. I. Mirabdullayev, pers. comm.), *A. iskrecensis*, *A. stygius deminutus*, *A. reductus*, and *A. milotai* n. sp.; absent in *A. propinquus* and not reported from *A. radevi*. The mandibular palp has 2 setae in

A. propinquus, *A. milotai* n. sp., and *A. reductus* but this character is rarely reported in the literature for the other species. One character has to be observed as being species-specific, i.e., the shape of the seminal receptacle, which is of taxonomic importance in the Cyclopidae (cf. Defaye et al., 2003). *A. milotai* n. sp. exhibits a symmetrical seminal receptacle, the anterior and posterior lobes with the distal edge rounded; the same shape is present in *A. kieferi*, *A. propinquus*, *A. reductus*, *A. petkovskii*, and *A. balcanicus*, whereas in *A. biarticulatus*, *A. hispanicus*, and *A. radevi*, the anterior lobe is larger; and in *A. iskrecensis* the anterior lobe is expanded laterally into two small horns.

Biogeographic remarks on *Acanthocyclops milotai* n. sp.

The habitat of the new species is similar to that of the other species of the *kieferi*-group, exclusively living in groundwater, mostly in caves (Pandourski, 1994, 1997; Iepure, 2001; Galassi & De Laurentiis, 2004). From a biogeographic point of view, *A. milotai* n. sp. has to be considered an endemic species, currently reported from caves in the Banat karst area (fig. 1). The new species was found in the south-west of Romania, while the known distribution of the *kieferi* species-complex is in the north-western part. In the Romanian groundwater, eight taxa from this complex are currently known: *A. kieferi*, *A. balcanicus bisaetosus*, *A. plesai*, *A. reductus*, *A. propinquus*, *A. stygius deminutus*, and *A. stygius stygius* in the north-western part; and *A. kieferi* and *A. milotai* n. sp. in the south-west (Pleşa, 1957, 1961, 1985; Iepure, 2001) (fig. 1). This newly described species of the *kieferi* complex, added to the already known data, makes Romania the first country in south-eastern Europe for the biodiversity of this group, Bulgaria being the second, with six species (Pandourski, 1994). We can hypothesize that all the species of the *kieferi*-group have differentiated from a common ancestor, when the geological events of the Miocene had created those isolated basins in subterranean waters.

Adaptive traits towards a groundwater habitat in the *kieferi* species-complex

Despite of the fact that the phenotype tends to be conservative in cyclopine copepods, several particular morphological adaptations are shown in species inhabiting subterranean habitats (Reid & Strayer, 1994; Galassi, 2001). Generally, the *kieferi* species-complex shows significant differences in body size (0.35-0.8 mm). However, one should note that most of the species of this complex reported from cave habitats (pools, percolation water, and interstitial habitats of subterranean rivers) are smaller-bodied than those found in interstitial habitats of the surface rivers. This is the case in *A. kieferi*, which is a relatively large-bodied species within the complex (body length ranging between 0.6 and 0.84 mm) (Pleşa, 1969). *A. kieferi* is widespread throughout the whole of Europe and

was reported, besides from cave habitats (pools and the interstitial of subterranean rivers), in interstitial spaces formed by the alluvial sediments of surface rivers (Lescher Moutoué, 1973; Pesce & Galassi, 1985; Pandourski, 1994; Moldovan et al., 2000). *A. milotai* n. sp. displays a small body length, ranging between 0.41 and 0.59 mm (females), the males being even smaller, with 0.376 mm.

Oligomerization of the appendages is a common feature in species that inhabit subterranean habitats. By its reduced segmentation of the antennules, *A. milotai* n. sp. resembles several other stygobitic species of the Cyclopinae (cf. Reid & Spooner, 1998; Galassi & De Laurentiis, 2004). This is considered an apomorphic state, as are the reduction of the number of setae on the antennary 2nd endopodal segment and the absence of any intercoxal sclerite ornamentation in P1-P4. Several genera display the same armature pattern of the antennary basis, without exopodal seta, such as *Diacyclops* (partim), *Itocyclops*, *Rheocyclops*, *Stolonicyclops*, and *Zealandcyclops*. This feature is not exclusively a privilege of the stygobites, but it is much more frequently absent in species that inhabit subterranean waters. Several stygophilic taxa from the *Diacyclops languidoides*-group also lack the exopodal seta as a result of convergence, or of parallel evolution, as has been suggested by Pospisil & Stoch (1999). Another important reduction occurs in the mouthparts: the mandible with a reduced palp, bearing two short setae in *A. milotai* n. sp. and *A. propinquus*, instead of the plesiomorphic two long and one short setae. A similar feature occurs in the species of *Itocyclops* and *Zealandcyclops*, in which the mandibular palp is tiny and bears a single seta (Reid & Ishida, 2000; Karanovic, 2005), and in *Stolonicyclops*, in which the palp bears two short setae (Reid & Spooner, 1998). This feature is recognized to occur usually in benthic and interstitial cyclopoids (Reid & Spooner, 1998).

The species and subspecies of the *kieferi*-group (sensu Pandourski, 1997), with the exception of *A. kieferi* and *A. petkovskii* (all 3-segmented) show a different reduction in the segmentation pattern of the swimming legs. The highest degree of reduction is shown in *A. biarticulatus*, which displays all endopods of the swimming legs as 2-segmented (Monchenko, 1972; Mirabdullayev & Kuzmetov, 1997), and in *A. agamus*, with all exo- and endopods 2-segmented (Galassi & De Laurentiis, 2004). The oligomerization process usually refers to the non-expression of the usual segmentation: thus, the enp2 of legs 1-2 shows an outer indentation, which marks the ancestral boundary of its segments 2 and 3. This pattern is present in legs 1-2 in *A. milotai* n. sp. and in leg 1 in *A. propinquus*, in both males and females, with no sexual dimorphism. The same pattern was stated for other species of the complex (*A. balcanicus bisaetosus*, *A. agamus*) and of the genus (*A. rhenanus* Kiefer, 1936) as well as in *Rheocyclops virginianus* (Reid, 1993), and has been interpreted as an unstable process that occurs late during the last copepodid moult (Reid, 1993; Galassi & De Laurentiis, 2004).

The *kieferi* species-complex displays a broad genital double somite, almost 2 times as broad as long, a feature also present in the newly described species, *A. milotai* n. sp. This characteristic is known for other *Diacyclops* species and was interpreted by Reid & Strayer (1994) as being related with the preferential reduction of the endopodites of the swimming legs, which allows the genital double somite to remain relatively large.

All features described above, usually occur in groundwater cyclopoids and are associated with the regressive adaptive traits of organisms adapting to subterranean habitats (Reid & Strayer, 1994; Reid & Spooner, 1998; Monchenko & Von Vaupel Klein, 1999; Galassi & De Laurentiis, 2004). Furthermore, the oligomerization process leads to new combinations of morphological structures, which are further tested by the species in order to favour the best adaptation to the conditions of subterranean life (Monchenko & Von Vaupel Klein, 1999).

ACKNOWLEDGEMENTS

This work was financially supported by the European Community Programme "Structuring the European Research Area" under SYNTHESIS at the Muséum national d'Histoire naturelle, Paris (MNHN, TAF no. 1250/2005). The work was financed also by grants No. 446/2003 (NURC Romania) and No. 76/2004 (Romanian Academy). We would like to thanks the two reviewers for their helpful comments on earlier drafts, and Shonil Bhagwat for checking the English of the final version of the manuscript.

REFERENCES

ALEKSEEV, V. R., 1998. Key to freshwater Cyclopidae of Russia and adjacent lands (Crustacea). Zoosystem. Rossia, **7**: 25-43.

APOSTOLOV, A. & I. PANDOURSKI, 2004. Crustacés des eaux souterraines karstiques des montagnes de Vitocha et de Golo Bardo (Bulgarie occidentale): composition taxonomique, caractéristique générale et répartition spatiale dans le karst. Hist. nat. Bulgariense, **16**: 47-57.

BJÖRKMAN, L., A. FEURDEAN & B. WOHLFARTH, 2002. Late-glacial and Holocene forest dynamics at Steregoiu in the Gutaiului Mountains, northwest Romania. Rev. Paleobiol. Palynol., **124**: 79-111.

BOXSHALL, G. A., D. EVSTIGNEEVA & P. F. CLARK, 1993. A new interstitial cyclopoid copepod from a sandy beach on the western shore of Lake Baikal, Siberia. Hydrobiologia, **268**: 99-107.

CHAPPUIS, P. A., 1924. Descriptions préliminaires de Copépodes nouveaux de Serbie. Bull. Soc. Sci. Cluj, **1-2**: 27-45.

— —, 1925. Sur les Copépodes et les Syncarides des eaux souterraines de Cluj et des monts Bihar. Bull. Soc. Sci. Cluj, **2**: 157-182.

DAMIAN-GEORGESCU, A., 1963. Copepoda. Fam. Cyclopidae (forme de apă dulce). Fauna Republicii Populare Romîne, **4**: 1-205. [Copepoda. Fam. Cyclopidae (freshwater species)], (Ed. Acad. R.P.R., Bucharest). [In Romanian.]

DANIELOPOL, D. L. & P. POSPISIL, 2002. Taxonomic diversity of Crustacea Cyclopoida in the Austrian "Danube floodplain" National Park. Vie et Milieu, **52**: 67-75.

DEFAYE, D., C. CUOC & R. M. BARTHÉLÉMY, 2003. A new interpretation of the female genitalia in *Macrocyclops albidus* (Copepoda, Cyclopidae). Acta Zool., Stockholm, **84**: 25-31.

DUSSART, B. H., 1969. Les Copépodes des eaux continentales d'Europe occidentale, **2**, Cyclopoïdes: 1-236. (Edition Boubée, Paris).

DUSSART, B. H. & D. DEFAYE, 2006. World directory of Crustacea Copepoda of inland waters, 2. Cyclopiformes: 1-354. (Backhuys Publishers, Leiden).

FIERS, F. & I. VAN DE VELDE, 1984. Morphology of the antenna and its importance in the systematics of the Cyclopidae. Crustaceana, **7**: 182-199.

GALASSI, D. M. P., 2001. Groundwater copepods: diversity patterns over ecological and evolutionary scales. Hydrobiologia, **453/454**: 227-253.

GALASSI, D. M. P. & P. DE LAURENTIIS, 2004. Little-known cyclopoids from groundwater in Italy: re-validation of *Acanthocyclops agamus* and redescription of *Speocyclops italicus* (Crustacea, Copepoda, Cyclopoida). Vie et Milieu, **54**: 203-222.

HUYS, R. & G. A. BOXSHALL, 1991. Copepod evolution: 1-468. (The Ray Society, London).

IEPURE, S., 2001. Cyclopides des monts Apuseni. I. *Acanthocyclops plesai* n. sp. et *Acanthocyclops balcanicus bisaetosus* n. ssp. (Crustacea, Copepoda). Studia Univ. Babes Bolyai, (Biol.) **2**: 31-41.

KARANOVIC, T., 2005. Two new genera and three new species of subterranean cyclopoids (Crustacea, Copepoda) from New Zealand, with redescription of *Goniocyclops silvestris* Harding, 1985. Contrib. Zool., Amsterdam, **74** (3/4): 223-254.

KIEFER, F., 1927. Versuch eines Systems der Cyclopiden. Zool. Anz., **73**: 302-308.

LESCHER-MOUTOUÉ, F., 1973. Sur la biologie et l'écologie des Copépodes cyclopides hypogés (Crustacés). Ann. spéléol., **28** (3): 429-502; (4): 581-674.

MAZEPOVA, G. F., 1978. Tsiklops ozera Baikal. Trudy limnol. Inst. Akad. Nauk SSSR, (Sibirskoe Otdel.) **28** (49): 1-144.

MIRABDULLAYEV, I. M. & D. DEFAYE, 2004. On the taxonomy of the *Acanthocyclops robustus* species-complex (Copepoda, Cyclopidae): *Acanthocyclops brevispinosus* and *A. einslei* sp. n. Vestnik. Zool., **38**: 27-37.

MIRABDULLAYEV, I. M. & A. R. KUZMETOV, 1997. Second record of *Acanthocyclops biarticulatus* Monchenko, 1972 and first description of its male (Crustacea: Copepoda). Bull. zool. Mus. Univ. Amsterdam, **16** (2): 1-14.

MOLDOVAN, O., S. IEPURE & A. FEKETE, 2000. Preliminary data concerning the groundwater fauna of the Somesul Cald basin (Transylvania, Romania). In: D. L. DANIELOPOL, C. GRIEBLER, J. GIBERT, H. P. NACHTNEBEL & J. NOTENBOOM (eds.), Groundwater ecology — A tool for management of water resources: 335-343. (Office for Official Publications of the European Communities, Luxembourg).

MONCHENKO, V. I., 1972. Tsiklopi (Copepoda, Cyclopidae) grountovykh vod pustyni Kyzylkum. [Subterranean water *Cyclops* (Copepoda, Cyclopidae) from Kisilkum]. Trudy zool. Inst., **51**: 78-97. [In Russian, with English summary.]

— —, 1974. Gnathostome cyclopoids, cyclopids (Cyclopidae). Fauna Ukraini, **27**: 1-449. (Naukova Dumka, Kiev).

— —, 1982. On two sympatric Black Sea estuarian cyclops of the genus *Diacyclops* (Crustacea, Copepoda). Zool. Zh., Moscow, **6**: 182-189.

MONCHENKO, V. I & J. C. VON VAUPEL KLEIN, 1999. Oligomerization in Copepoda Cyclopoida as a kind of orthogenetic evolution in the animal kingdom. Crustaceana, **72**: 241-264.

NAIDENOW, T. W. & I. PANDOURSKI, 1992. Zwei neue Höhlencyclopiden (Copepoda, Cyclopoida) aus den Grundgewässern Bulgariens. Fragm. Balcanica Mus. Macedonici Sci. nat., **15**: 7-14.

PANDOURSKI, I., 1992. *Acanthocyclops* (*Acanthocyclops*) *iskrecensis* sp. n. (Copepoda, Cyclopoida) des eaux souterraines de la Stara Planina d'ouest (Bulgarie). Bull. Mus. reg. Sci. nat., Torino, **10**: 401-405.

— —, 1993. Cyclopides des eaux souterraines de la Bulgarie. I. *Acanthocyclops* (*Acanthocyclops*) *radevi* sp. n. et *Diacyclops pelagonicus saetosus* ssp. n. (Crustacea, Copepoda). Mém. Biospéol., **20**: 165-168.

— —, 1994. Cyclopides (Crustacea, Copepoda) des eaux souterraines de la Bulgarie. III. Répartition des cyclopides des sous-familles Eucyclopinae et Cyclopinae et remarques sur la morphologie de quelques espèces rares. Hidrobiologiya, Sofia, **39**: 3-16.

— —, 1997. Composition, origine et formation de la faune cyclopidienne stygobie de Bulgarie et définition du groupe "*kieferi*" du genre *Acanthocyclops* (Crustacea, Copepoda, Cyclopoida). Bull. Mus. reg. Sci. nat., Torino, **15**: 279-197.

PESCE, L. G. & D. P. M. GALASSI, 1984. *Acanthocyclops kieferi kieferi* (Chappuis) di acque freatiche della Toscana: ciclopide nuovo per la fauna italiana (Crustacea Copepoda: Cyclopinae). Riv. Idrobiol., **23**: 159-164.

— — & — —, 1985. Due nuovi *Diacyclops* del complesso '*languidoides*' (Copepoda: Cyclopidae) di acque sotteranee di Sardegna e considerationi sul significato evolutivo dell'antenna nei copepodi stigobionti. Boll. Mus. civ. St. nat. Verona, **12**: 411-418.

PLEŞA, C., 1957. Eine neue Hohlencyclopidenform (*Acanthocyclops reductus* var. *propinqus* n. var.) aus Rumanien. Zool. Anz., **159**: 128-130.

— —, 1969. Cercetări asupra periodicităţii reproductive la unele crustacee cavernicole troglobionte: 1-180. [Research on reproductive periodicity of some cave crustaceans.] (Ph.D. Thesis, Bucharest). [In Romanian.]

— —, 1961. Redescription of the subterranean freshwater cyclopoid *Acanthocyclops reductus propinquus* Pleşa. Crustaceana, **3**: 47-55.

— —, 1985. Conspectul sistematic al cyclopidelor (crustacee, copepode) cunoscute până în prezent din R.P.R. Studia Univ. Babes Bolyai, (Biol.) **3**: 137-150. [In Romanian.]

PLEŞA, C., M. PINTEA-ALB, M. SĂLĂJAN & F. WILD, 1965. Date noi asupra ecologiei şi răspândirii geografice a ciclopidelor (Crustacea, Copepoda) în România. Lucr. Inst. Speol. «Emil Racoviţă», **4**: 141-154. [In Romanian.]

PLEŞA, C. & G. RACOVIŢĂ, 1973. Recherches sur les gours, en tant que milieux aquatiques souterrains. Note I. In: Livre du Cinquantenaire Inst. Spéol.: 487-502.

POSPISIL, P. & F. STOCH, 1999. Two new species of the *Diacyclops languidoides*-group (Copepoda, Cyclopoida) from groundwaters of Austria. Hydrobiologia, **412**: 165-176.

REID, J. W., 1993. *Diacyclops virginianus*, a new species of Cyclopoida (Crustacea, Copepoda) from Goose Creek, Virginia. The Maryland Naturalist, **37**: 36-45.

REID, J. W. & T. ISHIDA, 2000. *Itocyclops*, a new genus proposed for *Speocyclops yezoensis* (Copepoda: Cyclopoida: Cyclopidae). Journ. Crust. Biol., **20**: 589-596.

REID, J. W. & J. D. SPOONER, 1998. *Stolonicyclops heggiensis*, new genus, new species, from Georgia, U.S.A. (Copepoda: Cyclopoida: Cyclopidae). Journ. Crust. Biol., **18**: 405-411.

REID, J. W. & D. L. STRAYER, 1994. *Diacyclops dimorphus*, a new species of copepod from Florida, with comments on morphology of interstitial cyclopine cyclopoids. Journ. North American benthol. Soc., **13**: 250-265.

REID, J. W., D. L. STRAYER, J. V. MCARTHUR, S. E. STIBBE & J. J. LEWIS, 1999. *Rheocyclops*, a new genus of copepods from the southeastern and central U.S.A. (Copepoda: Cyclopoida: Cyclopidae). Journ. Crust. Biol., **19**: 384-396.

RYLOV, V. M., 1948. Rakoobraznye. Cyclopoida presnykh vod: 1-314. Fauna SSSR. (Academy of Sciences, U.S.S.R., Zoological Institute, Leningrad). [English translation.]

STOCH, F., 1995. The ecological and historical determinants of crustacean diversity in groundwaters, or: why are there so many species? Mém. Biospéol., **22**: 139-160.

— —, 1999. The new species of the *Diacyclops languidoides*-group (Copepoda, Cyclopoida) from groundwaters of Austria. Hydrobiologia, **412**: 165-176.

STOCH, F. & P. POSPISIL, 2000. The *Diacyclops languidoides*-group (Copepoda: Cyclopoida) in Austria, with redescription of *Diacyclops cohabitatus* Monchenko, 1980. Annls. Limnol., **36**: 21-29.

YEATMAN, H. C., 1959. Free-living Copepoda: Cyclopoida. In: W. T. EDMONDSON (ed.), Ward & Whipple's freshwater biology, **2**: 795-815. (Wiley, New York).

First received 10 July 2007.
Final version accepted 15 October 2007.