ANNALES ZOOLOGICI (Warszawa), 2011, 61(2): 427-438

A NEW *ACANTHOCYCLOPS* KIEFER, 1927 (COPEPODA: CYCLOPIDAE) FROM CAVES IN APUSENI MOUNTAINS (NORTH-WESTERN ROMANIA)

SANDA IEPURE[1] and ANDREEA OARGA[2]

[1]*Institutul de Speologie "Emil Racoviţă", Cluj Department, Clinicilor 400006, Cluj, Romania, e-mail: sanda.iepure@iser-cluj.org*
[2]*University of Nova Gorica, Vipavska 13, SI-5000 Nova Gorica, e-mail: dora_oarga@yahoo.com*

Abstract.— A new stygobite species *Acanthocyclops transylvanicus* **sp. nov.** is described from the north-western Romanian Carpathians (Apuseni Mountains) and is accommodated in the subterranean kieferi-group. *Acanthocyclops transylvanicus* **sp. nov.** resembles closely *A. biarticulatus* Monchenko, 1972 in the segmentation pattern of the swimming legs (3.2/3.2/3.2/3.2), but differs by the following characters: antennary second endopodite segment with 7 setae; distal articles of endopodites of P1 and P3 with 4 and 5 setae, respectively; absence of coxopodite ornamentation pattern in leg 4; leg 4 endopodite with 3-segmented appearance, but lacking a functional articulation between the second and third segment. We assume that the 2-segmented nature of the leg 4 endopodite as observed in both species is a convergent acquisition. The adult shape of the leg 4 endopodite in *A. transylvanicus* **sp. nov.** results from the simple suppression of an arthrodial membrane formation. *A. transylvanicus* is the ninth representative known in Romania which is accommodated in the *kieferi*-group. An identification key for the species of *kieferi*-group known to occur in Romania is given.

Key words.— Copepoda, Cyclopidae, *Acanthocyclops*, taxonomy, new species, caves, Romania.

INTRODUCTION

Since the initial exploration of the groundwater realm in Romania at the beginning of the former century a considerable number of groundwater cyclopids have been discovered and described (Chappuis, 1924, 1925; Botoşăneanu and Damian, 1955; Pleşa, 1956, 1957; Damian-Georgescu, 1963; Pleşa *et al.*, 1965; Pleşa, 1969; Pleşa *et al.*, 1985; Iepure, 2001; Iepure, 2007a, b; Iepure and Defaye, 2008). The Apuseni Mountains (also known as the Western Carpathians, Fig. 1) located in north-western part of Romania, is the most intensively investigated karst area so far, and became known as a hot-spot in groundwater biodiversity (Botoşăneanu, 1985). This mountain unit consists of several well-defined Mesozoic age limestone and dolomite massifs (e.g., Bihor and Pădurea Craiului) showing highly diversified karst landscape (Onac and Constantin, 2004).

Thus far, twenty six species and subspecies of cyclopines are known to occur in the Apuseni and the associated mountains (Iepure, 2007a). Among them, five species appear to be endemic for Romania and are exclusively known from subterranean habitats (Iepure, 2007). The genus *Acanthocyclops* Kiefer, 1927 appears to be the most diversified (Damian-Georgescu, 1963; Pleşa, 1985; Iepure, 2007) with five representatives currently assembled in the *kieferi*-group:

PL ISSN 0003-4541 © Fundacja *Natura optima dux*
doi: 10.3161/000345411X584889

A. deminutus (Chappuis, 1925), *A. kieferi* (Chappuis, 1925), *A. propinquus* Pleşa, 1967, *A. plesai* Iepure, 2001 and *A. balcanicus bisaetosus* Iepure, 2001 (Chappuis, 1925; Damian-Georgescu, 1963; Iepure, 2001) (Table 2; Fig. 1A).

Recent surveys of the fauna thriving in percolation water in several caves located in the Apuseni Mountains revealed the presence of several new copepod species (harpacticoids and cyclopids) (Meleg *et al.*, in press). The present contribution deals with the description of a new subterranean cyclopid of the *kieferi*-group in the genus *Acanthocyclops*. This new addition to the Romanian copepod fauna is discussed in terms of the diversity and distributional patterns of the *kieferi*-species group.

MATERIAL AND METHODS

The specimens were collected from the water percolating the vadose zone in Ungurului Cave (44°50’44”N; 22°24’38”E), Pădurea Craiului Mountains (north-western part of the Apuseni Mountains, north-western Romania) developed in Ladinian (middle Triassic) limestones (Orăşeanu 1991) (Fig. 1A). The sampling site was located at about 300 m from the cave entrance (Oarga 2006, 2008). Dripping water, directed through a funnel, was collected into a plastic container and subsequently filtered through a 100 μm mesh net. The sampling of percolating water was performed continuously (see Pipan 2005) during November 2005, May 2006 (Oarga 2006, 2008) and July 2008 (Meleg *et al.* in press). Collected specimens were preserved in 70% ethanol for long term storage. Additional material from dripping water and pools in four other caves have been used for comparison, two from Pădurea Craiului Mountains: Ciur Izbuc Cave (46°43’86’’N; 22°43’84’’E) (lower Cretaceous limestone) and Întorsuri Cave (46°52’94’’N, 22°34’53’’E) and two from Bihor Mountains: Coteţul Dobreştilor Cave (46°39’90’’N, 22°54’15’’E) and Poarta Alunului Cave (46°37’24’’N, 22°48’95’’E) (Fig. 1A, Table 2).

Observations on specimens, transferred from ethanol to a mixture of ethanol and glycerol, were made on a Leica DMLB® phase-contrast and ZEISS microscope. Illustrations were made using a drawing tube mounted on the former. Un-dissected specimens are preserved in 70% ethanol. Type specimens and additional material is stored in the copepod collection of ISER (Cluj, Romania). Roman and Arabic numerals used armature formulae refer to spines and setae, respectively. Abbreviations used in the text and figures are: ISER, Institute of Speleology “Emil Racoviţă”; ae, aesthetascs; exp1 – 3, first to third segment of exopodite; end1 – 3, first to third segment of endopodite; legs 1 – 6, first to sixth thoracopods; sp – spine.

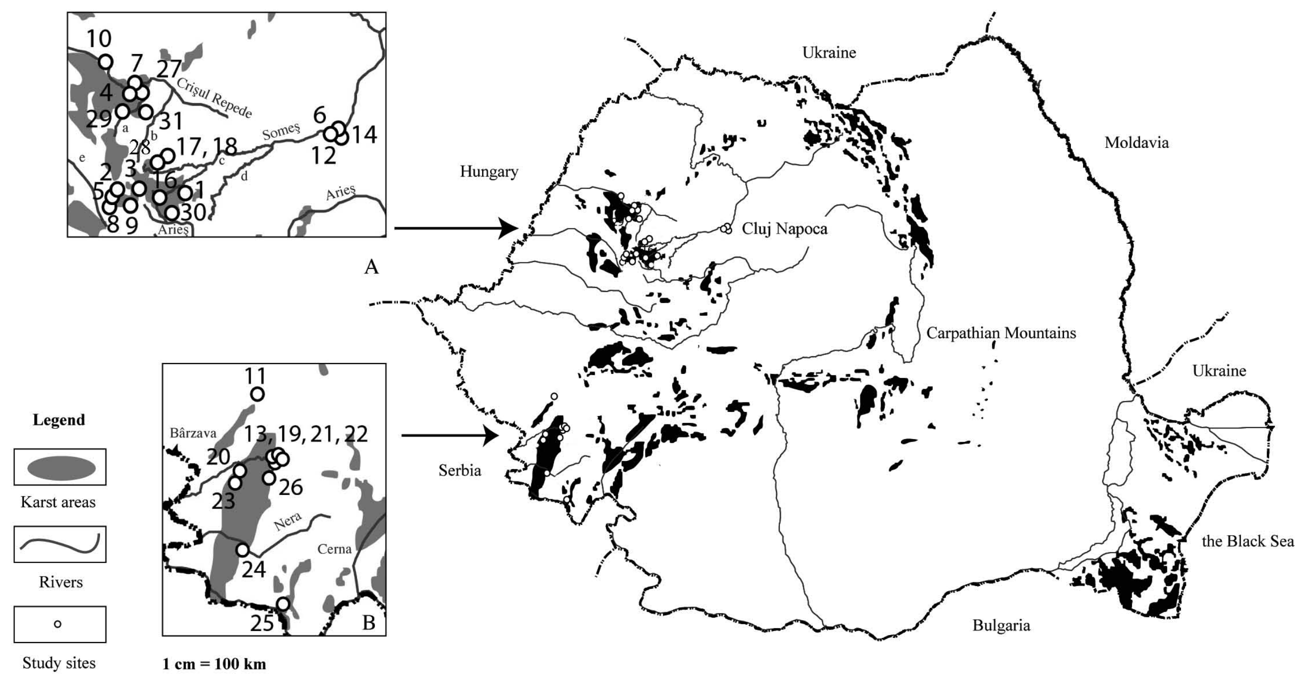

Figure 1. Distribution of the species from *Acanthocyclops kieferi*-group in groundwater habitats in Romania (see Table 2) (numbers refer to location description in Table 2; grey areas represent the limestone distribution in Romania). Letters refer to the following valleys: a – Iada Valley; b – Drăgan Valley; c – Someşul Cald River; d – Someşul Rece River; e – Crişul Alb River (Map modified after Onac & Constantin, 2003).

TAXONOMY

Acanthocyclops transylvanicus sp. nov.

Type locality. Ungurului Cave, Pădurea Craiului Mountains, Bihor County (Romania), in water percolating from the cave ceiling.

Type material. *Holotype*: a female dissected and mounted in glycerol, leg. S. Iepure and A. Oarga, 24.V.2006. *Allotype*: a male dissected and mounted in glycerol, leg. S. Iepure and A. Oarga, 24.V.2006. *Paratypes*: 1 ♂ collected from percolation water in Ungurului Cave, leg. S. Iepure and A. Oarga, 24.V.2006; 2 ♀♀ collected from percolation water in Ungurului Cave, leg. I. Meleg, 28.VII.2008; 42 ♀♀ and 10 ♂♂ collected from percolation water in Ciur Izbuc Cave, leg. I. Meleg, November 2007-March 2009; 2 ♀♀ and 1 ♂ from Întorsuri Cave, pools, leg. S. Iepure, 23.VI.2000.

Additional material examined. Bihorului Mountains (Bihor County, northwest Romania): 1 ♀, Cotețul Dobreştilor Cave, pools, leg. T. Brad, 18.III.2001; 1 ♀, Poarta Alunului Cave, pools, leg. O. Moldovan, 27.VIII.2001.

Etymology: From Transylvania, the Latin name of the region where Ungurului Cave, type-locality of the species, is located.

Description. Female. Habitus typical cyclopid shaped with a distinct constriction between prosome and urosome, and without surface ornamentation. Body length excluding caudal setae, 450 μm; cephalothorax width 116 μm (holotype) (Fig. 2A). Genital double-somite about 1.36 times wider than long. Seminal receptacle with anterior part expanded, but posterior part not (Fig. 2C). Copulatory canal spirally curved (Fig. 2C). Anterior receptacle nearly rectangular (Fig. 2C). The posterior margin of the urosomites lacking hyaline fringe. Anal somite with continuous row of spinules along the postero-ventral border (Fig. 2C); postero-dorsal margin smooth (Fig. 2D). Anal operculum crescent covering anal area but not particularly protruded (Fig. 2D).

Caudal rami (Fig. 2C, D) slightly divergent, 1.8 times longer than wide; anterolateral seta positioned in dorsal plane and accompanied with spinules at insertion; posterolateral seta as long as ramus with long spinules at insertion. Medial seta slightly longer than posterolateral one. Dorsal seta inserted near the caudal inner edge and slightly longer than rami (Fig. 2D). Terminal median setae with breaking planes (Fig. 2C, D).

Antennule (Fig. 2B) 11-segmented, not reaching posterior margin of cephalothorax. Surface of segments apparently smooth. Armament on segments I to XI: I (8), II (4), III (8), IV (4), V (1+sp), VI (2), VII (3), VIII (2+ae), IX (2), X (2+ae) XI (7+ae). Aesthetasc on segment VIII linguiform, reaching distal part of segment IX. Aesthetasc on segment X short, piliform. Aesthetasc of bithek on segment XI filiform. Antenna (Fig. 3A) typically cyclopid, lacking exopodite element. Praecoxopodite region broken. Coxopodites with 2 abexopodal setae, integument smooth. First to last endopodite segment with 1, 7, 7 setae, respectively. Mandible (Fig. 3B) with tiny palp bearing two short setae. Mandibular gnathobase devoid of ornamentation. Maxillulary arthrite (Fig. 3C) with 4 medio-apical spines and 7 medial elements (one element broken). Maxilla (Fig. 3D) with praecoxopodite and coxopodite fused that form the syncoxopodite. Syncoxal proximal endopodite of the maxilla bear 2 setae, median endopodite with one, and distal endopodite with 2 setae; basipodite with well-developed claw armed with fine teeth and bearing one accessorial claw and 1 setae. Endopodite 2-segmented, first segment with two serrate setae and second segment with two setiform setae and a robust one. Maxilliped (Fig. 3E) 4-segmented, with (from proximal to distal): 2, 2, 1 and 2 setae, respectively. Frontal surface of second and third segment ornamented with slender setules.

Legs 1–3 (Fig. 4) with 3-segmented exopodites and 2-segmented endopodites. Precoxal part (not illustrated) with smooth surface; medial setae of coxopodite present in all legs; basipodite of leg 1 with medial spine as long as the first endopodite segment, ornamented at insertion with long and slender spinules. Medial edge of basipodite rounded and hairy in legs P2-P4; spine formula 2/3/3/3 and setae formula 4/4/4/4 (Table 1).

Leg 4 endopodite (Fig. 4D) with 3-segmented appearance but lacking functional articulation between ancestral second and third podomere. Distal part of leg 4 endopodite coinciding with ancestral third

Table 1. Armament of legs 1–4 in the female of *Acanthocyclops transylvanicus* n. sp.

Legs	Coxopodite	Basipodite	Exopodite	Endopodite
Leg 1	0-1	1-I	I-0; I-1; II,2,2	0-1; 1,I+1,2
Leg 2	0-1	1-0	I-0; I-1; II,I+1,3	0-1; 1,I+1,3
Leg 3	0-1	1-0	I-0; I-1; II,I+1,3	0-1; 1,I+1,3
Leg 4	0-1	1-0	I-0; I-1; II,I+1,3	0-1; 0-2; 1,II,2*

* 3-segmented appearance, articulation between segments 2 and 3 not functional.

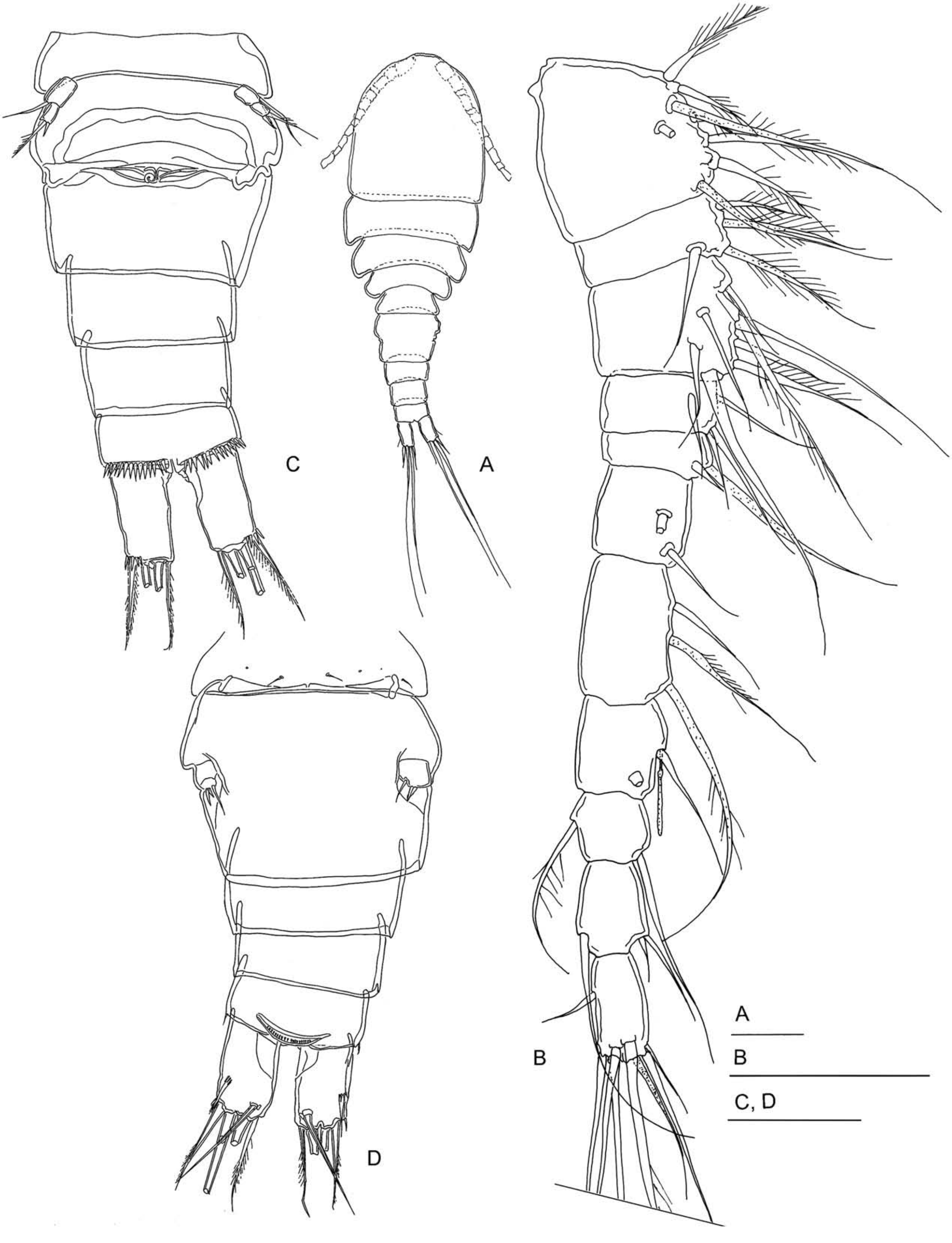

Figure 2. *Acanthocyclops transylvanicus* n. sp. (Ungurului Cave, Romania); A – habitus female (dorsal view); B – antennula (ventral view, holotype); C – urosome, female (ventral view, paratype, Ungurului Cave); D – urosome, female (dorsal view, paratype, Ungurului Cave) (A scale 100 μm; B, C, D scale 50 μm).

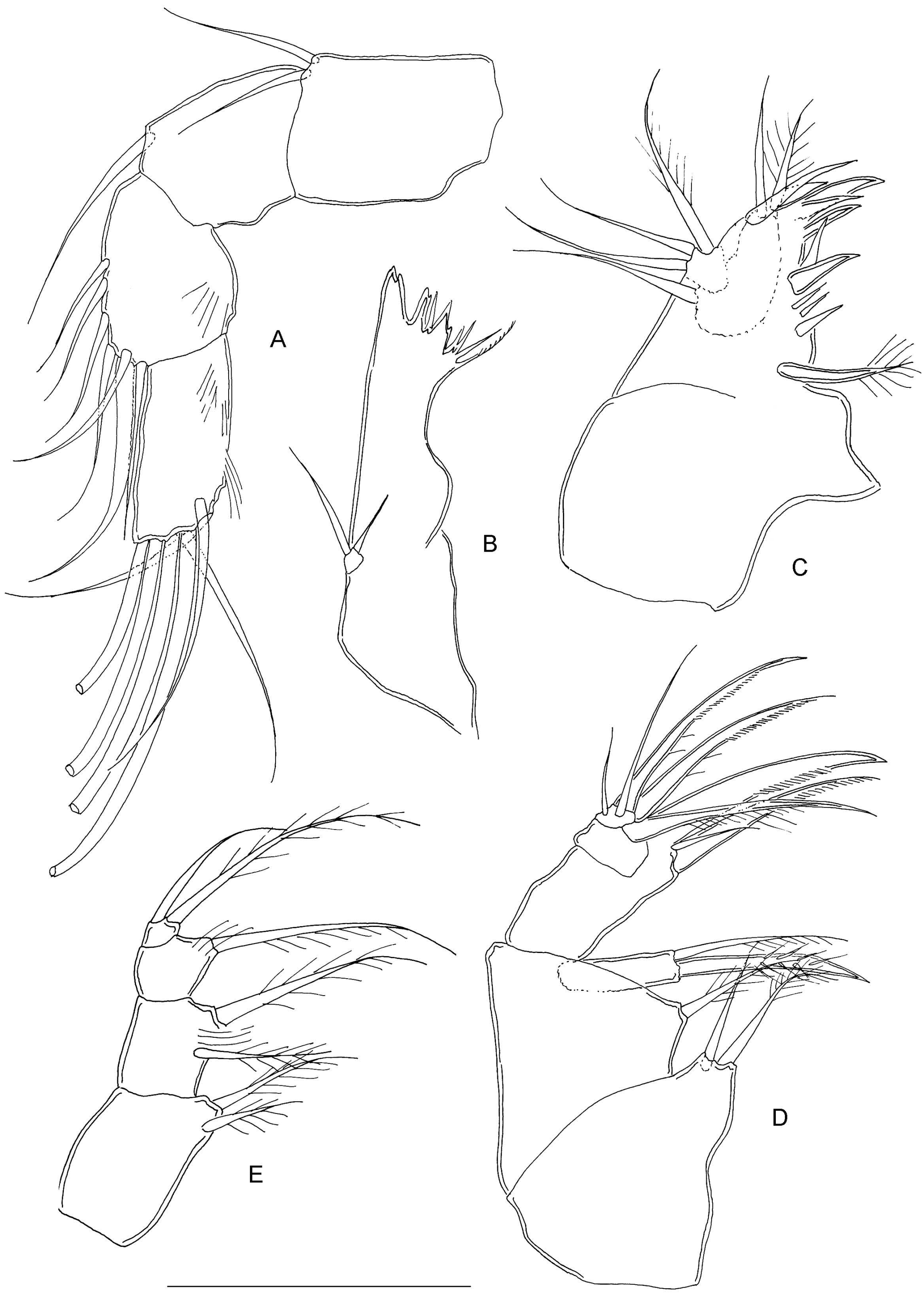

Figure 3. *Acanthocyclops transylvanicus* n. sp. (Ungurului Cave, Romania) (female holotype); A – antenna (frontal view); B – mandibula with the palp; C – maxillule (caudal view); D – maxilla (caudal view); E – maxilliped (frontal view) (scale 50 μm).

segment, 1.2 times longer than wide, bearing two terminal spines, inner apical spine 1.66 times as long as outer spine (Fig. 4D).

Leg 5 (Fig. 2C) 2-segmented, basal segment armed with outer seta; distal segment sub-quadrate, distinctly articulating on basal segment, bearing one external terminal seta 3.2 times longer than the subapical medial spine, the latter slightly shorter than the distal segment. Leg 6 armed with three elements: one medialmost long seta and two equally sized outer spines (Fig. 2D).

Male. Habitus as in the female, except for separated urosomites 2 and 3. Body length, excluding furcal setae, 400 μm, cephalothorax width 160 μm (allotype) (Fig. 5A). Posterior margin of anal somite completely set with uninterrupted row of spinules (Fig. 5B, C). Anal operculum rounded, not protruded, without ornamentation (Fig. 5B). Caudal rami and setal lengths similar as in female. Antennule 16-segmented, geniculated, with principal aesthetascs on segments I, IV, IX and XIII (not illustrated). Aesthetascs linguiform and relatively short. Cephalic appendages, legs 1–3 and leg 5 as in the female. The leg 4 endopodite with a reduced functional articulation between median and terminal segment (Fig. 5D). Ancestral third segment of leg 4, 1.06 times wider than long, the medial spine 1.7 times as long as lateral spine (Fig. 5D). Leg 6 bearing 3 elements: outer and median one setiform, inner one spiniform and rather robust (Fig. 5C).

Comments. *Acanthocyclops transylvanicus* n. sp. is attributed to *Acanthocyclops* according to the current definition of the genus: the fifth leg represented by two segments with the distal article armed with a small subapical medial spine (Einsle 1996, Dussart and Defaye 2001).

Acanthocyclops transylvanicus n. sp. shares the 11-segmented antennula with twenty six epigean and hypogean taxa currently ascribed to the genus (Einsle, 1996; Pandourski, 1997; Galassi and DeLaurentiis, 2004; Mercado, Suarez-Morales and Silva-Brano, 2006; Lee and Chang 2007; Iepure and Defaye, 2008). Fifteen obligate living subterranean taxa with this feature in common have been assembled by Pandourski (1997) in the *kieferi*-group. Three other species were discovered subsequently and assigned to this particular species group (Iepure 2001; Iepure and Defaye, 2008). *Acanthocyclops transylvanicus* n. sp. appears to be related to the *kieferi*-group by the following similarities: an 11-segmented female antennule, the shape of the genital receptacle as in *A. kieferi*, oligomerisation of the swimming legs and similar spine and seta formula of the exopodites (2.3.3.3 and 4.4.4.4, respectively). The main difference between the present species and the eight congeners present in the Apuseni Mountains is the segmentation pattern of the swimming legs in which all legs having a two-segmented endopodite (formula: 3.2/3.2/3.2/3.2). Although the leg 4 endopodite still displays the conventional form of a 3-segmented ramus and retained the spinular armament located at the transition between the former median and terminal segments, the 2-segmented nature of the ramus is obvious because of the absence of a clear articulation rim and the unaltered thickness of the integument.

Among the members of the *kieferi*-group only *A. biarticulatus* Monchenko, 1972 described from central Asia, displays a similar oligomerisation pattern (Monchenko, 1974; Mirabdullayev and Kuzmetov, 1997). However there are several significant differences between these two species. *Acanthocyclops transylvanicus* n. sp. differs from *A. biarticulatus* in the following characters: antennary second endopodal segment with 7 setae (9 in *A. biarticulatus*); distal articles of endopodites of P1 and P3 with 4 and 5 setae, respectively; (5 setae and 6 setae in *A. biarticulatus*); the leg 4 coxopodite surface without ornamentation on anterior part (ornamented with a row of spinules in *A. biarticulatus*) (according to Mirabdulayev and Kuzmetov, 1997); in *A. biarticulatus* the proximal exopodite segment of each swimming leg possesses an inner setae, while in *A. transylvanicus* n. sp. P1-P4 lack inner seta on the first exopodite (Fig. 4); and the distal segment of P5 is slightly longer than wide in *A. biarticulatus*, while in the new species the distal segment is sub-quadrate (Fig. 2C).

Since the species and subspecies of *kieferi*-group are morphologically extremely similar and their recognition requires a set of additional characters and micro-characters, our findings related to leg 4 endopodite development might be useful in their characterization. In *A. biarticulatus* the second and the third endopodites of the leg 4 are completely fused and have a compact appearance in which the original 3-segmented nature of the ramus is lost. In *A. transylvanicus* n. sp. the leg 4 endopodite retained the 3-segmented aspect but close examination clearly show that the original second and third endopodite segments are united (Fig. 4D). Consequently, it is assumed here that i) the 2-segmented nature of the leg 4 endopodite as observed in both species is convergently acquired character, and ii) while in *A. transylvanicus* the adult shape of the endopodite has resulted from a simple suppression of an arthrodial membrane formation, the shape of this ramus in the adult of *A. biarticulatus* seems to be related to the suppression of structural differentiation in the earlier developmental stages (for leg 4 in stage CIV).

Zoogeographic remarks. The diversification pattern of the subterranean *Acanthocyclops* species accommodated to *kieferi*-group has been proved to be high in the Alpine-Carpathian chain, within the Slovenian and Italian Alps (Kiefer, 1930; Kiefer, 1937; Ferreira, *et al*., 2007; Galassi *et al*., 2009), Romanian

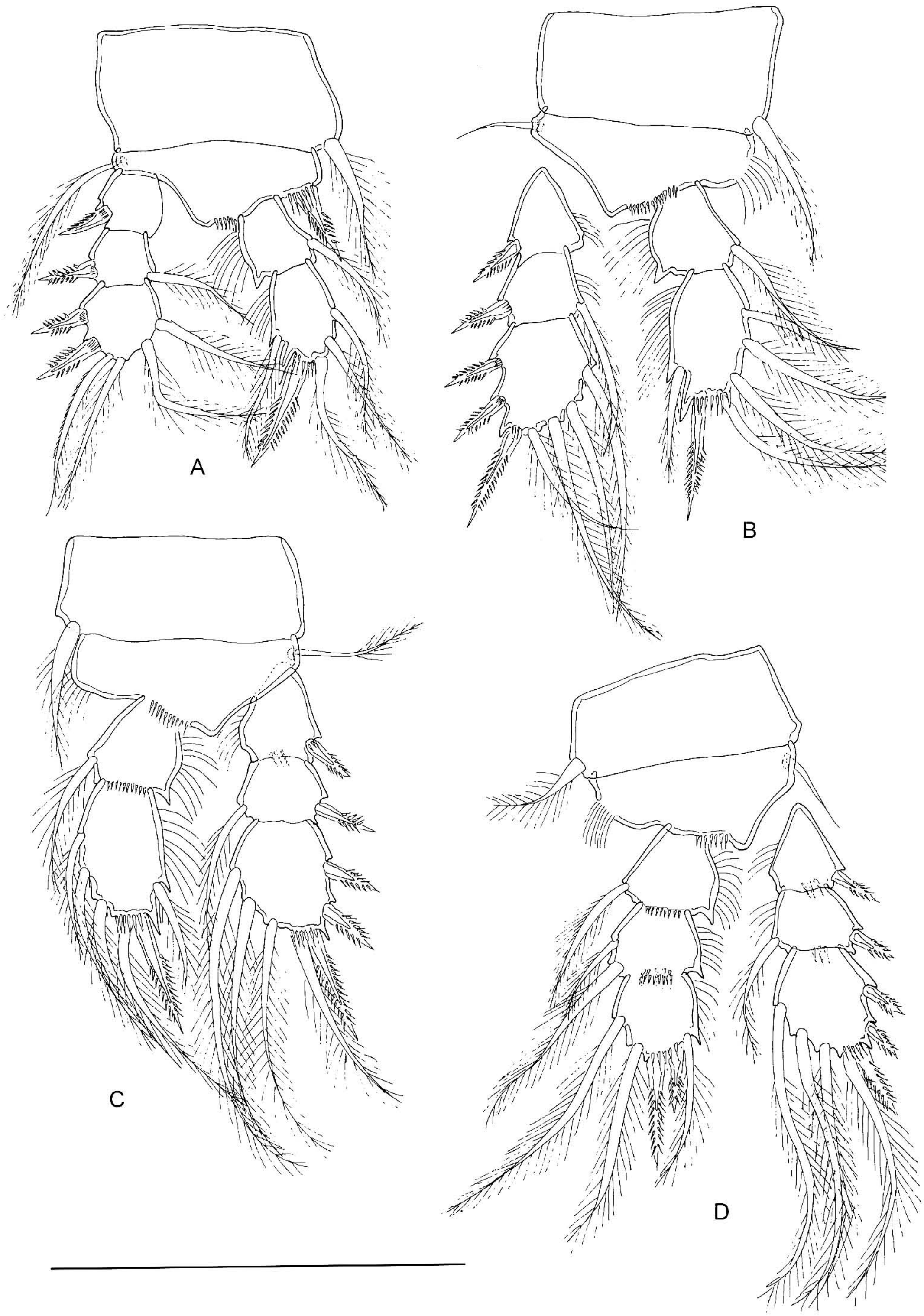

Figure 4. *Acanthocyclops transylvanicus* n. sp. (female holotype) (Ungurului Cave, Romania); A – leg 1; B – leg 2; C – leg 3; D – leg 4 (frontal view) (scale 100 μm).

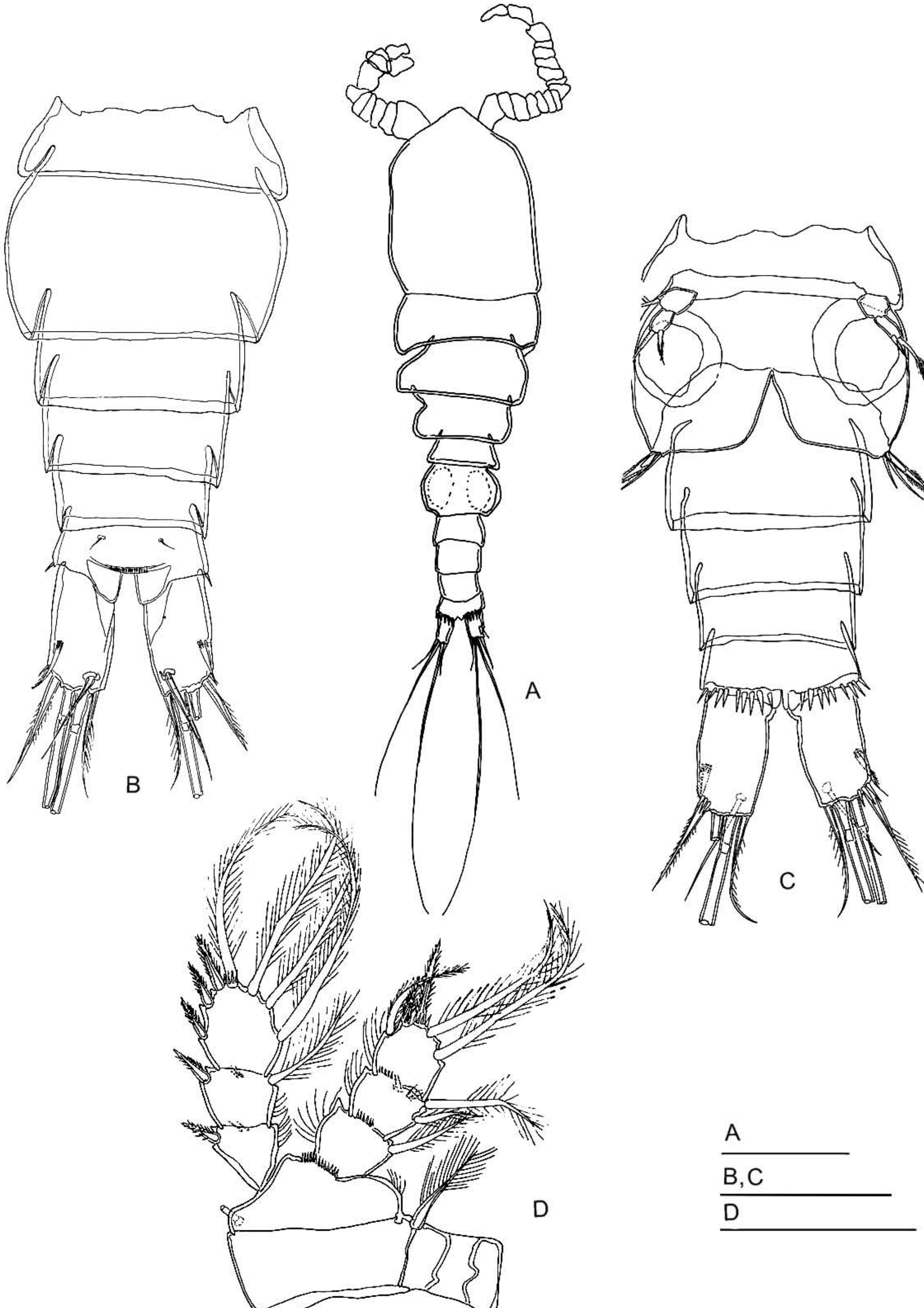

Figure 5. *Acanthocyclops transylvanicus* n. sp. (male) (Ungurului Cave, Romania); A – habitus male (ventral view); B – urosome (dorsal view); C – urosome (ventral view); D – leg 4 (frontal view) (A scale 100 μm; B – E, scale 50 μm).

Carpathians (Chappuis, 1925; Damian-Georgescu, 1963; Pleşa, 1985; Pleşa, 1969; Iepure, 2001; Iepure and Defaye, 2008) and the Balkan Mountains (or Stara Planina) in Serbia and Bulgaria (Pandourski, 1993, 1994, 1997, 2007; Pandourski and Bobič, 2008). The species from Western Europe have scattered distribution in specific sites from the Spanish and French Pyrenees (Kiefer, 1937; Stoch *et al*., 2004; Galassi *et al*., 2009). The only species found in Central Asia, *A. biarticulatus* (Monchenko, 1972, 1974; Mirabdulayev and Kuzmetov, 1997) appears to be the closest to the new species described herein.

Eastern and south-eastern Europe display so far the highest diversification of the group (Pandourski 1997, Iepure 2007, Iepure and Defaye 2008). In Romania the group has high level of radiation in northwestern part of the Carpathians, the Apuseni outcrop (Fig. 1A). *Acanthocyclops transylvanicus* is the ninth species accommodated to *kieferi*-group found in Romania, and the twelfth representant of the genus in subterranean habitats (Pleşa, 1985; Iepure, 2007). In this massif beside *Acanthocyclops transylvanicus* there are other five species belonging to the group, the distributional areas of which do not significantly overlap, but are immediately adjacent to each other. These species are found in caves and interstitial habitats of two major karst massifs of the Apuseni: Pădurea Craiului located in the north-western part of the Apuseni, and Bihor Mountains located in the south (Onac & Constantin, 2004) (Table 2; Fig. 1A).

The first karst massif, the Pădurea Craiului, hosts three cave species within the hydrographic basin of Crişul Repede (Table 2; Fig. 1). One species that was described from a cave pool by Pleşa (1956) from a tributary of Crişul Repede River (Iadului Valley) as *Diacyclops languidoides spelaeus* Pleşa, 1956 was placed in synonymy with *D.* (*Acanthocyclops*) *stygius deminutus* by Monchenko (1974) and undertaken by Dussart and Defaye (2006). However, the validity of this species needs to be reconfirmed. The second karst massif, the Bihor, harbors five stygobites of the group in caves and interstitial habitats of three hydrographical basins: the upper Crişul Negru basin (on the Sighiştel Valley) (3 species) (Damian-Georgescu, 1963; Pleşa, 1969); the upper part of the Arieş River (2); and the upper part of the Someşul Cald River (2) (Table 2, Fig. 1A). The endoreic basin of Bălileasa-Cetăţile Ponorului (in northern part of Bihor massif) hosts one cave endemic (Iepure, 2001). The tap water of the Institute of Speleology from Cluj Napoca town is the locality where P. A. Chappuis (1925) described three species accommodated to the *kieferi*-group: *A. reductus* (Chappuis, 1925), *A. kieferi* and *A. stygius* (Chappuis, 1924). Cluj Napoca is located at the conjunction between the Apuseni Mountains, the Someşan Plateau and the Transylvanian field (Fig. 1A). The town is crossed by the Someşul Mic River formed by the confluence of two headstreams coming from the Apuseni, Someşul Cald and Someşul Rece. In 1925 the water system of Cluj Napoca was supplied from the alluvial aquifer of the Someşul Mic River, 5 km upstream from the town. Nowadays, these species could not be found again in the type locality because of drinking water depuration, but they might probably still inhabit the Someşul Mic aquifer.

Within the southwestern part of the Romanian Carpathians, in the Banat, the radiation of the subterranean *Acanthocyclops* species is low in comparisonwith the previous karst outcrops (Fig. 1B, Table 2). Banat Mountains is dominated by Jurassic and Cretaceous limestones (Onac and Constantin 2004). Three species assigned to the *kieferi*-group are known only from caves especially from the vadose zone: one species is found in one cave from the northern Banat (cf. Pleşa 1965); one in the middle part (Petkovski 1972) (presence of which needs to be reconfirmed) and one from southern part of Banat, known from eight caves within two close aquifers, both tributaries to the Danube (Miniş and Cerna) (Iepure and Defaye, 2008) (Table 2, Fig. 1B).

The six south-easternmost European species accommodated to this group have a narrow distribution within the Balkan Mountains crossing eastern Serbia and northern Bulgaria respectively (Pandourski and Bobič, 1993; Pandourski, 1993, 1994, 1997; Pandourski and Bobič, 2008). They distributions are almost contiguous, comparable with those congeners from the Apuseni. The taxa have been recorded from various karst subterranean habitats (the vadose zone, springs and interstitial) with one exception, *A. propinquus* found in one site in mosses from Vitosha massif in central Bulgaria (Apostolov and Pandourski, 2004).

The Timok valley in eastern Serbia (part of Balkan Mountains) hosts two species in one single cave, *A. stygius* (cf. Pandourski and Bobič, 2008, needs to be reconfirmed) and *A. iskrecensis* (Chappuis, 1924; Pandourski and Bobič, 2008); the Balkan Mountains in northern Bulgaria contain three species, *A. iskrecensis*, *A. radevi* and *A. propinquus*; and the Rila-Rodopi chain in southern Bulgaria hosts two species, *A. balcanicus* and *A. strimonis* (Pandourski, 1997). All these massifs together with the Romanian Carpathians, are parts of the Alpine chain uplifted during the Alpine orogeny that begun in the late Mesozoic and ended during Tertiary (Moores and Fairbridge, 1998). The species from the *kieferi*-group is supposed to originate from a common ancestor that differentiates at the end of Pliocene and beginning of Quaternary when the epigean populations got extinct as a result of habitats loss during the repeated climate cooling (Pandourski 1997, Apostolov and Pandourski 2004). However, with the current knowledge of the species

Table 2. Distribution of the members of the *Acanthocyclops kieferi*-group in groundwater habitats in Romania (location numbers refer to these in Fig. 1) (Note: tap water is the former water distribution of Cluj in 1921 coming from the alluvial aquifer located at 5 km from the town).

Species	Author	Location	Mountains/Plateau	Habitat	Reference
A. deminutus	(Chappuis, 1925)	(1) Poarta lui Ionele Cave	Bihor	Cave	Chappuis, 1925, 1933
A. deminutus	(Chappuis, 1925)	(2) Sighiştel Valley	Bihor	Interstitial	Damian-Georgescu, 1963
A. deminutus	(Chappuis, 1925)	(3) Neagra din Groapa de la Barsa Cave	Bihor	Cave	Pleşa, 1969
A. deminutus	(Chappuis, 1925)	(4) Crişul Repede Valley	Pădurea Craiului	Interstitial	Damian-Georgescu, 1963
A. deminutus	(Chappuis, 1925)	(5) Corbasca Cave	Bihor	Cave	Pleşa, 1969
A. kieferi	(Chappuis, 1925)	(6) Cluj Napoca	Someşan Plateau	Tap water	Chappuis, 1925
A. kieferi	(Chappuis, 1925)	(7) Vadu Crişului Cave	Pădurea Craiului	Cave	Pleşa, 1969
A. kieferi	(Chappuis, 1925)	(8) Sighiştel Valley	Bihor	Interstitial	Damian-Georgescu, 1963
A. kieferi	(Chappuis, 1925)	(9) Băița (Crişul Negru Valley)	Bihor	Interstitial	Damian-Georgescu, 1963
A. kieferi	(Chappuis, 1925)	(10) Aleşd (Crişul Repede Valley)	Pădurea Craiului	Interstitial	Damian-Georgescu, 1963
A. kieferi	(Chappuis, 1925)	(11) Casa Lotrilor Cave	Banat	Cave	Pleşa, 1985
A. reductus	(Chappuis, 1925)	(12) Cluj Napoca	Someşan Plateau	Tap water	Chappuis, 1925, 1933
A. reductus	(Chappuis, 1925)	(13) Cheile Gârliştei Cave	Banat	Cave	Petkovski, 1972
A. stygius	(Chappuis, 1924)	(14) Cluj Napoca	Someşan Plateau	Tap water	Chappuis, 1925, 1933
A. propinquus	Plesa, 1957	(15) Magura Cave	Bihor	Cave	Pleşa, 1957
A. plesai	Iepure, 2001	(16) Fața Răchiții Cave	Bihor	Cave	Iepure, 2001
A. balcanicus bisetosus	Iepure, 2001	(17) Alun Cave	Bihor	Cave	Iepure, 2001
A. balcanicus bisetosus	Iepure, 2001	(18) Humpleu Cave	Bihor	Cave	Iepure, 2001
A. milotai	Iepure & Defaye, 2008	(19) Oase Cave	Banat	Cave	Iepure & Defaye, 2008
A. milotai	Iepure & Defaye, 2008	(20) Rol Cave	Banat	Cave	Iepure & Defaye, 2008
A. milotai	Iepure & Defaye, 2008	(21) Buhui Cave	Banat	Cave	Iepure & Defaye, 2008
A. milotai	Iepure & Defaye, 2008	(22) Ponor Uscata Cave	Banat	Cave	Iepure & Defaye, 2008
A. milotai	Iepure & Defaye, 2008	(23) Padina Popii Cave	Banat	Cave	Iepure & Defaye, 2008
A. milotai	Iepure & Defaye, 2008	(24) Boilor Cave	Banat	Cave	Iepure & Defaye, 2008
A. milotai	Iepure & Defaye, 2008	(25) Socolovăț Cave	Banat	Cave	Iepure & Defaye, 2008
A. milotai	Iepure & Defaye, 2008	(26) Găurile lui Miloi II	Banat	Cave	Iepure & Defaye, 2008
A. transylvanicus n. sp.	described herein	(27) Ungurului Cave	Pădurea Craiului	Cave	Present cotribution
A. transylvanicus n. sp.	described herein	(28) Poarta Alunului Cave	Bihor	Cave	Present contribution
A. transylvanicus n. sp.	described herein	(29) Intorsuri Cave	Pădurea Craiului	Cave	Present contribution
A. transylvanicus n. sp.	described herein	(30) Cotețul Dobreştilor Cave	Bihor	Cave	Present contribution
A. transylvanicus n. sp.	described herein	(31) Ciur Izbuc Cave	Pădurea Craiului	Cave	Present contribution

group (distribution and morphology) it is difficult to trace if the direct ancestor was an epigean or a hypogean form.

Finding a new species in the Carpathians, reveal the high speciation rate of this subterranean clade of which might be related on one side, to the small scale of species dispersion linked to the combination of biogeographic history of the group and, to the complex hydrologic history of the regions where they are found, on the other.

All the subterranean species of *Acanthocyclops* accommodated to *kieferi*-group share the following characters: 11-segmented antennules, antenna without exopodite seta, different degree of the oligomerisation of the swimming legs, spine and seta formulas of exopodites of legs 1-4 are 2.3.3.3 and 4.4.4.4 or 4.4.4.3, respectively. A key for identification of the nine species accommodated to *kieferi*-group recorded in Romania is as follows:

1. P1-P4 with 3-segmented endopodites . . . ***A. kieferi***
–. P1 endopodite 2-segmented, P2-P4 2- or 3-segmented . . . **2**
2. P2-P4 endopodites 3-segmented . . . **3**
–. P2 endopodite 2-segmented . . . **4**
3. Anal operculum crescent; second exopodite segment of P2-P4 with inner seta; terminal exopodite segment of P4 with 4 inner setae . . . ***A. balcanicus bisaetosus***
–. Anal operculum triangular; second exopodite segment of P1-P2 without inner seta, corresponding segment on P3-P4 with inner seta; terminal exopodite segment of P4 with 3 inner setae . . . ***A. plesai***
4. P3 endopodite 3-segmented . . . **5**
–. P3 endopodite 2-segmented . . . **7**
5. P5 distal segment with 2 equally long terminal elements, inner one as long as segment . . . ***A. stygius***
–. P5 distal segment with 2 unequal terminal elements (1 short spine and 1 long seta) . . . **6**
6. Anal operculum triangular; caudal rami 1.5-1.7 times longer than wide; medial terminal seta shorter than outer terminal spine and shorter than caudal ramus length . . . ***A. propinquus***
–. Anal operculum rounded, caudal rami 1.7-2.5 times longer than wide; medial and outer terminal elements equally long, and as long as caudal ramus length . . . ***A. milotai***
7. Anal operculum crescent, expanded caudally; P5 distal segment with minute inner element much shorter than segment length . . . ***A. reductus***
–. Anal operculum crescent, not expanded caudally; P5 distal segment with long medial element (at least as long as segment) . . . **8**
8. P4 endopodite with 3-segmented appearance but with confluent middle and terminal .segment; anal somite with continuous row of long spinules along postero-ventral border . . . ***A. transylvanicus***
–. P4 endopodite composed of 3 well defined and articulating segments; anal somite lacking (or very minute if present) spinules along posterior border . . . ***A. deminutus***

ACKNOWLEDGMENTS

This research received support from the SYNTHESYS Project http://www.synthesys.info/ which is financed by European Community Research Infrastructure Action under the FP6 "Structuring the European Research Area Program at the Museum of Natural History, Madrid (MNCN ES-TAF 2847/2007) and the Royal Belgian Institute of Natural History, Brussels (BE-TAF 5576-2009). The study was partially supported by the NURC Romania project IDEI 1051/2007-2010. Authors thank to the two reviewers for their helpful comments on the manuscript. This paper is a contribution to the *Physical, climatic, and anthropogenic changes archived in karst deposits and ecosystems of NW Romania*, Project of the "Emil Racovita" Institute of Speleology.

REFERENCES

Apostolov, A. and 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, Historia naturalis Bulgarica, 16: 47–57.

Botoşăneanu, L. and A. Damian. 1955. Cercetări hidrobiologice asupra izvoarelor din bazinul Arieşurilor (Munții Apuseni) cu privire specială asupra Copepodelor şi asupra cercurilor de forme. Buletin Ştiintific, Academia Republicii Populare Romîne, Biologie şi Stiințe Agricole (Zoologie), 7(2): 433–452.

Chappuis, P. A. 1924. Descriptions préliminaires de Copépodes nouveaux de Serbie. Buletinul Societății de Ştiințe din Cluj, 1–2: 27–45.

Chappuis, P. A. 1925. Sur les Copépodes et les Syncarides des eaux souterraines de Cluj et des Monts Bihar. Buletinul Societății de Stiințe din Cluj, 2: 157–182.

Chappuis, P. A. 1933. Copépodes. (Première série) Avec l'énumération de tous les copépodes cavernicoles connus en 1931. Archives de Zoologie Experimentale et Générale Biospeologica, 59: 1–57.

Damian-Georgescu, A. 1963. Copepoda. Familia Cyclopidae (Forme de apă dulce). Fauna Republicii Populare Romîne. Academia R.P.R. Bucureşti, 205 pp. (In Romanian).

Dussart, B. D. and D. Defaye. 2001. Introduction to the Copepoda. 2nd edition. Guides to the identification of Microinvertebrates of the Continental Waters of the World. The Hague, 344 pp.

Dussart, B. D. and D. Defaye. 2006. World Directory of Crustacea Copepoda of Inland Waters. II. Cyclopiformes. Backhuys, Leiden, 354 pp.

Einsle, U. 1996. Copepoda: Cyclopoida. Genera *Cyclops*, *Megacyclops*, *Acanthocyclops*, pp. 1–82. *In*: H. J. F. Dumont (ed.). Guides to the Identification of the Microinver-

tebrates of the Continental Waters of the World 10. SPB Academic Publishing, Amsterdam.
Ferreira, D., Malard, F., Dole-Olivier, M. J. and J. Gibert, 2007. Obligate subterranean fauna of France: diversity pattern and conservation implications. Biodiversity and Conservation, 16: 567–596.
Galassi, D. M. P. and 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 Millieu, 54: 203–222.
Galassi, D. M. P., F. Stoch, B. Fiasca, T. Di Lorenzo and E. Gattone. 2009. Groundwater biodiversity patterns in the Lessinian Massif of northern Italy. Freshwater Biology, 54: 830–847.
Iepure, S. 2001. Ciclopides du Parc National des Monts Apuseni. I. *Acanthocyclops plesai* n. sp. et *A. balcanicus bisaetosus* n. ssp. (Crustacea, Copepoda), Studia Universitatis Babeş-Bolyai, Biologia, 2: 31–41.
Iepure, S. 2007a. Micro-evolutionary aspects of speciation in groundwater populations of cyclopids and ostracods. PhD Thesis. Romanian Academy, 270 pp.
Iepure, S. 2007b. Ordinul Cyclopoida. pp. *In*: O. T. Moldovan (ed.). Checklist of Romanian Fauna (terrestrial and freshwater species), Casa Cărții de Ştiințe, Cluj.
Iepure, S. and D. Defaye. 2008. The *Acanthocyclops kieferi* complex (Crustacea: Copepoda) from South-Eastern Europe, with description of a new species. Crustaceana, 81: 611–630.
Kiefer, F. 1930. Beiträge zur Copepoden kunde. Zoologischer Anzeiger, 87: 118–124.
Kiefer, F. 1937. Cyclopiden (Crustacea, Copepoda) aus nordspanischen Höhlen. Zoologischer Anzeiger, 119: 226–233.
Lee, J. M. and C. Y. Chang. 2007. *Acanthocyclops fonticulus* (Cyclopoida, Cyclopidae) a new species of Cyclopoid Copepods from Mountain Springs in Korea. Integrative Biosciences, 11: 61–68.
Meleg, I., O. T. Moldovan, S. Iepure, F. Fiers and T. Brad. In press). Diversity patterns of fauna in dripping water of caves from Transylvania.
Mercado, N., E. Suarez-Morales and M. Silva-Brano. 2006. A new *Acanthocyclops* Kiefer, 1927 (Copepoda: Cyclopoida) from central Mexico with comments on the distribution of the genus in Middle America. International Review of Hydrobiology, 91: 148–163.
Mirabdullayev, I. M. and A. R. Kuzmetov. 1997. Second record of *Acanthocyclops biarticulatus* Monchenko, 1972 and first description of its male (Crustacea: Copepoda). Bulletin Zoölogisch Museum Universiteit Amsterdam, 16: 1–14.
Monchenko, V. I. 1972. [Subterranean water Cyclops (Copepoda, Cyclopoidae) from Kisilkum]. Tsiklopy (Copepoda, Cyclopoida) gruntovykh vod pustyni Kyzylkum, pp. 78–97. *In*: Fauna gruntovykh vod srednei Azii. Trudy Zoologicheskogo Instituta, Leningrad. (In Russian).
Monchenko, V. I. 1974. Cyclopidae. *In*: Fauna d'Ukraine. Kiev. 452 pp. (In Ukrainian).
Moores, E. M. and R. W., Fairbridge. 1998. Encyclopedia of European and Asian Regional Geology. Encyclopedia of Earth Sciences Series. London. 825 pp.
Oarga, A. 2006. Groundwater Faunal Assemblages in Different Habitats of Padurea Craiului Mountains (NW Romania). M.Sc. Thesis. Babes-Bolyai Univesity, Cluj-Napoca. Romania. 63 pp.
Oarga, A., 2010. Species richness in sediment-filled pools and drips in caves, 89 pp. *In*: A. Moškrič and P. Trontelj (eds.). Conference Abstract Book of 16th International Karstological School "Classical Karst", Karst sediments. Postojna, Slovenia.
Onac, B. P. and Constantin, S. 2003. Karst of Eastern Europe, pp. 328–331. *In*: J. Gunn (ed.), Encyclopedia of Caves and Karst Routledge. New York.
Bobič, M. 2003. Microfauna of percolating waters in Lazareva Cave (East Serbia, Yugoslavia), pp. 101–104. *In*: V. Ljubojevic (ed.). Proceeding of 4th Symposium on Karst Protection, Beograd.
Pandourski, I. 1993. Cyclopides des eaux souterraines de la Bulgarie. I. *Acanthocyclops* (*Acanthocyclops*) *radevi* sp. n. et *Diacyclops pelagonicus saetosus* ssp. n. (Crustacea, Copepoda). Mémoire de Biospéléologie, 20: 165–168.
Pandourski, I. 1994. Cyclopides (Crustacea, Copepoda) des eaux souterraines de la Bulgarie. III. Distribution et remarques morphologiques sur les cyclopides des sous-familles Eucyclopinae et Cyclopinae. Hydrobilogy, Sofia, 39: 3–16.
Pandourski, I. 1997. Composition, origine et formation de la faune cyclopidienne stygobie de Bulgarie et définition du groupe "*kieferi*" du genre *Acanthocyclops* (Crustacea, Copepoda, Cyclopoida). Bollettino del Museo regionale di Scienze naturali, Torino, 15: 279–197.
Pandourski, I. 2007.Stygofauna of the Fresh Waters in Bulgaria, pp. 527–536. *In*: V. Fet and A. Popov (eds.). Biogeography and Ecology of Bulgaria. Monographiae Biologicae, Springer, Berlin.
Pandourski, I. and M. Bobič, 2008. *Acanthocyclops iskrecensis* Pandourski, 1992 (Crustacea, Copepoda, Cyclopoida) from the Karstic Groundwaters of Eastern Serbia. Acta Zoologica Bulgarica, 60: 193–195.
Petkovski, T. K. 1972. Zur Copepodenfauna der Höhlen von Banat. Acta Musei Macedonici Scientiarum Naturalium, Skopje, 13: 21–38.
Pipan, T., 2005. Epikarst- a Promising Habitat. Ljubljana, ZRC Publishing. 101 pp.
Pleşa, C. 1956. Contribuþii la fauna ciclopidelor (Crustacee, Copepode) din Republica Populară Romînă. Analele Institutului de Cercetări Piscicole, 1: 363–372.
Pleşa, C. 1957. Eine neue Höhlencyclopidenform (Acanthocyclops reductus var. propinquus n. var.) aus Rumänien. Zoologischer Anzeiger, 159(5–6): 128–130.
Pleşa, C. 1969. Fauna cavernicolă acvatică din peăterile calcaroase. pp. 354–358. *In*: R. Călinescu (ed). Biogeografia României. Ed. Ştiinţifică, Bucureşti.
Pleşa, C. 1985. Conspectul sistematic al ciclopidelor (Crustacee, Copépode) cunoscute până în prezent din Republica Populară Română. Studia Universitatis Babeş et Bolyai, Biologia. 2–3: 137–150.
Pleşa, C., M. Pintea-Alb, I. Sălăjan and F. Wild, 1965. Date noi asupra ecologiei şi răspândirii geografice a ciclopidelor (Crustacea, Copepoda) în România. Lucrările Institutului de Speologie "Emil Racoviță", 4: 141–154.
Stoch, F., F. Malard, F. Castellarini and M. J. D.Olivier, 2004. Pascalis Deliverable for Workpackage 7: Statistical analyses and identification of indicators, 155 pp.

Received: July 14, 2010
Accepted: February 28, 2011