LITTLE-KNOWN CYCLOPOIDS FROM GROUNDWATER IN ITALY: RE-VALIDATION OF ACANTHOCYCLOPS AGAMUS AND REDESCRIPTION OF SPEOCYCLOPS ITALICUS (CRUSTACEA, COPEPODA, CYCLOPOIDA)

D. M. P. GALASSI, P. DE LAURENTIIS
Dipartimento di Scienze Ambientali, University of L'Aquila, via Vetoio, Coppito, 67100 L'Aquila, Italy
Corresponding author: diana.galassi@aquila.infn.it

ABSTRACT. – Recent stygobiological investigation devoted to the assessment and conservation of groundwater biodiversity in Italy resulted in the rediscovery of the stygobiont cyclopoids Acanthocyclops agamus and Speocyclops italicus. The occasion has been taken to redescribe them from both males and females. Ecological and biogeographical considerations are discussed for both species.

STUDY AREA

The Alburnian Massif represents a large calcareous ridge of Mesozoic age and is characterized by important epigean and hypogean karstic formations, which include both vertical and horizontal karstic conduits. Castelcivita Cave, 5 km in length, is located in the south-western part of the Alburnian massif, about 30 km inland from the Tyrrhenian coast, and has large cavities with horizontal development. Three different karstification levels are recognizable. The highest level reaches the maximum altitude of 110 m a.s.l. and represents the first and most ancient level of karstification, now completely fossil. The intermediate level is represented by the still-active sectors of the cave and develops at an altitude ranging from 60 to 70 m a.s.l. It is in equilibrium with the groundwater base flow. The lowest level is constituted by different karstic conduits, probably belonging to a different karstic circuit, in which the groundwater flows below the present sea level. The maximum discharge value of the Castelcivita system is 4 m³/s and the minimum discharge value 1 m³/s. The hydrological regime is that of a typical conductive system, with fast response to rain events.
Pertosa Cave (also named Grotta di San Michele Arcangelo) is located in the Alburnian Massif, and opens in the Vallo di Diano, at the opposite side of the massif from Castelcivita Cave. Pertosa Cave is 2.560 km in length. The active part consists of a subterranean river which emerges from a large resurgence, at 70 m above the Tanagro streambed. The subterranean river is presently dammed at the cave entrance, forming an artificial lake. The remaining part of the cave is fossil, with small, intermittent concretion pools.

Additional material of Acanthocyclops agamus was obtained by a drift sample from a large karstic spring of the Gizio River (Abruzzo, central Italy). This spring is the main outlet of the Genzana-Greco hydrogeological subunit (Genzana-Greco hydrogeological unit), and has a mean annual discharge of 4.5 m³/s. Another population of this species has been collected from the Mazzoccolo spring. This spring is managed for drinking purposes and represents one of the main outlets of the karstic system located in the Western Aurunci region (Central Italy). The spring is about 100 m far from the Tyrrhenian coast at the elevation of nearly 20 m a.s.l., predominantly fed by diffuse infiltration and partially by fracture and sinkhole fast-infiltration water. The Aurunci regional aquifer to which Mazzoccolo spring refers, is a low mountain karst with temperate climate, whose average altitude is about 600 m a.s.l. The Aurunci aquifer is karstified with diffuse landforms of sinkholes. The karstic habitats were sampled. Additionally drift samples were taken at Gizio and Mazzoccolo springs. The hyporheic habitats were sampled with a Bou-Rouch µm mesh net. Both epikarstic and saturated karstic habitats were sampled. Additional material of Acanthocyclops agamus was obtained by a drift sample from a large karstic spring of the Gizio River (Abruzzo, central Italy). This spring is the main outlet of the Genzana-Greco hydrogeological subunit (Genzana-Greco hydrogeological unit), and has a mean annual discharge of 4.5 m³/s. Another population of this species has been collected from the Mazzoccolo spring. This spring is managed for drinking purposes and represents one of the main outlets of the karstic system located in the Western Aurunci region (Central Italy). The spring is about 100 m far from the Tyrrhenian coast at the elevation of nearly 20 m a.s.l., predominantly fed by diffuse infiltration and partially by fracture and sinkhole fast-infiltration water. The Aurunci regional aquifer to which Mazzoccolo spring refers, is a low mountain karst with temperate climate, whose average altitude is about 600 m a.s.l. The Aurunci aquifer is karstified with diffuse landforms of sinkholes. The flow regime is heavily influenced by rainfalls (mean precipitation of 1300 mm year⁻¹), therefore it is quite variable along the whole hydrologic cycle. The total groundwater discharge at the Mazzoccolo spring ranges from 0.4 to 1 m³/s, with a mean annual value of 0.6 m³/s.

MATERIAL AND METHODS

Specimens were collected from different karstic habitats by filtering the groundwater close to the bedrock with a 100 µm mesh net. Both epikarstic and saturated karstic habitats were sampled. Additionally drift samples were taken at Gizio and Mazzoccolo springs. The hyporheic habitats were sampled with a Bou-Rouch pump (Bou & Rouch 1967).

Specimens were preserved in 7% formalin solution and dissected in polyvinyl lactophenol. Drawings and measurements were made using a camera lucida on a Leica DMLB phase-contrast microscope. The descriptive terminology of Huys & Boxshall (1991) is adopted. Abbreviations used in the text and figures are: P1-P6, first to sixth thoracopods; exp, exopod; enp, endopod; exp (enp) 1- (-2 -3 ) to denote the proximal (middle, distal) segment of a ramus.

SYSTEMATICS

Family Cyclopidae Burmeister, 1834

Subfamily Cyclopinae Dana, 1913, emend. Kiefer, 1927

Genus Acanthocyclops Kiefer, 1927

Acanthocyclops agamus Kiefer, 1938

Material examined: δ holotype, dissected and mounted on 3 slides (n. 3898-3899-3900) deposited at Staatliches Museum für Naturkunde Karlsruhe (Germany). Topotypes: 1 δ, completely dissected and mounted in polyvinyl lactophenol; 6 ♀♂ ♀ mounted in the same medium; Castelcivita Cave (Salerno, southern Italy); coordinates: 40°29′32″ N, 15°12′32″ E; altitude 94 m a.s.l.; small pool with siphon connection to a phreatic lake, about 3.5 km from the entrance; small amount of sediment on calcareous bottom; depth: 0.3-1.5 m; 30 September 1995; coll D Galassi, G Maj & G Costantino. 3♂ δ, completely dissected and mounted in the same medium, basal karstic spring of the Gizio River (Abruzzo, central Italy); coordinates: 41°58′07″N, 13°57′19″E; altitude 620 m a.s.l.; 25 January 1999; 18 October 2000; coll T Di Lorenzo. 76 specimens (♀♀♂♂) preserved in alcohol, Mazzoccolo spring (Latium, central Italy); coordinates: 41°15′17″N, 13°36′53″E; altitude: 20 m a.s.l.; April, May, June, August, September, October, November, December 2003; coll D Cipriani. One male and one female deposited at the Natural History Museum, London. Remaining material in D Galassi collection at the Dipartimento di Scienze Ambientali, University of L’Aquila, Italy.

Description

Male. Body length, excluding caudal setae, ranging from 451 µm in very contracted specimens to 554 µm in extended specimens (with mean of 506 µm based on 4 individuals). Prosome/urosome ratio: 1.63. Habitus slender in dorsal view (Fig. 1a), without surface ornament except for dorsal sensilla. Posterior margins of all prosomites and first urosomite smooth. Hyaline frills of abdominal somites with widely spaced indentations. Anal somite ornamented with spinules along posterior margin; surface naked except for paired dorsal sensilla. Anal operculum quadrate, smooth, protruding past distal margin of anal somite.

Caudal rami (Fig. 1a, b) about 2.8 times longer than wide, with 6 plumose setae. Seta I absent; seta II (lateral) with a row of spinules at its insertion; terminal accessory seta (VI) about 1.73 times longer than posterolateral seta (III); seta IV and V well-developed; dorsal seta (VII) slightly longer than ramus and inserted on small protuberance.

Rostrum (Fig. 1c) subtriangular in frontal view, with 2 sensilla.

Antennule (Fig. 1d) geniculate, with 16 segments; surfaces of segments apparently smooth.
Fig. 1. – Acanthocyclops agamus Kiefer, 1938 (male); a. habitus (topotype); b. abdomen (holotype); c. rostrum (topotype); d. antennule (topotype); e. labrum (topotype).
Setation formula as follows: 1- [8+2ae], 2-[4], 3-[2], 4-[2], 5-[2], 6-[2], 7-[2], 8-[1], 9-[2], 10-[2], 11-[1], 12-[1 spine], 13-[0], 14-[3 spines+1 striated element], 15-[1+1 striated element], 16- [10+1ae].

Antenna (Fig. 2a): coxa reduced and unarmed; basis ornamented with row of spinules on outer margin and bearing only 1 seta on anterolateral distal corner; exopod absent; endopod 3-segmented, first segment with 1 inner medial seta, second segment with 7 setae, third segment with 7 apical setae; all segments with a row of setules on outer margin.

Labrum (Fig. 1e) with teeth of different size on posterior margin and paired rows of long setules on ventral surface.

Paragnaths (Fig. 2b) consisting of paired lobes ornamented with usual rows of fine setules and 4 spines, one stouter, and row of small spinules along the inner margin.

Mandible (Fig. 2c) with well-developed coxal gnathobase, armed with teeth of different size and 1 bipinnate seta at dorsal corner; transverse row of long spines inserted subdistally on cutting blade; palp reduced, bearing 3 plumose setae, one very short.

Maxillule (Fig. 3a) comprised of praecoxa and 2-segmented palp. Praecoxal arthrite with 3 spines fused to segment and 1 articulated spine on apex; inner surface of arthrite armed with 4 spiniform setae and 1 densely plumose element. Palp composed of basis bearing stout pectinate spine and 2 bipinnate setae on inner margin, and 1 proximal outer seta representing exopod; distal segment of palp, representing endopod, armed with 3 long bipinnate setae.

Maxilla (Fig. 3b) 5-segmented; praecoxa with single distal endite armed with 2 plumose setae, coxa armament consisting of 1 plumose seta inserted on proximal endite and 1 seta and 1 spine, the spine ornamented with strong spinules, on well-developed distal endite; basis with a well-developed claw armed with spines on concave border, 1 bipinnate element and 1 naked seta. Endopod 2-segmented: first segment with 2 spiniform setae, second segment with 3 setae, one of these setae stout and spiniform.

Maxilliped (Fig. 3c) 4-segmented, comprising syncoxa, basis and 2-segmented endopod; syncoxa armed with 2 spinulose setae of different length; basis bearing 2 spinulose setae and a row of spinules along the inner margin; first endopedal segment with single spinulose element; second endopedal segment with 1 long spinulose seta and 1 shorter naked seta.

Segmental pattern of P1-P4 exopods and endopods: 2.2/2.2/3.2/2.2. Great variation has been observed in the segmentation patterns of different specimens. The male holotype shows P1-P2 (Fig. 4a-b) with 2-segmented exopod and endopod, P3 (Fig. 4c) with 3-segmented exopod and 2-segmented endopod; P4 (Fig. 5a) with 3-segmented exopod and 2-segmented endopod. Legs 1-4 (holotype) with spine formula 3.4.3.3. Nevertheless incomplete suture lines are still observable in the P2 exopod between the presumptive segments 2 and 3; and on the posterior surface of P3 exopod, in which the suture line is complete on the anterior surface and incomplete on the posterior surface. An asymmetrical presence of the suture line is evident in the P4 endopod, absent on the left leg and incipient on the right one. Examination of topotypic material and of additional material from Gizio and Mazzoccolo springs resulted in additional patterns observed. In particular, P1 and P2 are always 2-segmented in both exopod and endopod. The only topotypic male from Castelcivita Cave shows P3 and P4 exopods 2-segmented on posterior surface and with lateral and incomplete suture line on anterior surface; the P3-P4 endopods are always 2-segmented. Additionally the P4 (Fig. 5b) is anomalous in the absence of the inner seta on the exp-1, and asymmetrical in the armature of enp-2, bearing 2
apical spines on the orthodox ramus, and 1 spine
and 1 seta on the anomalous one. The populations
from Gizio and Mazzoccolo springs are more ho-
mogeneous: the males show P1-P4 with 2-seg-
mented rami (Figs. 4d; 5c).

Leg 1 (Fig. 4a) intercoxal sclerite, coxa and ba-
sis with smooth surfaces. A spinule row along the
coxo-basis boundary. Spinule rows present on the
free distal margin of the basis between the inser-
tions of the exopod and endopod and along the
inner margin. Coxa with inner bipinnate seta.
Basipodal outer seta very long. Exopod and
endopod 2-segmented. Exp-1 with outer spine and
a short inner seta; exp-2 with incipient suture on
the anterolateral side; armature consisting of
3 outer spines, 1 apical spine and 1 apical seta, and
4 inner setae. Enp-1 with 1 inner seta; enp-2 with
outer indentation marking original segmentation
between former segments 2 and 3; armature con-
sisting of 1 outer seta; 1 apical spine and 1 apical
seta, and 3 inner setae.

Leg 3 (Fig. 4c) intercoxal sclerite, coxa and ba-
sis with smooth surfaces. A spinule row inserted
along the coxo-basis boundary. Spinule rows pres-
ent on the free distal margin of the basis between
the insertions of the exopod and endopod and along
the inner margin. Coxa with inner bipinnate seta.
Basipodal outer seta relatively long and slender.
Exopod 3-segmented; endopod 2-segmented. Exp-
1 with outer spine and short inner seta; exp-2 with
outer spine and 1 inner seta; exp-3 with 2 outer
spines, 1 spine and 1 apical seta, and 3 inner setae.
Enp-1 with 1 inner seta; enp-2 with outer indenta-
tion marking original segmentation between former
segments 2 and 3; armature consisting of 1 outer
seta; 1 apical spine and 1 apical seta, and 4 inner
setae.

Leg 4 (Fig. 5a) intercoxal sclerite, coxa and ba-
sis with smooth surfaces. Spinule row along the
coxo-basis boundary. A spinule row present along
the inner margin of basis. Coxa with inner bipin-
nate seta. Basipodal outer seta short and tiny.
Exopod 3-segmented. Exp-1 with outer spine and
short inner seta; exp-2 (when distinct) with outer
spine and 1 inner seta; exp-3 with 2 outer spines,
1 apical spine and 1 apical seta, and 3 inner setae.
Enp-1 with 1 inner seta; enp-2 with 1 outer seta,
2 apical spines, 4 inner setae.

P5 (Fig. 5d): proximal segment well-defined at
base, bearing short basipodal outer seta; exopod
long, distinctly articulated to the proximal seg-
ment, bearing 1 long apical seta and a very short
subapical spine. P6 (Fig. 1b) inserted at the distal
part of the second urosomal somite, in ventrolateral
position, armed with 2 outer setae and 1 inner
spine.

Female. Body length, excluding caudal setae,
ranging from 480 to 609 µm (with mean of 554 µm
based on 6 individuals). Prosome/urosome ratio:
1.93. Habitus broad in dorsal view, without surface
ornament except for dorsal sensilla (Fig. 6a). Pos-
terior margins of all prosomites and first urosomal
segments. Genital double-somite (Fig. 6b) completely fused in both dorsal and ven-
Fig. 4. – *Acanthocyclops agamus* Kiefer, 1938 (male); a. P1 (holotype); b. P2 (holotype); c. P3 (holotype); d. P3 (Gizio spring, Italy).
Fig. 5. – *Acanthocyclops agamus* Kiefer, 1938 (male); a. P4 (holotype); b. P4 (topotype); c. P4 (Gizio spring, Italy); d. P5 (holotype).
tral surfaces, enlarged in proximal half, flattened dorsoventrally. Genital field developed in proximal half of genital double-somite (Fig. 6b).

Antennule (Fig. 6d) with 11 segments; surface of segments apparently smooth. Setation formula as follows: 1- [8], 2-[4], 3-[8], 4-[4], 5-[2], 6-[2], 7-[3], 8-[2+ae], 9-[2], 10-[3], 11-[7+ae].

Antenna, labrum, paragnaths, mandible, maxillule, maxilla, maxilliped as in male.

Segmental pattern of P1-P4 2.2/2.2/2.2/2.2. Spine formula: 3.4.4.4; setal formula: 5.5.5.5. P1 distinctly 2-segmented, as in male. P2-P4 (Fig. 7a-c) with incomplete anterolateral suture line marking original segmentation in exopods and an indentation, more or less evident, indicating original boundary between segments 2 and 3 is recognizable on the second segment of all endopodal rami.

Armature formula as in Table I [Roman numerals indicating spines, Arabic numerals representing setae; according to Sewell (1949)].

P5 (Fig. 7d): proximal segment well-defined at base, bearing short basipodal outer seta; exopod long, distinctly articulated to proximal segment, bearing 1 long apical seta and 1 very short subapical spine. P6 (Fig. 6b) inserted at middle of the genital
Remarks

Acanthocyclops agamus was originally described by Kiefer (1938) from one male from Castelcivita Cave (the type locality). The simple description, based only on one male, and the lack of females raised doubts as to the generic attribution of the species (Kiefer 1938, Dussart 1969, Dussart & Defaye 1985, Einsle 1996). The collection of a more consistent population from a siphon lake of Castelcivita Cave and the discovery of the female led us to expand the original description and assess the systematic position of the species in a more accurate way.

The genus Acanthocyclops is presently composed of some 58 species and subspecies, mostly holarctic in distribution. The total number of species is approximate, due to the continual transposition of species from this genus to Diacyclops Kiefer, 1927 and vice-versa. As a matter of fact, the systematic status of Acanthocyclops is complicated by its close affinity with the genus Diacyclops. According to present knowledge, the only difference between these genera supporting a vague and somewhat subjective taxonomic distinction is the morphology of the leg 5 (Kiefer 1929). In both genera it is composed of a distinct basipodal segment that is not fused [sometimes fused in Diacyclops according to Kiefer (1929)] to the somite, bearing 1 outer seta; and one free distal segment bearing 1 spine and 1 seta; in the genus Acanthocyclops the spine is short in respect to the length of the P5 distal segment and inserted in a subapical inner position, whereas in Diacyclops it is longer and apical or subapical. Nevertheless, the position and the relative length of this spine have been reported as variable within the same species [see Pandourski (1997) for variations observed in Acanthocyclops iskrecensis, 1992, A. balcanicus Naidenow & Pandurski, 1992, and A. strimonis (Pandourski 1994)]. Moreover, different degrees of development of this spine, as well as intermediate positions of the spine along the inner margin of the distal segment reported for several species that are presently assigned to both genera, make this character further confused or at least not always diagnostic (Rylov 1948, Damian-Georgescu 1963, Mazepova 1978, Boxshall et al. 1993, Reid & Strayer 1994). Recently, Mirabdullayev & Kuznetov (1997: 13) upon redescribing Acanthocyclops biarticulatus Monchenko, 1972 noted that “the morphology of P5 of A. biarticulatus corresponds to the genus Acanthocyclops, as well as to the genus Diacyclops, resulting in difficulties in separating these genera”. In this regard the validity of both genera was discussed and questioned by several authors, never reaching a final judgement by eventually proposing the synonymy of Diacyclops with Acanthocyclops [except for the position taken by Rylov (1948)], or alternatively by separating these genera on emended diagnoses. Characters, such as the segmental pattern of antennules and swimming legs, are not of great help to support monophyly since oligomerization occurred more than once in the evolutionary history of Cyclopinae as a whole, and, apparently, among lines currently assigned to Acanthocyclops and Diacyclops. Moreover, differences in microcharacters, such as the spinule patterns on the antennary basis (Fiers & Van De Velde 1984), as well as ornamentation of intercoxal sclerites, coxa and basis of legs 1-4, which in other cyclopids have been fruitful tools to recognize phylogenetic affinities among genera or at least for taxonomic distinction, proved to be unreliable in several Diacyclops and Acanthocyclops species, which lack most spinule series or any trace of ornamentation, and this situation further compounds the problem.

Difficulties in assessing the status of these genera, and their position in respect to related taxa (Rheocyclops Reid, 1999, Itocyclops Reid & Ishida, 2000, Reidycyclops Karanovic, 2000) arise from the low standard of descriptions of most species, as well as from circumstantial difficulties in tracing robust apomorphies. For instance, some species assigned to the genus Diacyclops and Acanthocyclops possess 2 setae instead of 1 seta and 1 spine on the distal segment of P5. This is the case for Diacyclops biceri Boxshall, Evstigneeva & Clark, 1993; D. neglectus Mazepova, 1984; D. arenosus (Mazepova, 1950); Acanthocyclops intermedius Mazepova, 1952; D. incolotenia Mazepova, 1950. The generic names of the species mentioned are given according to Dussart & Defaye (1985), although they are to be considered provisional, because it has been never stated which taxonomic criteria were adopted to transfer species from one genus to another. In a similar way, Morton (1985) transferred Acanthocyclops michaelseni (Mrázek, 1901), A. mirnyi Borutzky & Vinogradov, 1957, and A. skottsbergi Lindberg, 1949 to Diacyclops. Diacyclops hypogeus was transferred by Petkovski & Brancelj (1985) to the genus Acanthocyclops. Additionally, the former Diacyclops virginianus, showing 2 setae on the distal segment

Table I. – Armature formula of P1-P4 in Acanthocyclops agamus (female).

<table>
<thead>
<tr>
<th>Coxa</th>
<th>Basis</th>
<th>Exopod</th>
<th>Endopod</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>0-1</td>
<td>1-1</td>
<td>I-1; II,2,3</td>
</tr>
<tr>
<td>P2</td>
<td>0-1</td>
<td>1-0</td>
<td>I-1, III,1+1,4</td>
</tr>
<tr>
<td>P3</td>
<td>0-1</td>
<td>1-0</td>
<td>I-1, III,1+1,4</td>
</tr>
<tr>
<td>P4</td>
<td>0-1</td>
<td>1-0</td>
<td>I-1, III,1+1,4</td>
</tr>
</tbody>
</table>

double-somite, in ventrolateral position, consisting of small chitinous lamella bearing 2 stout spines and short inner seta.
Fig. 7. – *Acanthocyclops agamus* Kiefer, 1938 (female toptype); a. P2; b. P3; c. P4; d. P5.
of P5, is now formally transferred to the genus *Rheocyclops*, on the basis of the major autapomorphy represented by the basipodal segment of P5 fused to the somite and the distal segment bearing 2 setae (Reid et al. 1999). On the same grounds, Karanovic (2000) established the genus *Reidycyclops* on the basis of certain characters (shared also by other genera within the subfamily), the major autapomorphy being a distinct proximal segment of leg 5 together with a distal segment bearing 2 setae of about the same length. The same author included in this genus *Reidycyclops imparilis* (Monchenko 1985). This new combination is in our opinion not well grounded, especially because the structure of the leg 5, as described and figured by Monchenko (1985), is typical of the genus *Diacyclops* (Reid & Ishida 2000).

Moreover, in his diagnosis, great emphasis is given to the sexual dimorphism in the segmental pattern of legs of the species belonging to this group, but similar patterns are traceable in *Rheocyclops virginianus*, *R. hatchiensis* and in *Acanthocyclops agamus* also, although in the latter species the boundaries marking the original segmentation are also, although in the latter species the boundaries marking the original segmentation are variable and more or less pronounced in different individuals. Sexual dimorphism in the segmentation of legs occurred more than once in the evolutionary history of the Cyclopinae (Graeteriella brehni Lescher-Moutoué, 1968, G. rouchi Lescher-Moutoué, 1968, *Rheocyclops virginianus* (Reid, 1993), *Metacyclops planus* (Gurney, 1909), *Reidycyclops dimorphus* (Reid & Strayer, 1994), *Reidycyclops trajani* (Reid & Strayer, 1994), *Rheocyclops virginianus* (Reid, 1993), *Rheocyclops hatchiensis* Reid & Strayer, 1999, and members of *Bryocyclops* Kiefer, 1927, *Haplocyclus* Kiefer, 1952, and *Hesperocyclops* Herbst, 1984). In general, the male legs are less oligomerized (Reid & Strayer 1994, Reid et al. 1999). This situation occurs also in *Acanthocyclops agamus*. In this species the female possesses P1-P4 with 2-segmented rami; whereas an unstable situation is observable in males, in which the segmentation is variable between specimens and sometimes is also asymmetrical. Oligomerization is essentially the result of heterochrony, and it is very probable that the arrested development occurred at different times during growth in the two sexes. The female shows an arrested development early in respect to the male and consequently undergoes a more drastic and stable reduction in segmentation. Sometimes oligomerization is accompanied by reduction in leg setation (Reid & Strayer 1994). This is not the case for *A. agamus*, in which spine and setal patterns of all legs are typical of 3-segmented rami, although segments 2 and 3 are not separated.

Pending revision of the *Acanthocyclops-Diacyclops* complex, *Acanthocyclops agamus* is assigned to the genus *Acanthocyclops* on the only character presently available: the unsatisfactory leg 5 structure. Although "the structure of the leg 5 is considered conservative and is the base character for the present systematic arrangement of the Cyclopidae" (Reid & Ishida 2000: 594), several genera show a common "ground-structure" with few perceived differences, or these differences are distributed in a morphological continuum, bridging the gap between species belonging to different genera.

The 11-segmented antennule and the 2-segmented P1-P4 in the female place this species in the more derived group of *Acanthocyclops*. In this regard, Pandourski (1997) established a "kieferi-group" on the basis of the following characters: 11-segmented female antennule, 3-segmented P1-P4 exopods, spine formula: 2.3.5.3.; setal formula: 4.4.4.4. The same author excluded from the above group some other species showing the same characters on the basis of differences in body length (several Balkanian species) without any argumentation (*Acanthocyclops parvulus* Strayer, 1988, *Acanthocyclops exilis* Coker, 1934). Additionally, the inclusion of several *Diacyclops* species belonging to the stygius-group (Naidenow & Pandurski 1992b) deserves further analysis.

Our re-examination of *Acanthocyclops* species with 11 antennulary segments revealed that several species share these characters (Table II), and the kieferi-group may be polyphyletic or at least paraphyletic. From a mere taxonomic point of view, among the species showing 11 antennulary segments (Table II), *A. agamus* is unique in showing 2-segmented rami in P1-P4 legs (female) and a more labile segmental pattern in the male (P1-P2 always 2-segmented, and P3-P4 with 3- or 2-segmented exopod or with incipient segmentation line between segments 2 and 3 on anterior or posterior surfaces. In one male also the P4 endopod still shows a partial segmentation on the anterior surface, between the former segments 2 and 3). Nevertheless the total number of spines, as well as their topology in P1-P4 exp-3, is typical of 3-segmented exopods, and incipient indentation of segments is observable in P1 exp-2 (both male and female), in male P2 exopod (between former segments 2 and 3), in male P3 endopod, in female P2 endopod, and in female P3-P4 (both exopod and endopod). This pattern indicates that the arrested development is unstable and occurs late, during the last copepodid moult (CV). Within the genus *Acanthocyclops*, a tendency toward an incomplete segmentation between segments has also been reported as an anomaly in *Acanthocyclops rhenanus* by Kiefer (1957). Similarly, *Rheocyclops virginianus* shows the same instability (Reid 1993).

*Acanthocyclops agamus* is also reduced in the armature of the antennary basis, the exopodal seta being missing. The exopodal seta is absent in several derived species belonging to different genera of Cyclopidae and obviously also the genus *Acanthocyclops*. Confining the discussion to strictly related species of the same genus, *Acanthocyclops balcanicus* lacks the exopodal seta, but shows the
remaining 2 distomedial setae [only 1 seta was reported in the original description (Naidenow & Pandourski 1992a), two setae in the redescription given by Pandourski (1994)]. The exopodal seta is missing in A. propinquus (Plesa, 1957), A. biarticulatus Monchenko, 1972, as redescribed by Mirabdullayev & Kuzmetov (1997), A. plesai lepoure, 2001 and A. balcanicus bisaetosus Iepure, 2001.

Acanthocyclops agamus lacks also the inner medial seta of the antennary basis, a reduction in setation shared with a few members of the subfamily Cyclopinae (Diacyclops harryi Reid, 1992; Itocyclops yezoensis (Ito, 1953); Rheocyclops virginianus, Rheocyclops carolinianus Reid, 1999; R. hatchiensis Reid & Strayer, 1999; Rheocyclops talladega Reid & Strayer, 1999).

Acanthocyclops agamus seems to be only distantly related to A. petkovskii Pesce & Lattinger, 1983, in respect to the relative length of the apical spines on female P4 exp-2, as originally described by Pesce & Lattinger (1983), although great differences are observable with the redescription of the same species given by Einsle (1996). Among the more derived species within the genus, A. agamus shows also feeble affinities with A. biarticulatus in the tendency to oligomerization of the swimming legs (3.2/3.2/3.2/3.2), the length of the caudal rami, the length ratio between setae III and VI, the length ratio between apical spines of P4 exp-2, and the presence of indentations on P1-P2 exp-2, marking the original segmentation between former segments 2 and 3. With Acanthocyclops hypogaeus (Kiefer, 1930) A. agamus shares the relative length of apical spines of P4 exp-3, the relative length of caudal rami, the armature of leg 5, bearing a seta and a very small inner subapical spine, and a trend toward oligomerization of P1-P4 (2.2/3.2/3.3/3.3). With Acanthocyclops reductus Chappuis, 1925 and A. agamus shares the presence of a protruding anal operculum, although rounded and not serrate. Nevertheless, a developed anal operculum is shared also by other Acanthocyclops species (e.g., A. parvulus, A. strimonis Pandourski, 1994, Diacyclops chappusi Naidenow & Pandourski, 1992).

The species shows an interesting convergence with Rheocyclops virginianus in the identical chaetotaxy of P1-P2 exp-2 and of P3-P4 exp-3, in which the original armature of a 3-segmented exopod is maintained. In the same way, the female shows P1-P4 2-segmented rami (as in A. agamus) and the male shows the same instability of A. agamus (2-segmented P1-P2, P1-P4 with 2- or 3-segmented exopods and 2-segmented endopods).

### Table II. – Acanthocyclops species with 11-segmented antennule.

<table>
<thead>
<tr>
<th>Species</th>
<th>P1-P4 segmental pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthocyclops agamus Kiefer, 1938</td>
<td>2.2/2.2/2.2/2.2</td>
</tr>
<tr>
<td>Acanthocyclops balcanicus Naidenow &amp; Pandourski, 1992</td>
<td>3.2/3.3/3.3</td>
</tr>
<tr>
<td>Acanthocyclops balcanicus bisetosus lepore, 2001</td>
<td>3.2/3.3/3.3</td>
</tr>
<tr>
<td>Acanthocyclops biarticulatus Monchenko, 1972</td>
<td>3.2/3.2/3.2/2.2</td>
</tr>
<tr>
<td>Acanthocyclops exilis (Coker, 1934)</td>
<td>3.3/3.3/3.3</td>
</tr>
<tr>
<td>Acanthocyclops hispanicus Kiefer, 1937</td>
<td>3.2/3.2/3.3</td>
</tr>
<tr>
<td>Acanthocyclops hypogaeus (Kiefer, 1930)</td>
<td>2.2/3.2/3.3</td>
</tr>
<tr>
<td>Acanthocyclops iskrecensis Pandourski, 1992</td>
<td>3.2/3.2/3.3</td>
</tr>
<tr>
<td>Acanthocyclops kieferi (Chappuis, 1925)</td>
<td>3.3/3.3/3.3</td>
</tr>
<tr>
<td>Acanthocyclops michaelis (Mrazek, 1901)*</td>
<td>3.3/3.3/3.3</td>
</tr>
<tr>
<td>Acanthocyclops notabilis Mazepova, 1950</td>
<td>3.3/3.3/3.3</td>
</tr>
<tr>
<td>Acanthocyclops parvalus Strayer, 1988</td>
<td>3.3/3.3/3.3</td>
</tr>
<tr>
<td>Acanthocyclops petkovskii Pesce &amp; Lattinger, 1983</td>
<td>3.3/3.3/3.3</td>
</tr>
<tr>
<td>Acanthocyclops plesai lepoure, 2001</td>
<td>3.2/3.2/3.3</td>
</tr>
<tr>
<td>Acanthocyclops profundos Mazepova, 1950</td>
<td>3.3/3.3/3.3</td>
</tr>
<tr>
<td>Acanthocyclops profundos tomito Mazepova, 1950</td>
<td>3.3/3.3/3.3</td>
</tr>
<tr>
<td>Acanthocyclops propinquus (Plesa, 1957)</td>
<td>3.2/2.2/3.3</td>
</tr>
<tr>
<td>Acanthocyclops radevi Pandourski, 1993</td>
<td>3.2/3.2/3.3</td>
</tr>
<tr>
<td>Acanthocyclops reductus (Chappuis, 1925)</td>
<td>3.2/2.3/3.3</td>
</tr>
<tr>
<td>Acanthocyclops sambugarae Kiefer, 1981</td>
<td>3.3/3.3/3.3</td>
</tr>
<tr>
<td>Acanthocyclops similis Flössner, 1984</td>
<td>3.3/3.3/3.3</td>
</tr>
<tr>
<td>Acanthocyclops strimonis (Pandourski, 1994)</td>
<td>3.2/3.2/3.3/3.3</td>
</tr>
</tbody>
</table>

* MENU MARQUE (1991) synonymized A. scotsbergi with this species.

Speocelops italicus Kiefer, 1938

Material examined: 1♀, 2 copepods, completely dissected and mounted in polyvinyl lactophenol; Castelcivita Cave (Salerno, southern Italy); coordinates: 40°29'42" N, 15°12'32" E; altitude: 94 m a.s.l.; phreatic lake, about 3.5 km from the entrance; large amount of silt on calcareous bottom; depth: 4 m; 25.6.1995; coll D Galassi & G Costantino. 5 ♀ ♂, 2 copepods, completely dissected and mounted in polyvinyl lactophenol; stream Calore, upwelling areas, close to the entrance of Castelcivita Cave, same hydrological system of Castelcivita cave. 5 ♀ ♂, 4 copepods, completely dissected and mounted in polyvinyl lactophenol; stream Calore, upwelling areas, close to the entrance of Castelcivita Cave, same hydrological system of Castelcivita cave. 5 ♀ ♂, 1 ♀ ♂, 1 copepodid, Pertosa Cave (Salerno, southern Italy); concretional pools; coordinates: 40°32'15" N, 15°27'20" E; altitude: 263 m a.s.l.; 26.6.1995; coll D Galassi. 1 ♀ ♂, 1 ♀ ♂ deposited at the Natural History Museum (London). Material consulted: ♀ holotype marked TYPUS (slides nos. 3893, 3894 and 3895), deposited at Staatliches Museum für Naturkunde Karlsruhe (Germany) (mouthparts not observable); 1 slide (3896) with 2 undissected ♀ ♀ (deteriorated); 1 slide (3897) with 1 undissected ♀ ♀ (deteriorated).

Additional material examined: 5 ♀ ♀, 1 ♀ ♀ completely dissected and mounted in polyvinyl lactophenol, hyporheic habitat, Mugnone stream, Olmo, Firenze (Italy); ♀ ♀, completely dissected and mounted in polyvinyl lactophenol, hyporheic habitat, Candigliano stream, Piobbico, Pesaro-Urbino (Italy); 1 ♀ ♂, completely dissected and mounted in polyvinyl lactophenol, hyporheic habitat, Velino River, Antrodoco, Rieti (Italy); ♀ ♀, completely dissected and mounted in polyvinyl lactophenol, phreatic habitat (well), Madonna del Piano, Monte Castello Vibio, Perugia (Italy).

Description

Female. Body length, excluding caudal setae, with mean of 483 µm, range 392 to 540 µm (based on 8 individuals). Habitus compact in dorsal view (Fig. 8a) with irregular pitting and dorsal sensilla. Prosome only slightly wider than urosome. Prosome: urosome ratio 1.65. Hyaline frills of cephalothorax and somites bearing P2-P5 smooth. Genital and first abdominal somites distinct on dorsal surface, completely fused on ventral side; with a posterior serrate fringe both dorsally and ventrally. Dorsal suture line between genital segment and first abdominal somite feebly indent. Genital field (Fig. 8b) located on the second third of genital double-somite. Anterior part of genital field bilobed, hardly observable. Copulatory pore located on distal third of genital double-somite. Anal somite ventrally ornamented with strong spines; paired sensilla located dorsally; anal operculum ovate, with irregularly toothed margin (Fig. 8a, c).

Caudal rami (Fig. 8 a-c) slightly longer than wide, with 6 bipinnate setae; hind margin with spines ventrally. Seta I absent; seta II located near middle of outer margin; posterolateral seta (III) about 1.74 times longer than terminal accessory seta (VI); seta IV and V well-developed; dorsal seta (VII), inserted on dorsal chitinous ridge, about 1.58 times longer than caudal ramus and with a row of spines of its insertion.

Rostrum (Fig. 8a) subtriangular in frontal view, with 2 sensilla.

Antennule (Fig. 8d) 11-segmented; surfaces of segments smooth, except for ventral spineule row on segment 1. Armature formula: 1- [8], 2- [4], 3- [8], 4- [2], 5- [2], 6- [2], 7- [3], 8- [2+ ae], 9- [2], 10- [2+ae], 11- [7+ae]; most setae sparsely plumose.

Antenna (Fig 9a): coxa reduced; basis bearing 2 bipinnate setae on antero-distal angle; exopodal seta absent; endopod 3-segmented, first segment with 1 inner medial seta, second segment with 7 setae and a row of setules on outer margin, third segment armed with 7 setae and 2 rows of setules on outer margin.

Labrum (Fig. 9b) with 12-14 blunt central teeth between produced lateral corners; paired transverse rows of long setules on ventral surface.

Paragnaths (Fig. 9c) consisting of paired lobes bearing 4 well-developed setae, one seta stouter and spiniform, and a row of spinules along the inner margin.

Mandible (Fig. 9d) with complex biting edge formed by sharp teeth and 2 bipinnate setae; transverse row of long spinules inserted subdistally on cutting blade; palp absent.

Maxillule (Fig. 9e) composed of well-developed praecoxa and 2-segmented palp. Praecoxa bearing 3 spines fused to segment and 1 articulated spine on apex of arthrite; inner surface of arthrite armed with 4 naked setae, 1 spine and 1 densely plumose element. Palp comprising coxobasis with 1 unipinnate spine and 2 setae distally, and 1 bipinnate seta (vestigial exopod) on outer margin; distal segment of palp, representing endopod, armed with 3 long bipinnate setae.

Maxilla (Fig. 9f) comprising syncoxa, basis and 2-segmented endopod. Syncoxa with 3 endites, proximal endite armed with 2 plumose setae, medial endite represented by single plumose seta, distal endite bearing 2 spiniform setae; basis with a well-developed claw armed with very fine teeth, 1 stout unipinnate element and 1 naked seta. Endopod segments 1 and 2 not well distinguishable; first segment with 2 spiniform setae, second segment with 1 stout element plus 2 bipinnate setae.

Maxilliped (Fig. 9g) 4-segmented, comprising syncoxa, basis and 2-segmented endopod; syncoxa bearing 1 spinulose seta; basis with 1 spinulose seta and a row of spinules along the inner margin; first endopodal segment bearing single spinulose element and spinules around base; second endopodal segment partially incorporated into the first segment, with 1 spinulose and 1 shorter naked setae.
Fig. 8. – *Speocyclops italicus* Kiefer, 1938 (female); a. habitus (Pertosa Cave, southern Italy); b. abdomen (topotype); c. anal somite and caudal rami (topotype); d. antennule (topotype).
Fig. 9. – *Speocyclops italicus* Kiefer, 1938 (female, Pertosa Cave, southern Italy); a. antenna; b. labrum; c. paragnath; d. mandible; e. maxillule; f. maxilla; g. maxilliped.
Fig. 10. – *Speocyclops italicus* Kiefer, 1938 (female topotype); a. P1; b. P2; c. P3; d. P4; e. P5; f. P6.
P1-P4 (Fig. 10 a-d) with 2-segmented rami; enp-1 and enp-2 similar in length; exp-2 about 2 times longer than exp-1. P4 enp-2 about 1.33 times longer than wide; apical spine about 1.27 times longer than segment. Intercoxal sclerites without ornamentation. Coxa armed with inner plumose seta and a row of minute spinules along distal margin; posterior surface with a row of spinules near outer margin. Basis with outer seta plumose, ornamented by a row of spinules near articulation with endopod and a row of setules along inner margin. P1 basis with bipinnate spine inserted at inner corner, reaching distal edge of proximal endopodal segment. Armature formula as in table III (Roman numerals indicating spines, Arabic numerals representing setae).

Table III. – Armature formula of P1-P4 in *Speocyclops italicus* (female and male).

<table>
<thead>
<tr>
<th></th>
<th>Coxa</th>
<th>Basis</th>
<th>Exopod</th>
<th>Endopod</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>0-1</td>
<td>1-1</td>
<td>I-0; II,2,3</td>
<td>0-1; I,1,2</td>
</tr>
<tr>
<td>P2</td>
<td>0-1</td>
<td>1-0</td>
<td>I-0; II,3,1,3</td>
<td>0-1; I,1,2</td>
</tr>
<tr>
<td>P3</td>
<td>0-1</td>
<td>1-0</td>
<td>I-0; II,3,1,3</td>
<td>0-1; I,1,3</td>
</tr>
<tr>
<td>P4</td>
<td>0-1</td>
<td>1-0</td>
<td>I-0; II,1,2</td>
<td>0-1; I,1,2</td>
</tr>
</tbody>
</table>

P5 (Fig. 10e): exopod not defined at base, still distinguishable, incorporated into the P5-bearing somite, armed with 2 bipinnate setae similar in length; basipodal outer seta inserted on small prominence, adjacent to the reduced exopod, incorporated into the P5-bearing somite.

P6 (Fig. 10f) represented by chitinous lamella apparently fused to the somite, armed with 1 outer bipinnate seta and 2 spines, the inner spine stouter.

Male. Body length, excluding caudal setae, ranging from 422 to 470 µm, with mean of 439 µm (based on 3 individuals). Prososome: urosome ratio 1.44 (Fig. 11a). Urosome (Fig. 11b) with six somites. General ornamentation of body somites and caudal rami as in female. Sexual dimorphism in body size, abdominal segmentation, antennule and P6. Antennule (Fig. 11c) geniculate, with 16 segments; surface of segments smooth, except for ventral spinule row on segment 1. Setation formula as follows: 1- [8+2 ae], 2-[4], 3-[2], 4-[2], 5-[2], 6-[2], 7-[1], 8-[1], 9-[2], 10-[1], 11-[2], 12-[1+spine], 13-[0], 14-[1 striated element], 15-[1+striated element], 16-[8+ae]; most setae sparsely plumose.

P6 (Fig. 11b) represented by chitinous lamella articulated with the somite, bearing 1 inner stout spine and 2 setae subequal in length.

The male is identical to the female in all other respects.

Remarks

*Speocyclops italicus* was originally described by Kiefer (1938) from Castelcivita Cave. Following this discovery, the species has been reported from several localities in central and southern Italy, from both phreatic and hyporheic habitats (Kiefer 1938, Pesce 1980, Pesce & Galassi 1983, Pesce et al. 1987, Pesce & Maggi 1979). Re-examination of specimens from the populations assigned to this species confirmed their attribution to *S. italicus*, which shows a wide geographical range in central and southern Italy. Some variability was observed in the following characters: a) relative length of setae III and VI of caudal rami (from 1.26 to 2.33 in specimens collected from Castelcivita and Pertosa caves and Calore stream; from 2.05 to 2.12 from Umbria, from 1.33 to 1.94 from Tuscany); b) appearance of the dorsal suture line of the genital double-somite (more or less serrate depending on the individual specimen examined); c) degree of fusion between segments forming the genital double-somite in the female. The common situation is the presence of a dorsal suture marking the original segmentation vs. ventrally fused; however in the populations from Tuscany a variable degree of fusion was observed: from specimens in which apparently the suture line is absent to those in which it is evident only on the dorsolateral sides. A questionable specimen has been discovered in Latium (Velino River) in which the suture line is incomplete (only along the dorsolateral sides). A questionable specimen has been discovered in Latium (Velino River) in which the suture line is incomplete (only along the dorsolateral sides). A questionable specimen has been discovered in Latium (Velino River) in which the suture line is incomplete (only along the dorsolateral sides).

ECOLOGICAL AND BIOGEOGRAPHICAL CONSIDERATIONS

It is becoming evident that groundwater biodiversity is higher than previously thought. Until now this kind of biodiversity has been neglected for several reasons, and especially for the low level of knowledge of the groundwater ecosystems, and related fauna, that prevent any kind of further elaboration of data. Copepods are one of the major components of the groundwater fauna (Galassi 2001) and surely may be considered as a focal group *sensu* Hammond (1995) in the evaluation of the qualitative status of these ecosystems, as well as to propose new tools for conservation priorities of the groundwater habitats. Conservation strategies should take into account not only the overall biodiversity of a given stygohabitat but also the taxonomic dispersion of such a diversity (Gaston 1996, Danielopol et al. 2002, Danielopol & Pospisil 2002). As a rule, groundwater harbours poor com-
Communities, in terms of both species richness and abundance. This is the case for the saturated karst of Castelcivita Cave, as well as for the Genzana Massif and the Western Aurunci range aquifers, from which *Acanthocyclops agamus* has been collected. The species appears with a low number of individuals, and the associated fauna is composed of a few species, all in low abundances. In Castelcivita Cave, *A. agamus* lives together with *Speocyclops italicus*, *Diacyclops zschokkei* (Graeter, 1910) and an undescribed species of *Eucyclops* Claus, 1893, belonging to the "subterraneus-group" according to Pesce & Galassi (1983), and with the ectinosomatid harpacticoid *Pseudectinosoma kunzi* Galassi, 1997. In the karstic spring of the Gizio River (Genzana Massif) it lives together with

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![Fig. 11. – *Speocyclops italicus* Kiefer, 1938 (male); a. habitus (Pertosa Cave, southern Italy); b. urosome (Calore brook, southern Italy); c. antennule (Calore brook, southern Italy).](image_url)
Pseudectinosoma reductum Galassi & De Laurentiis, 1997; Moraria stankovitchi Chappuis, 1923; Diacyclops paolae Pesce & Galassi, 1987; Nitocrella kunzi Galassi & De Laurentiis, 1997; Nitocrella sp.; and Parastenocaris sp. In the Mazzoccolo karstic spring, A. agamus co-occurs with a new species of Pseudectinosoma Kunz, 1935 and Parastenocaris oricina Chappuis, 1938. In these cases, the biological diversity is low, but the taxonomic dispersion of the diversity is relatively high.

In particular, in Castelcivita Cave, both Acanthocyclops agamus and Pseudectinosoma kunzi are to be considered ancient colonizers of this karstic system, although of quite different origin, because the cyclopoid A. agamus has a direct freshwater origin, whereas the harpacticoid P. kunzi has marine origin. Whereas a paleogeographical scenario has been illustrated to explain the phyleogeography of P. kunzi and related species (Galassi et al. 1999), the origin of A. agamus is more difficult to reconstruct; firstly, because the relationships of this species within the genus Acanthocyclops are quite unclear; secondly, this species shows an enigmatic disjunct distribution in three different karstic aquifers in central and southern Italy, which have been separated since the Tertiary. Dispersion events leading to the present-day distribution are unlikely, since the species is strictly confined to saturated karst and no records are available from other stygohabitats, also in the same cave and in the same aquifer. For instance, extensive sampling campaigns in Castelcivita Cave, in different stygohabitats (concretion pools, tricklets, gours) resulted in the discovery of quite different copepod assemblages, but A. agamus has never been collected in those habitats. An alternative hypothesis may be that these three populations are cryptic sister-species. However, accurate examination, on microcharacters also, did not provide any morphological difference.

It is difficult to evaluate the degree of adaptation of this species to the groundwater environment but it seems to be very high. A. agamus is depigmented and anophthalmic, it shows a compact body (especially the female), a large genital double-somite relative to the body, and especially it seems to have originated by heterochrony from a surface ancestor, a widespread event in several stygobiont taxa belonging to different phylogenetic lineages. Heterochrony is considered adaptive to the groundwater life, because it is frequently linked to oligemerization and miniaturization (Reid & Strayer 1994, Galassi 2001). From a biogeographical point of view, A. agamus is to be considered a relic element, presently recorded from Gizio and Mazzoccolo springs, and Castelcivita Cave; in these systems it appears with relatively small populations, more frequently linked to the capacitive annex sub-systems, and consequently it should be considered critically endangered according to the IUCN (2000).

Contrarily to A. agamus, Speocyclops italicus is widespread in central and southern Italy. Its wide geographical distribution is presumably linked to a wider ecological tolerance, as the species has been collected from different stygohabitats (saturated karst, epikarstic and vadose zones, hyporheos, phreatic waters).

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