# ACANTHOCYCLOPS MAGISTRIDUSSARTI SP. NOV., FROM GROUND WATERS OF PENINSULAR ITALY, WITH COMMENTS ON THE INTRASPECIFIC VARIABILITY OF THE ANTENNARY BASIS ORNAMENTATION (COPEPODA, CYCLOPOIDA, CYCLOPIDAE)

ΒY

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#### ABSTRACT

A new species of cyclopid copepod, Acanthocyclops magistridussarti sp. nov., is described herein and attributed to the Acanthocyclops vernalis-group. Within this species-group, Acanthocyclops magistridussarti sp. nov. shows the closest affinities with Acanthocyclops cf. orientalis sensu Petkovski, 1975, collected from ground waters in Macedonia and Bosnia, and Acanthocyclops orientalis sensu Lee et al., 2007 from Korean caves, characterized by long setae of the distal segment of the leg 4 endopod. The new species can be distinguished from all other species of the group by the peculiar ornamentation of the antennary basis, which, however, shows a surprising variability in the pattern of presence/absence of the group of spinules proximal to the insertion of the exopodal seta, of the oblique row of spinules inserted in the middle of the first 1/3 of the segment on the frontal surface, and of the oblique row of short spinules below the two distal setae. This latter row of spinules has never been recorded in the Acanthocyclops vernalis-group. Notwithstanding such variability, one diagnostic microcharacter showed to be constant: the group of six long spinules near the outer margin on the caudal side of antennary basis, which are inserted to form a tuft rather than a row, as is usual in the Acanthocyclops of the vernalis-group. The new species is so far known only from groundwater habitats, having been collected in caves, hyporheic habitats, and springs in the Apennines (peninsular Italy).

# RÉSUMÉ

Une nouvelle espèce de copépode cyclopide Acanthocyclops magistridussarti sp. nov., est décrite ici et attribuée au groupe Acanthocyclops vernalis. À l'intérieur de ce groupe d'espèces,

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Acanthocyclops magistridussarti sp. nov. présente de grandes affinités avec Acanthocyclops cf. orientalis sensu Petkovski, 1975, collecté dans les eaux souterraines de Macédoine et de Bosnie, et avec Acanthocyclops orientalis sensu Lee et al., 2007, de grottes de Corée, caractérisés par les longues soies du dernier segment de l'endopodite de P4. La nouvelle espèce se distingue de toutes les autres espèces du groupe par l'ornementation particulière du basipodite antennaire, qui, cependant, présente une surprenante variabilité du patron de présence/absence pour le groupe de spinules situé proximalement par rapport à l'insertion de la soie exopodale, et du rang oblique de spinules inséré au milieu du premier tiers du segment sur la face frontale, et le rang oblique de courtes spinules situé sous les deux soies distales. Ce dernier rang de spinules n'a jamais été trouvé dans le groupe vernalis. Exception faite de cette variabilité, un microcaractère diagnostique est apparu constant : le groupe de six longues spinules près du bord externe sur la face caudale du basipodite de l'antenne, qui forment une touffe au lieu d'une rangée comme habituellement chez les Acanthocyclops du groupe vernalis. La nouvelle espèce est jusqu'à présent, seulement connue des habitats souterrains, ayant été collectée dans les grottes, les habitats hyporhéiques et les sources des Apennins (péninsule Italienne).

#### INTRODUCTION

The freshwater cyclopid genus Acanthocyclops Kiefer, 1927 has a worldwide distribution and comprises more than 70 nominal species and subspecies (Pesce, 1999-2009; Dussart & Defaye, 2006), 12 of them being present in Italy (Stoch, 2006). The genus has been the subject of numerous investigations, especially concerning the two most widespread species, Acanthocyclops vernalis (Fischer, 1853) and Acanthocyclops robustus (G. O. Sars, 1863). Their identification remained problematic for a long time, and has largely been discussed during the last decades, because the morphological characters used for their identification vary with differing ecological conditions (see Mirabdullayev & Defaye, 2004 for the corresponding literature review). The Acanthocyclops vernalis-species group was defined by Petkovski (1975) to accommodate the two above-mentioned species, including their subspecies and other infraspecific forms; one of them (A. vernalis orientalis Borutzky, 1966) was raised to species level. Petkovski (1975) attributed two populations from Macedonia and Bosnia to A. cf. orientalis. In a paper published a year later, Kiefer (1976) revised the "robustus-vernalis"-group within the genus Acanthocyclops, excluding from it A. orientalis, which he considered either a member of the A. venustus-group, or close to Diacyclops antrincola Kiefer, 1967. Finally, some populations collected in South Korean limestone caves were recently described (Lee et al., 2007; Chang, 2009) and attributed to A. orientalis.

In recent years, the *A. vernalis* species-group has been extensively investigated. *Acanthocyclops robustus* has been shown to be a species complex (Mirabdullayev & Defaye, 2002, 2004) represented by four species: A. robustus, A. brevispinosus (Herrick, 1884), A. trajani Mirabdullayev & Defaye, 2002, and A. einslei Mirabdullayev & Defaye, 2004. Acanthocylops vernalis has been shown to be a complex of cryptic species with variable karyotypes and levels of reproductive isolation, but very difficult to discern morphologically (Dodson et al., 2003; Grishanin et al., 2005, 2006; Bláha et al., 2010). Other species, which are closer to A. vernalis due to the presence of 17segmented antennulae, smooth caudal rami, and an inner seta on the last (= terminal) endopodal segment of leg 4, are A. smithae Reid & Suárez Morales, 1999 and A. rebecae Fiers, Ghenne & Suárez-Morales, 2000, distributed in Central America. In addition, Mercado-Salas et al. (2006) and Dussart & Defaye (2006) included in this group A. dodsoni Mercado-Salas, Suárez-Morales & Silva-Briano, 2006 from Mexico, although this species has a lower number of antennulary segments as well as pilose caudal rami, and thus does not fit the definition of the group as given by Petkovski (1975) and subsequently Kiefer (1976). The differences between the species of the A. vernalis-group can be assessed taking into account microcharacters, such as the ornamentation of the antennary basis (see for instance Fiers & Van de Velde, 1984; Reid & Suárez Morales, 1999; Fiers et al., 2000; Mirabdullayev & Defaye, 2002, 2004).

During recent investigations carried out in peninsular Italy, several groundwater populations of the *Acanthocyclops vernalis*-group were collected in caves, springs, and hyporheic habitats along the Apenninic and Pre-Apenninic mountain ranges. These specimens are similar to those defined as *A*. cf. *orientalis* by Pesce & Maggi (1979) and Petkovski (1975), as well as to those attributed by Lee et al. (2007) to *A. orientalis*; they belong to an undescribed species, characterized by a peculiar ornamentation of the antennary basis. The aim of this paper is, therefore, to describe the new species and to highlight the taxonomic value of the spinulation pattern of the antennary basis within the *A. vernalis*-species group, while discussing its intraspecific variability in the new species.

### MATERIAL AND METHODS

Acanthocyclops magistridussarti sp. nov. was collected in cave, hyporheic, and spring habitats. Specimens were collected in three caves: two in the Northern Apennines, i.e., the "Tana della Mussina di Borzano" cave (Emilia-Romagna region, Reggio Emilia province; Messinian gypsum cave, cadastral number 2 ER, 275 m a.s.l.) (Stoch, 2001), and the "Rio Borsa" cave (Liguria

region, La Spezia province; limestone cave, cadastral number 980 Li, 636 m a.s.l.); and one in central Italy in the Vulsini Mountains: "Il Bucone" cave (Latium region, Viterbo province; travertine cave, cadastral number 923 La, 171 m a.s.l.). In all caves, specimens were collected by stirring the sediment of streams and pools, and collecting the dislodged organisms with a plankton net with 100  $\mu$ m mesh size.

Hyporheic specimens were collected using the Karaman–Chappuis method (Delamare Deboutteville, 1960) on the banks of the Secchia River and "dei Tramonti" brook, in the Northern Apennines (Emilia-Romagna region, Reggio Emilia province; alluvial sediment, 427 m a.s.l., and marly-arenaceous flysch, 645 m a.s.l., respectively) (Stoch et al., 2009).

Further specimens were collected by filtering the water of a reservoir fed by a spring along the "delle Fontane" brook (Emilia-Romagna region, Reggio Emilia province; marly-arenaceous flysch, 668 m a.s.l.) with a 100  $\mu$ m mesh size plankton net (Stoch et al., 2009), and from the spring Lagdei in the Parma Stream watershed in the Northern Apennines (Emilia-Romagna region, Parma province; sandstone and moraine deposits, 1260 m a.s.l.) by using traps partially dug into the sediment and left in situ for seven days (Bottazzi et al., 2008).

All samples were fixed in the field with 5% buffered formalin or 70% ethanol; specimens were sorted under a dissecting microscope. Selected specimens were dissected in water or glycerin, and mounted on slides in Faure's, CMC<sup>®</sup>, or glycerine medium. Slides were sealed with nail polish. The remaining specimens were stored in 70% ethanol with 10% glycerine added. Drawings were made at  $1000 \times$ , with the aid of a camera lucida, using a Zeiss Axioskop<sup>®</sup> compound microscope equipped with Nomarski differential contrast. All measurements were taken with an ocular micrometer. The terminology related to the external morphology follows Huys & Boxshall (1991). The terms used by Fiers & Van de Velde (1984) and Hołyńska & Dahms (2004) for the antennary basis, and those used by Einsle (1985) for the ornamentation of the caudal surface of the leg 4 coxa, are also adopted.

The holotype and selected paratypes are deposited in the Muséum National d'Histoire Naturelle in Paris (MNHN); the remaining material is located in the senior author's collection (FS).

# TAXONOMIC ACCOUNT

Family CYCLOPIDAE Rafinesque, 1815 Genus Acanthocyclops Kiefer, 1927

### Acanthocyclops magistridussarti sp. nov. (figs. 1-5)

Acanthocyclops cf. orientalis: Stoch, 2001: 142, 143. Acanthocyclops aff. trajani: Bottazzi et al., 2008: 58, 59, 60. Acanthocyclops sp. aff. orientalis: Stoch et al., 2009: 154, 155, 158, 159.

Material examined. — Lagdei spring (Aemilian Apennines), leg. E. Bottazzi, 14 June 2007 and 30 August 2007, 6 specimens; reservoir fed by a spring along "delle Fontane" brook (Aemilian Apennines), leg. F. Stoch & G. Tomasin, 28 October 2006, 93 specimens; Secchia River near Poiano (Aemilian Apennines), hyporheos, leg. F. Stoch & G. Tomasin, 28 October 2006, 4 specimens; "dei Tramonti" brook (Aemilian Apennines), hyporheos, leg. F. Stoch & G. Tomasin, 29 October 2006, 2 specimens; "Tana della Mussina di Borzano" cave (Aemilian Apennines), hypogean brook, leg. F. Stoch, 9 May 1999, 10 specimens; "Rio Borsa" cave (Ligurian Apennines), hypogean pools, leg. M. Bodon, 21 August 1993, 2 females; "Il Bucone" cave (Vulsini Mountains), residual pool of the hypogean brook, leg. F. Stoch & A. Terazzi, 4 November 2009, 45 specimens.

Type-series. — Holotype, female, dissected and mounted on slide labelled "Acanthocyclops magistridussarti female, holotype, Lagdei spring 30 August 2007 E. Bottazzi leg". (MNHN-Cp6033). Allotype, male, dissected and mounted on slide labelled "Acanthocyclops magistridussarti male, allotype, Lagdei spring, 30 August 2007, E. Bottazzi leg". (MNHN-Cp6034). Paratypes: one female (paratype 1), dissected and mounted on slide labelled "Acanthocyclops magistridussarti female paratype, Lagdei spring, 14 June 2007, E. Bottazzi leg". (MNHN-Cp6035); one female (paratype 2), dissected and mounted on slide labelled "Acanthocyclops magistridussarti female paratype, Lagdei spring, 30 August 2007, E. Bottazzi leg". (MNHN-Cp6036); two males (paratypes 3, 4), each dissected and mounted on a slide labelled "Acanthocyclops magistridussarti male paratype, Lagdei spring, 30 August 2007, E. Bottazzi leg". (MNHN-Cp6037 and MNHN-Cp6038, respectively); one female and one male (paratypes 5, 6), each dissected and mounted on a slide, labelled "Acanthocyclops magistridussarti paratype, spring along "delle Fontane" brook, 28 October 2007, F. Stoch & G. Tomasin leg". (FS); five males and five females from spring along "delle Fontane" brook, preserved in vials in 70% ethanol labelled "Acanthocyclops magistridussarti 5 female paratypes, 5 male paratypes, spring along "delle Fontane" brook, 28 October 2007, F. Stoch & G. Tomasin leg". (MNHN-Cp6039).

Description of female. — Total body length 0.93-1.25 mm (mean  $\pm$  standard deviation = 0.99  $\pm$  0.07, n = 30) measured from anterior end of cephalothorax to posterior margin of caudal rami. Body colourless. Hyaline frills of posterior margin of urosomites smooth; ventral and dorsal surfaces of urosomites without ornamentation. Lateral margins of prosomite 5 posteriorly produced (fig. 1A). Cephalothorax representing 38-40% of total body length. Antennules not reaching end of prosome.

Genital double-somite (fig. 1A) broadest in anterior half. Seminal receptacle divided in a broad anterior and a smaller posterior lobe. Copulatory pore located ventrally about halfway along length of genital double-somite; copulatory ducts straight, slightly indistinct in their lateral part. Anal and preanal somites equal-sized. Anal somite with one row of spinules along ventral distal



Fig. 1. Acanthocyclops magistridussarti sp. nov. A, female paratype, spring along "le Fontane" brook; B, C, D, E, F, holotype female, Lagdei spring. A, fifth prosomite and genital double somite; B, anal somite and caudal rami, ventral view; C, antennule; D, antenna (ornamentation of basis not shown); E, mandible; F, maxillule.

margin (fig. 1B), bearing two sensilla on dorsal surface; anal operculum broad, slightly convex, and weakly sclerotized. Proctodeum with setular rows. Paired egg-sacs (present in three females) carrying six or seven eggs each.

Caudal ramus (fig. 1B) 4.7 to 5.6 times as long as wide, inner margin bare. Terminal accessory seta 1.4-1.6 times as long as posterolateral seta, inserted at about 0.7 of caudal ramus. Dorsal seta very long, approximately 0.5-0.8 times as long as caudal ramus. Inner terminal seta about 1.7 times as long as outer terminal seta.

Antennule (fig. 1C): 17-segmented in all specimens; first segment with short row of spinules. Aesthetasc (ae) on segment 12 reaching to the end of segment 14 (fig. 2J–L). Armature formula: 1-[8], 2-[4], 3-[2], 4-[6], 5-[4], 6-[2], 7-[2], 8-[1], 9-[1], 10-[0], 11-[1], 12-[1 + ae], 13-[0], 14-[1], 15-[2], 16-[2 + ae], 17-[7 + ae].

Antenna (fig. 1D): coxa unarmed; basis bearing two distal setae; exopodal seta present. Caudal side of antennary basis (letters following Hołyńska & Dahms, 2004; fig. 2A, B, D, F, H) with: (a) outer proximal group of 3-4 long spinules; (b) group of 3-6 long spinules, located slightly distal and medial with respect to previous one; (c) inner proximal group of 4-8 spinules; (d) group of 6 long spinules distal to previous group, near outer margin; (e) oblique row of short spinules between previous group and two distal setae; group f of Hołyńska & Dahms (2004) lacking. Frontal side of antennary basis (not described in Hołyńska & Dahms, 2004; fig. 2C, E, G, I) with: (g) inner proximal longitudinal row of spinules; (h) oblique row of 3 long spinules in the middle of the first 1/3 of the segment, and between groups g and i; (i) group of 5-7 spinules at half of outer margin; (j) group of 5 spinules proximal to the insertion of exopodal seta. Groups e, h, and j not present in some specimens (see discussion). Endopod segment 1 with one inner seta; endopod segment 2 with nine inner setae; endopod segment 3 with seven terminal setae. All endopodal segments with outer longitudinal spinule row.

Mandible (fig. 1E): gnathobase with strongly chitinized teeth. Palp reduced, with two long plumose setae and one short seta.

Maxillule (fig. 1F): precoxal arthrite with one proximal tooth, and six spines on the frontal side, the proximalmost and the apicalmost pinnate. Arthrite ending with three chitinized claws. Palp segment 1 (coxobasis) without spinules, bearing apically two naked setae and one pinnate spine. Palp segment 2 (endopod) bearing three setae; exopodal seta present.

Maxilla (fig. 3A): precoxa and coxa not fused; precoxal endite armed with two strong, pinnate setae. Coxa with proximal endite represented by single spinulose seta; coxal caudal surface bare; distal endite well developed, with two apical setae of different lengths. Basis prolonged into strong claw bearing coarse spinules along middle part of inner margin; accessory armature



Fig. 2. Acanthocyclops magistridussarti sp. nov. A, B, C, female, "Il Bucone" cave; D, K, holotype female, Lagdei spring; E, female paratype, Lagdei spring; F, G, L, female paratype, spring along "le Fontane" brook; H, I, J, female, "Rio Borsa" cave. A, B, D, F, H, antennary basis ornamentation, caudal side (A, right antenna and B, left antenna, same individual); C, E, G, I, antennary basis ornamentation, frontal side; J, K, L, 12<sup>th</sup> and 13<sup>th</sup> antennulary segment with aesthetasc.

consisting of strong spine and thin seta. Endopod almost indistinctly twosegmented; first segment with two strong spiniform setae, second segment with two short, slender setae and one strong, spiniform seta.

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Fig. 3. Acanthocyclops magistridussarti sp. nov. A, D, F, G, H, female paratype, Lagdei spring;
B, C, E, holotype female, Lagdei spring. A, maxilla; B, maxilliped; C, D, E, leg 1–leg 3, frontal view;
F, G, H, leg 1–leg 3 intercoxal sclerite, caudal view.

Maxilliped (fig. 3B): four-segmented. Syncoxa with three spiniform setae along inner margin, the middle one longest. Basis with two spiniform setae, and two longitudinal rows of spinules. Endopod reduced, 2-segmented, first

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	Coxa	Basis	Exopodite	Endopodite
Leg 1	1-0	1-1	1-I; 1-I; 2, 2, III	1-0; 1-0; 3, 1-I, 1
Leg 2	1-0	0-1	1-I; 1-I; 3, 1-I, III	1-0; 2-0; 3, 1-I, 1
Leg 3	1-0	0-1	1-I; 1-I; 3, 1-I, III	1-0; 2-0; 3, 1-I, 1
Leg 4	1-0	0-1	1-I; 1-I; 3, 1-I, III	1-0; 2-0; 2-II, 1

TABLE I

Armature of swimming legs 1-4 (spines in Roman numerals, setae in Arabic) of *Acanthocyclops* magistridussarti sp. nov. Sequence follows inner to outer positions

segment with pinnate seta and longitudinal row of spinules; second segment with two pinnate and one naked setae.

Leg 1-4 with three-segmented exopod and endopod, intercoxal sclerite and coxa as in figs. 3F–H and 4D; armature as in table I; spine formula of the third exopodal segments 3444.

Leg 1 (fig. 3C). Basis with one slender seta on outer margin, inner margin moderately expanded, with seta reaching the end of second endopodal segment, with spinules at its insertion. Endopod slightly longer than exopod.

Leg 2 (fig. 3D). Basis with one slender seta on outer margin with small spinules near its insertion. Endopod slightly longer than exopod.

Leg 3 (fig. 3E). Basis with one slender seta on outer margin with small spinules near its insertion. Endopod slightly longer than exopod.

Leg 4 (fig. 4A). Ornamentation on the caudal surface of coxa (letters following Einsle, 1985; fig. 4D–E) represented by: (a) short proximal transveral row of spinules in the middle of the coxa; (b) oblique row of elongated spinules near proximal outer corner; (c + d) distal transversal row of spinules of different lengths; (e) distal group of thin spinules near outer distal corner; (f) group of spinules along outer distal margin. Basis with one slender seta on outer margin with small spinules near its insertion. Endopod longer than exopod, with notch on outer margin of first segment, third segment 2.7-2.8 times as long as wide. Inner apical spine 1.1-1.2 times as long as outer apical spine and 0.6-0.7 times as long as segment. Outer lateral spine inserted at 65-74% of the segment length.

Leg 5 (fig. 4B). Consisting of two free segments; basal segment subrectangular, 0.6 times as long as broad, with long outer seta. Distal segment about 2.3 times as long as broad, with long apical seta and short inner subapical spine 0.6 times as long as segment.

Leg 6 (fig. 4C). Represented by one long pinnate seta and two short, blunt spines.



Fig. 4. Acanthocyclops magistridussarti sp. nov. A, B, D, female paratype, Lagdei spring; C, holotype female, Lagdei spring; E, paratype female, spring along "le Fontane" brook. A, leg 4, frontal view; B, leg 5; C, leg 6; D, E, leg 4 intercoxal sclerite, coxa, basis, and first endopodal segment, caudal view.

Description of male. — Total body length 0.80-0.89 mm (mean  $\pm$  standard deviation = 0.84  $\pm$  0.03, n = 30) measured from anterior end of cephalothorax to posterior margin of caudal rami. Mouthparts, leg 1–leg 4 basis, coxa, and intercoxal sclerite ornamentation, as well as leg 1–leg 5 segmentation and armature, all identical to those of female.

Caudal ramus (fig. 5A) slightly shorter than in female, 4.3 times as long as wide, bearing the same ornamentation as in female.

Antennule (fig. 5B–D): 17-segmented in all specimens, 16<sup>th</sup> and 17<sup>th</sup> segments partially merged; sheath on segment 10; ridged element with pore

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Fig. 5. *Acanthocyclops magistridussarti* sp. nov. A, E, paratype male, Lagdei spring; B, C, D, paratype male, spring along "le Fontane" brook. A, anal somite and caudal rami, ventral view; B, antennule, segments 1-13, anteroventral view; C, antennule, segments 14-17, anteroventral view; D, antennule, distal geniculation between segments 14 and 15; E, fifth prosomite, leg 5, leg 6, and genital somite, ventral view.

on segment 14; two ridged elements on segment 15. Armature formula: 1-[8+3ae], 2-[4], 3-[2], 4-[2+ae], 5-[2], 6-[2], 7-[2], 8-[2], 9-[2+ae], 10-[2], 11-[2], 12-[2], 13-[2+ae], 14-[2], 15-[1+ae], 16-[4+ae], 17-[7+ae].

Leg 6 (fig. 5E) armed with a stout spine and two setae; inner seta as long as spine, outer seta twice as long as inner one.

Etymology. — The new species name is derived from the Latin "magister", meaning "teacher, mentor", and is dedicated to the late Professor Bernard Dussart, in appreciation of his outstanding contribution to the study of copepod diversity and because most of our studies on copepods were based on his work. The specific epithet is a noun in singular masculine genitive.

Remarks. — Acanthocyclops magistridussarti sp. nov. belongs to the Acanthocyclops vernalis-group as defined by Petkovski (1975) based on the 17segmented antennule of the female, the shape of the seminal receptacle, the morphology of the leg 4 endopod, and the smooth surface of the caudal rami. It can be distinguished from all other species of the group by the peculiar ornamentation of the antennary basis (see Fiers & Van de Velde, 1994; Einsle, 1996; Alekseev et al., 2002; Mirabdullayev & Defaye, 2002, 2004), and the unique combination of the following features: ornamentation of intercoxal sclerites and leg 4 coxa; shape, length of setae as well as of terminal spines of leg 4 third endopodal segment; shape and armature of leg 5; and caudal rami. Within the A. vernalis-group, Acanthocyclops magistridussarti sp. nov. has the closest affinities with Acanthocyclops cf. orientalis sensu Petkovski, 1975 and with A. orientalis sensu Lee et al., 2007. Petkovski (1975) considered the Macedonian and Bosnian groundwater populations to be close to A. orientalis, known from a single cave in Primorsky Krai, in the extreme southeast of Russia (Borutzky, 1966) based only on the length of the inner setae of the third endopodal segment of leg 4, which reaches past the end of the terminal spines. More recently, Lee et al. (2007) and Chang (2009) attributed to A. orientalis the specimens collected from five limestone caves and a spring in South Korea. Those authors list, among others, the following characterizing features of their specimens: inner spine on third endopodal segment of leg 4 longer than outer spine; elongate setae on the same segment which much exceed the terminal spines; intercoxal sclerite of leg 4 with continuous transverse row of spinules; spinule row on coxa of leg 4 interrupted by a gap (i.e., two distinct groups of spinules, c and d in our terminology); dorsal caudal seta about 1.5 times as long as outer caudal seta. Based on theses characters, Lee et al. (2007) discriminated the Korean and Russian specimens from the Balkanic ones. However, comparisons of the drawings and descriptions of Lee et al. (2007) and Chang (2010) with those of Borutzky (1966), show some important differences: the setae on the third endopodal segment of leg 4 are much longer in Borutzky's drawing (as long as twice the inner terminal spine), the third endopodal segment of leg 4 is shorter and stouter, the distal segment of leg 5 is shorter, with a shorter subapical spine, and the length of the caudal

rami and the ratios of their setae differ as well. Based on these differences, we regard the South Korean populations as not belonging to *A. orientalis*.

Acanthocyclops magistridussarti sp. nov. can be distinguished from A. orientalis, as well as from Acanthocyclops cf. orientalis sensu Petkovski, 1975 and A. orientalis sensu Lee et al., 2007 based on the following features: (1) length of the inner setae of the third endopodal segment of leg 4: reaching the end of the inner terminal spine in Acanthocyclops magistridussarti sp. nov., more than twice the length of the inner terminal spine in A. orientalis and in A. cf. orientalis sensu Petkovski, 1975; (2) ratio between the terminal accessory seta and the posterolateral seta of the caudal rami: measuring 1.4-1.6 in Acanthocyclops magistridussarti sp. nov., 2.1 in A. orientalis and 1.3 in A. cf. orientalis sensu Petkovski, 1975; (3) shape of leg 5 proximal segment: shorter and stouter in A. orientalis and in A. orientalis sensu Lee et al., 2007, than in Acanthocyclops magistridussarti sp. nov.; (4) shape of leg 5 distal segment: more elongated in A. cf. orientalis sensu Petkovski, 1975 and A. orientalis sensu Lee et al., 2007 than in Acanthocyclops magistridussarti sp. nov., shorter and stouter in A. orientalis; (5) leg 5 subterminal spine: short and stout in A. orientalis, long, "Diacyclops-like" in A. magistridussarti sp. nov. and in the Balkanic and Korean populations. Moreover, A. orientalis sensu Lee et al., 2007 differs from A. magistridussarti sp. nov. in the absence of the notch on the first endopodal segment of leg 4; the different armature of the intercoxal sclerite and of the caudal side of the coxa of the same leg; the spine formula of the third exopodal segments of legs 1-4 (2333 vs. 3444); and the different ratio between the caudal setae.

Unfortunately, further differences can not be detected due to the lack of drawings and description of other taxonomically informative characters for *A. orientalis* Borutzky, 1966, and the unavailability of the type material. Moreover, due to the very poor description, the assignment of *A. orientalis* to the *A. vernalis*-group as proposed by Petkovski (1975) remains doubtful; as already mentioned above, Kiefer (1976) suggested to assign it to the *A. venustus*-group (characterized by five setae on the third exopodal segment of legs 1-4) or even to transfer it to the genus *Diacyclops*.

For these reasons, we suggest that the Apenninic, Balkanic, and Korean stygobiotic populations of *Acanthocylops* do not belong to *A. orientalis*; it will be necessary to examine further material and in particular the ornamentation of the antennary basis of the Balkanic and Korean populations to establish their taxonomic status in relation to *A. magistridussarti* sp. nov.

As regards Italy, Pesce & Maggi (1979) and Pesce (1980) reported a population of A. cf. *orientalis* from ground waters of the Marche region

(Central Apennines, Italy) as being very close to *A*. cf. *orientalis* sensu Petkovski, 1975. Einsle (1996) redrew the same specimens and attributed them to *A. orientalis*. Unfortunately, Einsle's drawings markedly differ from those of Pesce & Maggi (1979), although they are based on the same material, and some important features such as the spinulation pattern of the antennary basis, the mouthparts, and the ornamentation of some of the intercoxal plates and coxae are not shown nor described. Moreover, *A. magistridussarti* sp. nov. does not show the variable spine formula reported by Einsle (1966) for *A. orientalis* (from 2333, as in the Marche region, to 3444, as in some males described by Petkovski, 1975). Unfortunately, the material deposited in Pesce's collection in L'Aquila University (Italy) could not be found while screening the collection. Therefore, based on the more recent and detailed drawings given by Einsle (1996), we can not attribute with certainty the material from the Marche region to *A. magistridussarti* sp. nov.

#### DISCUSSION

One of the distinguishing features of *Acanthocyclops magistridussarti* sp. nov. from its congeners, is the peculiar ornamentation of the antennary basis, represented by groups of spinules with fixed relative position on the caudal and frontal sides of the segment. This set of microcharacters is highly diversified among and within genera of the family Cyclopidae, and has successfully been used as a diagnostic character at the species level (see for example Fiers & Van de Velde, 1984; Van de Velde, 1984; Hołyńska & Dahms, 2004). For the members of the Acanthocyclops vernalis-group it was described in detail by Fiers & Van de Velde (1984), Reid & Suárez Morales (1999), Fiers et al. (2000), Alekseev et al. (2002), and Mirabdullayev & Defaye (2002, 2004). Mirabdullayev & Defave (2002, 2004) recently reviewed the taxonomy of the Acanthocyclops robustus species complex, and pointed out that A. robustus is the only species of this complex to possess a group of spinules proximal to the insertion of the exopodal seta (group j in our description). This microcharacter was the first couplet in the dichotomous key to the females of the Acanthocyclops robustus species complex (Mirabdullayev & Defaye, 2004). Another microcharacter considered to have diagnostic value is the oblique row of spinules in the middle of the first 1/3 of the segment on the frontal surface (group h), used to distinguish A. vernalis (Fischer, 1853) from Acanthocyclops robustus s. l. (cf. Fiers & Van de Velde, 1984).

A detailed examination of the ornamentation of the antennary basis in *A. magistridussarti* sp. nov. (fig. 2A–I) revealed a surprising variability in the pattern of presence/absence of the above-mentioned two groups of spinules (j and h), and in group e (i.e., the oblique row of short spinules below the two distal setae on the caudal side, so far never recorded in the *A. vernalis*-group) within and among populations, and even in the same individual. We recorded the following differences:

- a) group j is missing in some specimens of the springs in the Parma Stream watershed; the group is lacking on the left antenna of one specimen from "Il Bucone" cave;
- b) group h is missing in the population of the springs in the Parma Stream watershed and in the single specimen collected in the "Rio Borsa" cave;
- c) group e is reduced to a few spinules in the population of the spring along "delle Fontane" brook; the group is lacking on the right antenna of the above-mentioned specimen from "Il Bucone" cave.

Notwithstanding such variability, a diagnostic microcharacter that showed to be constant and thus characterizes A. magistridussarti sp. nov., is the group of six elongated spinules near the outer margin on the caudal side (group d), which are inserted to form a tuft instead of a row, as is usual in Acanthocyclops. A tuft is present in A. sensitivus (Graeter & Chappuis, 1914) and A. parasensitivus Reid, 1998, which are not members of the A. vernalis species group; in fact Kiefer (1957) created for A. sensitivus the new subgenus Rhenocyclops and Pospisil (1999) raised doubts on the inclusion of A. sensitivus in the genus Acanthocyclops due to its peculiar morphology. Another important feature is the presence of group e, which, albeit not present in all specimens, is, as far as we know, absent in other Acanthocyclops apart from the aberrant species A. sensitivus and A. parasensitivus (cf. Pospisil, 1999; Reid, 1998), and almost constantly present in the closely related genus Cyclops (cf. Hołyńska & Dahms, 2004), as well as in some other genera, mainly belonging to the subfamily Eucyclopinae (cf. Fiers & Van de Velde, 1984). For this reason, the presence of this spinule group in A. magistridussarti sp. nov. should be considered a plesiomorphic character, in our opinion.

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