Observations on the “tenuis group” (Eutardigrada, Macrobiotidae) and description of a new Macrobiotus species

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Abstract
A new species of the tardigrade genus Macrobiotus is described. The species, designated M. ciprianoi n. sp., was isolated from a mixture of Provence broom leaf litter and mosses, and from rock mosses collected in the Sierra de Guadarrama, Madrid (Spain). Given that Macrobiotus ciprianoi n. sp. shares several characters to members of the “tenuis group”, we assessed the taxonomic homogeneity of the group. The new species differs from those of the “tenuis group” according to a unique set of characters related with claw shape, features of the buccal-pharyngeal apparatus, and egg morphology. Our analysis of holotypes and/or paratypes of “tenuis group” species and other Macrobiotus species with similar characters (M. bondavallii and M. caelicola) reflects the heterogeneity of this group of species as currently described.

Keywords: Eutardigrada, Iberian Peninsula, Macrobiotus ciprianoi n. sp., Sierra de Guadarrama, tenuis group

Introduction
Maucci (1987a) identified a group of Macrobiotus species, which he subsequently formalized and called the “tenuis group”, and indicated that “it often proves difficult to discriminate between these species [tenuis group] in the absence of eggs, which are, on the contrary, always easy to identify”. The common characters of these species are: (1) a yellowish or light brown colour; (2) large size (over 600 μm long); (3) short ventral lamina (strengthening bar); (4) styllet support inserted a long distance from the posterior end of the buccal tube; (5) two macroplacoids, the first with a middle constriction that can be very deep; in such cases, the first macroplacoid can be interpreted as two; (6) slanting cuticular bars (between the apophyses and first macroplacoid) inside the pharynx; (7) long and thin double-claws, with secondary branches much shorter than primary branches, connected at
roughly right angles; (8) hind claws always clearly larger than the others; and (9) large lunules, those of the fourth pairs larger than any other and with more or less clear dentations. Maucci (1987a) identified the following species within this group: _Macrobiotus tenuis_ Binda and Pilato, 1972, _Macrobiotus willardi_ Pilato, 1977, _Macrobiotus mongolicus_ Maucci, 1987b, _Macrobiotus ariekammensis_ Węglarska, 1965, _Macrobiotus hyperonyx_ Maucci, 1982, _Macrobiotus higginsi_ Maucci, 1987a, and _Macrobiotus hystricogenitus_ Maucci, 1978. In 1999, Biserov added to this group _Macrobiotus kozharai_ Biserov, 1999. Tumanov (2005, 2007), besides defining the “tenuis-type” of claw excluding the group _M. ariekammensis_, added to this group _Macrobiotus danilovi_ Tumanov, 2007, _Macrobiotus tenuiformis_ Tumanov, 2007, and _Macrobiotus voronkowi_ Tumanov, 2007.

The polyphyletic status of the genus _Macrobiotus_ (one of the largest tardigrade genera) (Guidetti and Bertolani 2001; Guidetti et al. 2005, 2007) and the finding of a new _Macrobiotus_ species with several characters common to the “tenuis group” prompted us to examine known species of this group, to evaluate their homogeneity as a taxonomic group with a view to the future systematic re-organization of the genus.

In the Iberian Peninsula, 23 _Macrobiotus_ species have been identified (out of 150 species of the entire genus), but only 14 have been observed in the Madrid province (Guil 2002, 2004; Guil and Guidetti 2005). This new _Macrobiotus_ species found in a xerophilous shrub landscape of the granite mountain range of Sierra de Guadarrama, Madrid (Spain) thus adds to the number of species in this area.

**Materials and methods**

**New species**

Seventeen specimens and five eggs (none with an embryo) of the new species were isolated from samples of a mixture of Provence broom (_Cytisus purgans_ (L.)), leaf litter and mosses (_Isothecium myosuroides_ Bridel, 1827; _Hypnum cupressiforme_ Hedwig, 1801) and from samples of rock mosses (_Tortella tortuosa_ (Hedwig) Limpricht, 1888), all collected from Sierra de Guadarrama (Madrid) in central Spain. Litter and moss samples were placed in water and left to soak overnight, after which samples were agitated, squeezed, and poured through two sieves (1.0 mm and 80.0 μm meshes). Sediment was collected into a Petri dish and then fixed with Carnoy liquid (three parts ethanol: one part acetic acid). Individual animals and eggs were mounted on microscope slides using Faure mounting medium (Ramazzotti and Maucci 1983).

**Further material examined**

Neotype of _Macrobiotus hufelandi_, C. A. S. Schultzze, 1834 (slide C455-s65), paratype of _Macrobiotus bondavallii_ Manicardi, 1989 (slide C990-s19), and a specimen of _M. ariekammensis_ from Russia (slide C.T.1566(7)) obtained from Bertolani’s collection at the Animal Biology Department of the University of Modena and Reggio Emilia, Italy; holotypes of _M. hyperonyx_ (slide C.T.9981), _M. hystricogenitus_ (slide C.T.5049), _M. mongolicus_ (slide C.T.12852), and _M. higginsi_ (slide C.T.12621); paratypes of _M. hyperonyx_ (C.T.9982), _M. higginsi_ (slide C.T.12621), _M. willardi_ (slide C.T.7326), and _M. adelges_ Dastych, 1977 (slide C.T.11142; this last species has been described by Dastych 1985 as a synonym of _M. ariekammensis_); specimens of _M. tenuis_ from Italy (slides C.T.2039, C.T.2040), all belonging to Maucci’s collection of the Museum of Natural History of
Verona (Italy); and four paratypes of *Macrobiotus caelicola* Kathman, 1990 (slides A62/95 35-38) from the Zoologisches Museum Hamburg (Germany).

Measurements were made at the highest magnification (×100) using a light microscope (LM) equipped for oil immersion differential interference contrast (DIC) and/or phase contrast (PhC) microscopy. The *pt* ratio reported in Tables I and II is the ratio of the length of a given structure to the length of the buccal tube (measured from the medio-dorsal ridge of the buccal armature to the end of the buccal tube), expressed as a percentage (Pilato 1981).

**Species description**

**Class** EUTARDIGRADA Marcus, 1927  
**Order** PARACHELA Schuster, Nelson, Grigarick, and Christenberry, 1980  
**Family** MACROBIOTIDAE Thulin, 1928  
**Genus** Macrobiotus Thulin, 1928  
*Macrobiotus ciprianoi* new species  
(Figures 1–3, 4A)

**Material examined**

**Holotype.** Holotype 535 μm long (see Table I), sex undetermined. Deposited in the non-insect invertebrate collection of the Natural History Museum of Madrid, Spain (Consejo Superior de Investigaciones Científicas, CSIC) (catalogue no. 23.00/2).

**Locus typicus.** Xerophilous shrub landscape close to the road from Redueña to Cabanillas at 880 m a.s.l. in Madrid (Spain; UTM 30T0449010-4519466); samples contained a mixture of Provence broom (*Cytisus purgans* (L.)), leaf litter and mosses (*Isothecium myosuroides* Bridel, 1827, and *Hypnum cupressiforme* Hedwig, 1801) and were collected by N. Guil (8 November 2001). Associated tardigrade fauna: *Diphascon (Diphascon) recamieri* (Richters, 1901).

**Paratypes.** Three specimens (see Table I) deposited in the non-insect invertebrate collection of the Natural History Museum of Madrid, Spain (Consejo Superior de Investigaciones Científicas, CSIC).

**Further material.** Samples of rock mosses (*Tortella tortuosa* (Hedwig) Limpricht, 1888) collected in the same area by N. Guil contained 13 specimens of the new species and four eggs. Seven specimens and three eggs were identified in samples collected in spring (12 May 2001) and six specimens and one egg in samples collected in autumn (8 November 2001). Associated fauna were: *Macrobiotus richtersi* Murray, 1991 (adults and eggs) in spring; and *Echiniscus canadensis* Murray, 1910 in autumn.

**Diagnosis**

Smooth cuticle lacking pearls (pores); colourless; dots present in the external base of all legs; two macroplacoids, the first with a deep middle constriction, and a microplacoid; claws with secondary branch inserted in main branch at a right angle; large lunules, those of the hind legs larger and with dentate or irregular margins; eggs with cone-shaped processes, the latter with a reticular network on their surface.
Table I. Measurements (μm) for each specimen of *Macrobiotus ciprianoi* n. sp.: holotype and paratypes were recovered from Provence broom leaf litter mixed with mosses collected in autumn; specimens 1–3 are from rock mosses collected in spring; and specimens 4–9 were collected from rock mosses in autumn (four of the 13 specimens from rock mosses collected in spring were folded, so measurements are not included in the tables).

| Position | tbl | btl | vl | pt-vl | ssi | pt-ssi | btd | prl | fpl | spl | m | pb2-3 | pt-pb2-3 | sb2-3 | pb4 | pt-pb4 | sb4 |
|----------|-----|-----|----|--------|-----|--------|-----|-----|-----|-----|---|-------|---------|-------|-----|-------|-----|
| Holotype | 535.0 | 41.2 | 20.8 | 50.4 | 31.4 | 76.2 | 3.9 | 22.5 | 10.8 | 4.9 | 2.9 | 10.8 | 26.2 | 5.9 | 11.8 | 28.6 | 6.9 | Ventral |
| Paratype 1 | 544.5 | 38.2 | – | – | 23.5 | 61.5 | 3.9 | 22.5 | 10.8 | 5.4 | 2.9 | – | – | – | – | – | L-V |
| Paratype 2 | 554.4 | 41.2 | 31.6 | 76.7 | 25.5 | 61.9 | 3.9 | 25.0 | 12.7 | 6.9 | 3.9 | – | – | – | – | – | Lateral |
| Paratype 3 | 297.0 | 34.3 | – | – | 26.5 | 77.1 | 2.9 | 14.7 | 7.3 | 3.9 | 2.0 | 8.8 | 25.7 | 3.9 | – | – | – | L-V |
| Specimen 1 | 207.9 | 27.4 | – | – | 20.0 | 73.0 | 3.9 | 14.7 | 5.8 | 2.0 | – | – | – | – | – | – | L-V |
| Specimen 2 | 356.4 | 43.1 | 28.7 | 66.5 | 33.3 | 77.3 | 3.9 | 22.5 | 10.8 | 5.4 | 2.9 | – | – | – | – | – | Lateral |
| Specimen 3 | 435.6 | – | 31.6 | – | – | – | – | 22.5 | 10.3 | 5.9 | 3.4 | – | – | – | – | – | Lateral |
| Specimen 4 | 341.6 | 36.3 | 22.7 | 62.6 | 22.5 | 62.2 | 4.9 | 23.5 | 10.3 | 5.4 | 2.0 | 8.8 | 24.3 | 6.9 | – | – | – | Lateral |
| Specimen 5 | 207.9 | – | – | – | – | – | 2.9 | – | – | – | – | 6.9 | 3.4 | – | – | – | Ventral |
| Specimen 6 | 475.2 | 44.1 | 23.7 | 53.8 | 34.8 | 78.9 | 3.4 | 21.6 | 10.3 | 5.4 | 2.9 | 11.8 | 26.7 | 7.8 | 13.7 | 31.1 | 8.3 | Lateral |
| Specimen 7 | 524.7 | 46.1 | 26.7 | 57.9 | 35.8 | 77.7 | 3.9 | 26.5 | 12.3 | 5.9 | 3.9 | – | – | – | – | – | Lateral |
| Specimen 8 | 514.8 | 48.0 | 33.6 | 70.0 | 37.2 | 77.6 | 4.4 | 25.5 | 11.8 | 6.9 | 3.9 | – | – | – | 16.7 | 34.7 | 8.8 | Lateral |
| Specimen 9 | 544.5 | 45.1 | – | – | 35.8 | 79.4 | 3.9 | 27.4 | 13.8 | 6.4 | 3.9 | – | – | – | – | – | L-V |
| Range | 207.9–274.4 | 20.8–274.4 | 53.8–200.0 | 61.5–297.0 | 2.9–14.7 | 5.8–20.0 | 6.9–25.7 | 3.4–11.8 | 28.6–6.9 | 14.1–31.5 | 3.8–8.8 |
| Mean | 426.1 | 40.5 | 27.4 | 62.6 | 29.7 | 73.0 | 3.8 | 22.4 | 10.6 | 5.4 | 3.1 | 9.4 | 25.7 | 5.6 | 14.1 | 31.5 | 8 |

tbl, total body length; btl, buccal tube length; vl, ventral lamina (strengthening bar); pt-vl, pt ratio of ventral lamina; ssi, stylet support insertion; pt-ssi, pt ratio of stylet support insertion; btd, inner buccal tube diameter; prl, macroplacoid row length; fpl, first macroplacoid length; spl, second macroplacoid length; m, microplacoid length; pb2-3, claw length of the second to third pair of legs; pt-pb2-3, pt ratio of claw length of the second to third pair of legs; sb2-3, length of the secondary branch of the second to third pair of legs; pb4, claw length of the fourth pair of legs; pt-pb4, pt ratio of claw length of the hind legs; sb4, length of the secondary branch of the hind legs; L-V, latero-ventral position of the specimen; –, missing measurements.
Description of the holotype (see measurements in Table I): body colourless, length 535.0 μm. Cuticle smooth without pearls (Figure 1), but with dots at the external base of each leg (more visible on hind legs). Ocular spots present. Buccal tube length 41.2 μm, internal diameter 3.9 μm (Figures 2A, 3A). Mouth terminal with peribuccal lamellae not well distinguishable on LM. Buccal armature comprises fine transverse ridge systems visible in the caudal region of the buccal cavity consisting of three dorsal and three ventral transverse ridges, very slender (Figure 2B, C). Dorsal central crest arched with its convexity turned backwards (Figure 2B). Stylet supports inserted in the buccal tube at a distance corresponding to 76.2% of its length (Table I).

Table II. Comparison of measurements among species of the “tenuis” group, other similar Macrobiotus species, and the type species of the genus (Macrobiotus hufelandi).

| Species                  | btl  | ptvl | ptss | ptpb1-3 | ptpb4 | ptpb4 – ptpb1-3 |
|-------------------------|------|------|------|---------|-------|-----------------|
| M. ariekammensis       | 54.7 | 61.8 | 73.3 | 45.2    | 65.4  | 20.3            |
| M. ariekammensis (Poland)| 48.5 | –    | 71.4 | 55.0    | 71.5  | 16.5            |
| M. ariekammensis (Poland)| 49.5 | 68.1 | 71.9 | –       | –     | –               |
| M. ariekammensis (Russia)| 49.5 | 64.0 | 73.9 | 51.9    | 88.1  | 36.2            |
| M. hyperonyx            | 55.7 | 54.6 | 73.4 | 31.5    | 50.9  | 19.4            |
| M. hyperonyx (holotype) | 51.5 | 63.5 | 76.9 | 40.4    | 67.4  | 27.0            |
| M. hyperonyx (paratype) | 55.4 | 66.2 | 75.1 | –       | –     | –               |
| M. caelicola (paratype) | 56.4 | –    | 73.8 | 44.0    | –     | –               |
| M. caelicola (paratype) | 57.4 | 60.5 | 72.5 | 41.5    | 65.5  | 24.0            |
| M. caelicola (paratype) | 48.5 | –    | 73.4 | 32.6    | 51.1  | 19.0            |
| M. caelicola (paratype) | 56.4 | 54.4 | 73.8 | 42.2    | 58.0  | 16.0            |
| M. caelicola            | 66.0 | ND   | ca   | 21.0    | 18.9  | 30.3            |
| M. higginsi             | 55.5 | 55.0 | 75.3 | 23.6    | 31.2  | 7.6             |
| M. higginsi (holotype)  | 57.4 | 51.7 | 74.2 | 34.5    | 43.2  | 8.7             |
| M. higginsi (paratype)  | 46.5 | 59.6 | 76.6 | –       | –     | –               |
| M. hystricogenitus      | 51.7 | 45.3 | 73.8 | 29.9    | 34.1  | 4.2             |
| M. hystricogenitus (holotype) | 53.5 | 44.4 | 70.4 | 29.6    | 33.3  | 3.7             |
| M. mongolicus           | 58.4 | 45.2 | 73.2 | 26.0    | 31.7  | 5.7             |
| M. mongolicus (holotype) | 55.4 | 44.6 | 73.2 | 28.5    | 35.7  | 5.0             |
| M. tenuis               | 55.2 | 55.0 | 72.8 | 23.9    | 30.8  | 6.9             |
| M. tenuis (Italy)       | 59.4 | –    | 75.0 | 26.6    | 30.0  | 3.4             |
| M. tenuis (Italy)       | 59.4 | 53.4 | 73.4 | 26.6    | 30.0  | 3.4             |
| M. willardi             | 47.9 | 51.5 | 74.8 | 19.9    | 25.8  | 5.9             |
| M. willardi (paratype)  | 55.4 | 57.2 | 66.1 | 27.0    | 30.2  | 3.2             |
| M. willardi (holotype)  | 58.4 | 59.3 | 74.6 | 25.7    | 29.1  | 2.0             |
| M. bondavallii (paratype) | 56.4 | 58.0 | 77.3 | 32.3    | 35.1  | 3.2             |
| M. kosharai (holotype)  | 43.0 | –    | 76.7 | –       | 34.9  | –               |
| M. kosharai (mean)      | –    | –    | 74.7 | –       | 29.4  | –               |
| M. tenuiformis (holotype) | 63.6 | –    | 74.4 | 34.9    | –     | –               |
| M. danilovi (holotype)  | 55.5 | –    | 77.3 | 25.3–29.3 | 34.7  | 5.4–9.4         |
| M. voronkovi (holotype) | 60.7 | –    | 74.4 | 24.4–29.3 | –     | –               |
| M. ciprianoi n. sp. (holotype) | 41.2 | 50.4 | 76.2 | 26.2    | 28.6  | 2.4             |
| M. ciprianoi n. sp. (total) | 40.4 | 64.6 | 73.5 | 25.7    | 31.5  | 5.8             |
| M. hufelandi (neotype)  | 39.6 | 62.6 | 80.1 | 22.5    | 27.5  | 5.0             |

btl, buccal tube length; ptvl, pt ratio of the ventral lamina; ptss, pt ratio for stylet support insertion; ptpb 1-3, pt ratio for claw length of one of the first three pairs of legs; ptpb 4, pt value for claw length of hind leg; ptpb4 – ptpb1-3, difference in pt ratios between claws of the first three pairs of legs and hind leg. *Mean values from Maucci (1987a, 1987b); data from Kathman (1990); ‘data from Pilato (1977); ‡data from Biserov (1999); §data from Tumanov (2007); ‡type specimens plus moss specimens.
Pharyngeal bulb contains apophyses, two macroplacoids, and a microplacoid (Figures 2A, 3B). First macroplacoid bears a deep middle constriction and the second macroplacoid a posterior globular expansion, microplacoid evident close to the second macroplacoid. Slanting cuticular bars (between the apophyses and the first macroplacoid; Figure 2A) present. Placoid row length including microplacoid 22.5 μm. First macroplacoid length 10.8 μm (Figures 2A, 3B), second macroplacoid 4.9 μm, and microplacoid 2.9 μm. Stylet furcae as in other *Macrobiotus* species.

Claws with secondary branch inserted in main branch at a right angle (Figures 2D, E, 3C, 4A). Very short peduncle. Short common tract with basal septum delineating a very small basal portion (Figure 2D, E). Fine accessory points on main claw branches. Large lunules at the base of claws of all legs. Lunules on legs I–III are smooth, those of the hind legs larger and dentate with small teeth (Figures 2D, E, 3D). Cuticular bars on legs absent.

In paratypes (lateral view), a long ventral lamina with a wide ventral crest is visible.

Five eggs found were ascribed to *Macrobiotus ciprianoi* n. sp. on the basis that the other tardigrade species in the samples do not lay free, ornamented eggs (*D. recamieri* and *E. canadensis*) or show different egg ornamentation (*M. richtersi*).

Eggs laid free, spherical in shape and with processes on their surface (Figure 3E–G). Processes are long conical or funnel-shaped with a network on their surface (Figure 3F, G). Egg shell surface between processes is not visible on LM. Diameter of the eggs 99.8–103.7 μm. Process heights 11.9–19.8 μm (mean 15.6 μm) and diameters of process bases 6.9–9.9 μm (mean 8.2 μm).

**Variability**

Ranges and means of the morphometric characters are reported in Table I. Only two paratypes have ocular spots (in the other types, ocular spots are not visible probably due to interference by the mounting medium). In smaller paratypes, probably juveniles, the constriction in the first macroplacoid is shallower.

In *M. ciprianoi* n. sp. specimens collected from rock mosses, ocular spots visible in eight of the 13 fixed specimens. In smaller specimens, most likely juveniles: first macroplacoid has a shallower constriction, and first macroplacoid is larger than the second (Table I). The second macroplacoid has a widening in its posterior zone, which is not visible in smaller specimens.

**Etymology**

The new species was named after the late Dr Cipriano López Almeida, grandfather of the first author (N. Guil).
Figure 2. *Macrobiotus ciprianoi* (holotype) structures. (A) Bucco-pharyngeal apparatus in latero-ventral view; (B) buccal armature in dorsal view; (C) buccal armature in ventral view; (D) claw of the third pair of legs; (E) claw of the fourth pair of legs. Scale bars: 5 μm.
Taxonomic remarks

*Macrobiotus ciprianoi* n. sp. can be easily distinguished from the other *Macrobiotus* species according to a set of morphological characters: claw shape, bucco-pharyngeal apparatus characteristics, and egg morphology. The new species shares several characters with species of the “*tenuis* group” (first macroplacoid with a deep constriction, secondary branch inserted in main branch at a right angle, and large lunules on all legs), yet differs with respect to its claw shape, presence of more rounded macroplacoids, and eggs with cone-shaped processes, whose surfaces are reticulate. *Macrobiotus tenuis* (Figure 4H) and *M.
kozharai are the most similar species to Macrobiotus ciprianoi n. sp. Nevertheless, M. ciprianoi n. sp. differs from M. tenuis in that it has larger lunules on the claws of the hind leg, a shorter common claw tract, and in the shape of the egg processes (the eggs of M. tenuis bear truncated cone-shaped processes), and from M. kozharai in that its claws and common

Figure 4. Claws. (A) Macrobiotus ciprianoi (paratype; third claws); (B) M. willardi (paratype; first claws); (C) M. ariekammensis (second claws); (D) M. hyperonyx (paratype; second claws); (E) M. hystricogenitus (holotype; first claws); (F) M. caelicola (paratype; third claws); (G) M. higginsi (holotype; second claws). (H) M. tenuis (second claws); (I) M. mongolicus (holotype; second claws); (J) M. bondavallii (holotype; second claws). (A, B, D–J) Phase contrast microscopy; (C) differential interference contrast microscopy. Scale bars: 10 µm.
claw tract are shorter, and it lacks a posterior band of teeth. Other *tenuis* species with two macroplacoids (first with a deep constriction) similar to *M. ciprianoi* n. sp. are *M. danilovi*, *M. tenuiformis*, and *M. voronkovi* (Tumanov 2007). These three species have a different buccal armature, slender and larger claws of the *tenuis*-type, and their macroplacoids are larger and thinner compared to those of *M. ciprianoi* n. sp. *Macrobiotus danilovi* has similar shaped egg processes to the new species, although the conical processes of *M. ciprianoi* n. sp. are longer and more slender. *Macrobiotus tenuiformis* and *M. voronkovi* have completely different shaped egg processes to *M. ciprianoi* n. sp. such that their distinction is unmistakable.

If the first, deeply constricted macroplacoid is interpreted as two macroplacoids (i.e. three macroplacoids in total), then *M. ciprianoi* n. sp. could be mistaken for *M. wilardi* (Figure 4B). This *tenuis* species has three macroplacoids and eggs bearing cone-shaped processes with elongated tips. Nevertheless, *Macrobiotus willardi* and *Macrobiotus ciprianoi* n. sp. can be clearly differentiated since the new species has a fine buccal armature with no visible band of teeth (both anteriorly and posteriorly), shorter macroplacoids, a smaller microplacoid closer to the third macroplacoid, and smaller accessory points. Moreover, the secondary branch of the claws of *M. ciprianoi* n. sp. is almost straight and forms a right angle with the main branch (Figures 2D, E, 3C), whereas in *M. willardi*, the secondary branch is curved and the angle made with the main branch is more acute (Figure 4B).

**Morphological remarks regarding Macrobiotus species of the “tenuis group” or similar species**

In this section, we list some of the characters observed in species of the “*tenuis* group” (or related species) that have not been previously described or were erroneously indicated in their original descriptions.

**Macrobiotus ariekammensis**

*(Figures 4C, 5B, D)*

Characters previously not cited in descriptions of *M. ariekammensis* and/or *M. adelges* (We˛glarska 1965; Dastych 1977, 1985) are: cuticular pearls over the entire surface of the animal, larger pearls in anterior (mainly around the mouth opening) and caudal dorsal regions; buccal tube with a clear bend in an anterior position (immediately behind the mouth opening). At this level, the animal has a large dorsal tooth on the internal surface of the buccal tube (Figure 5B, D); in lateral view, the ventral portion of the buccal tube is shorter and ends at the level of the stylet sheaths; ventral lamina has a wide ventral crest characterized by two lobes in lateral view (Figure 5D). Contrary to that reported by Dastych (1985), the buccal armature contains a posterior band of small teeth (transverse crests absent).

**Macrobiotus hyperonyx**

*(Figures 4D, 5C)*

Buccal tube has a clear bend in its anterior portion (just after the mouth opening). At this level, the animal has a large dorsal tooth on the inner surface of the buccal tube (Figure 5A, C), which was not cited in the original description (Maucci 1982). In lateral view, the ventral portion of the buccal tube is shorter and ends at the stylet sheaths.
Figure 5. Bucco-pharyngeal apparatus (arrows indicate large dorsal tooth). (A) *Macrobiotus hyperonyx*, lateral view (paratype); (B) *M. ariekammensis*, lateral view; (C) *M. hyperonyx*, dorsal view (paratype); (D) *M. ariekammensis*, dorsal view; (E, F) *M. caelicola* (paratypes): (E) dorsal view; (F) lateral view. (A, C) Phase contrast microscopy; (B, D–F) differential interference contrast microscopy. Scale bars: 7 µm.
Unlike the original description, the buccal armature comprises a posterior band of small teeth (transverse crests absent).

*Macrobiotus hystricogenitus*  
(Figure 4E)  
Contrary to the description by Maucci (1978), the buccal armature comprises a posterior band of small teeth (due to the position of the buccal tube, it was not possible to detect the presence of transverse crests).

*Macrobiotus caelicola*  
(Figures 4F, 5E, F)  
Buccal armature consists of a posterior row (two rows in a large specimen) of quite large, rounded teeth. At the position of the lateral transverse crests, two rounded teeth occur at both dorsal and ventral locations; central transverse crest is absent. Internal surface of the anterior portion of the buccal tube has a large dorsal tooth (not cited in the original description; Kathman 1990) (Figure 4E, F). Slanting cuticular bars (between apophyses and first macroplacoid) can be observed in the pharynx.

**Discussion**

Table II compares the measurements and/or pt ratios of some of the sclerified structures of the “*tenuis* group” species and *M. hufelandi* (as type species of the genus) with those reported by Maucci (1987a). Our data are generally similar (or within the standard error ranges) to those reported by this author (Maucci, 1987b). The only large discrepancies were the pt ratios for claw length (Table II).

According to data reported in the taxonomic remarks (Table II) and those provided in the original descriptions of the species, the common characters identified by Maucci (1987a, 1987b) for the “*tenuis* group” should be reconsidered as follows:

- Yellowish or light brown colour: this character is not present in most of the “*tenuis* group” species (*M. willardi, M. hystricogenitus, M. kosharai, M. tenuis, M. danilovi, M. tenuiformis, and M. voronkovi*). Ramazzotti and Maucci (1983) reported that specimens from a *M. tenuis* population were yellowish in colour but in the original description by Binda and Pilato (1972), the type specimens are colourless.
- Large size: specimens of the “*tenuis* group” can attain a large size (over 600 μm in length; Maucci 1987b) yet this is also true of other *Macrobiotus* species.
- Short ventral lamina: given the subjective and relative nature of the terms “long” or “short” for ventral lamina length and stylet support insertion point on the buccal tube, we used the figures reported by Claxton (1998) for *Minibiotus* species as reference measurements. Claxton (1998) reported pt ratios for ventral lamina length and distance to stylet support insertion point of ≤62 and ≤73, respectively, for *Minibiotus* species (generally considered to have a short ventral lamina and stylet support inserted a long distance from the posterior end of the buccal tube). Most species of the “*tenuis* group” have ventral lamina pt ratios that are lower or similar to those of *Minibiotus* species (Table II). Higher pt ratios were recorded, however, for specimens of *M. ariekammensis* and *M. hyperonyx* (Table II).
Stylet support inserted a long distance from the posterior end of the buccal tube: only *M. tenuis* has a *pt* ratio for the stylet support insertion point lower than that of the *Minibiotus* species, but all species of the “*tenuis* group” show low *pt* values, which are clearly lower than for *Macrobiotus hufelandi* (type species of the genus) (Table II).

Slanting cuticular bars (between the apophyses and first macroplacoid) inside the pharynx: these cuticular bars appear in all species of the “*tenuis* group”, but this character is often neglected in the description of the species, e.g. see taxonomic remarks and descriptions of *Macrobiotus bondavallii* (Figure 1 in Manicardi 1989), *Macrobiotus kurasi* Dastych, 1980 (Figure 2 in Dastych 1980), and *Macrobiotus marlenae* Kaczmarek and Michalczyk, 2004 (Figure 3 in Kaczmarek and Michalczyk 2004). This could indicate the observation of this character in species not belonging to the “*tenuis* group”. Moreover, its presence is also recorded in species of different genera (Tumanov 2005).

Hind claws always clearly larger than others: all species of the “*tenuis* group” have longer primary claw branches on the hind leg than on the other pairs of claws (this character is not clearly specified for *M. kosharai*; Biserov 1999). *Macrobiotus hystricogenitus*, *M. higinsi*, *M. willardi*, *M. tenuis*, *M. danilovi*, and *M. mongolicus* (see Table II) differ amongst themselves in terms of their *pt* ratios for hind claw length compared to the length of other pairs of claws. These ratios are similar to or a little larger than values for *M. hufelandi*. In contrast, ratios for *M. ariekammensis* and *M. hyperonyx* differ largely.

Large lunules, those of the fourth pair larger than others and with more or less clear dentations: character present in all species of the “*tenuis* group” yet common to other *Macrobiotus* species (e.g. *Macrobiotus walteri* Biserov, 1999 and *Macrobiotus crenatus* Maucci, 1987a).

Tumanov (2005, 2007) defined the *tenuis*-type claw as: “slender claws with main and secondary branches well differentiated … long thin common tract (secondary branch diverging at about half of the claw length) … distal part of basal portion typical of genus *Macrobiotus*, and … the stalk, connecting the claw base with the lunules, forming a prominent frontal appendage on the claw base”. This “frontal appendage” is not clearly evident in any of the pictures or figures provided by this author. It is therefore hard to recognize in *M. ciprianoi* n. sp. or other species of the *tenuis* group examined here.

Although some morphometric characters (large size, short ventral lamina, stylet support insertion distance, hind claws larger than the rest) and morphological features (slanting cuticular bars between the apophyses and the first macroplacoid, large lunules on the fourth pair with clear dentations) seem to be common to almost all species of the “*tenuis* group”, this group of species is far from homogeneous, with many of these characters also occurring in other species which may or may not belong to the genus *Macrobiotus*. As pointed out by Tumanov (2005), these characters can be considered of small phylogenetic significance according to current knowledge. Indeed, the morphometric and morphological characters mentioned above are also evident in *Macrobiotus* species that do not have characteristics associated with the claw shape of the “*tenuis* group” (Tumanov 2005, 2007). Moreover, certain features such as cuticular pearls, microplacoids, and a large tooth in the buccal tube are not common characters within the “*tenuis* group”.

The presence of long, thin double-claws with much shorter secondary branches than primary ones, which roughly form at right angles to them, is a feature that better characterizes the “*tenuis* group” of species, but is also present in other species such as *Macrobiotus ciprianoi* n. sp., *Macrobiotus bondavallii*, and *Macrobiotus caelicola* (Figure 4A–J). Nonetheless, these three species (*Macrobiotus ciprianoi* n. sp., *Macrobiotus bondavallii*, and *Macrobiotus caelicola*) share other characteristics with species from the “*tenuis* group” (see
taxonomic remarks, original descriptions, and Table II). Thus, *Macrobiotus bondavalli* could be assigned to the “*tenuis group*” (as defined in Maucci 1987b). *Macrobiotus ciprianoi* n. sp. and *M. caelicola* show independent characters that, if incorporated in this group, would render the “*tenuis group*” even more heterogeneous. *Macrobiotus ciprianoi* n. sp. has a peculiar claw structure (with the secondary claw branch forming a true right angle with the primary branch; Figures 2D, E, 3C, 4A) compared to other *Macrobiotus* species and to those of the “*tenuis group*” (in which the angle is more acute, such as in *M. willardi*; Figure 4B). *Macrobiotus caelicola* has a particular sclerified bar below the claws on the first three pairs of legs and cuticular pearls, which are lacking in most “*tenuis group*” species. In effect, within the “*tenuis group*”, only *M. ariekammensis* and *M. hyperonyx* have cuticular pearls. Moreover, these two species and *M. caelicola* have several common characters that distinguish them from other species of the “*tenuis group*” and from other *Macrobiotus* species: cuticular pearls, a large dorsal tooth on the inner surface of the buccal tube, a long ventral lamina, and notably long claws, which are even longer on the hind legs.

Tumanov (2005) proposed a new group of species within *Macrobiotus* designated the “*ariekammensis*” group, based on claw structure. This *ariekammensis* group is composed of *M. ariekammensis* and *Macrobiotus kirghizicus* Tumanov, 2005. *Macrobiotus kirghizicus* shares with *M. hyperonyx*, *M. ariekammensis*, and *M. caelicola* the features of a long ventral lamina, long claws and longer claws on the hind legs compared to the other legs, but differs in that it lacks cuticular pearls and a large dorsal tooth on the inner surface of the buccal tube.

Our findings indicate that the “*tenuis group*”, as defined by Maucci (1987a, 1987b), does not represent a homogeneous group of species. Further taxonomic revisions and phylogenetic analyses of *Macrobiotus* species are needed to define and clarify the status of the “*tenuis*” (Maucci 1987b; Tumanov 2005, 2007) and “*ariekammensis*” (Tumanov 2005) groups of species. Moreover, the entire genus would have to be taxonomically revised and analysed to understand and clarify the phylogenetic relationships among these taxa, and discover how and why they evolved.

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