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New records of Tardigrada from Cyprus with a description of the new species *Macrobiotus marlenae* (*hufelandi* group) (Eutardigrada: Macrobiotidae)

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ABSTRACT. A new species, *Macrobiotus marlenae* n. sp., is described from Cyprus. The new species is similar to *Macrobiotus persimilis* BINDA & PILATO, 1972, *M. patagonicus* MAUCCI, 1988 and *M. polonicus* PILATO et al., 2003 but it differs from them mainly by: shorter placoids, lower number of processes on the circumference of the egg and the processes are larger and have undulations under the terminal disc. The new species differs from others also by some morphometric characters of the adults and eggs. Besides *M. marlenae* n. sp., four other species are reported from Cyprus: *Echiniscus testudo* (DOYÈRE, 1840), *Macrobiotus* cf. *richtersi* MURRAY, 1911, *Milnesium tardigradum* (DOYÈRE, 1840) and *Ramazzottius* cf. *oberhaeuseri* (DOYÈRE, 1840). All are new to Cyprus. A key to identification of the species of the *hufelandi* group that have a 'smooth' egg surface between processes is also given.

Key words: Tardigrada, Macrobiotidae, *hufelandi* group, *Macrobiotus marlenae*, new species, Cyprus

INTRODUCTION

Tardigrades from Cyprus are almost completely unknown. Until now only three tardigrade species have been recorded from Cyprus: *Macrobiotus hufelandi* SCHULTZE 1840, *M. recens* CUÉNOT, 1932 and *M. reinhardti* MICHALCZYK & KACZMAREK, 2003 (BERTOLANI 1975, MICHALCZYK & KACZMAREK 2003). In this paper we report on three species that are new records for Cyprus, and one *Macrobiotus marlenae* n. sp. that is new to science. The new species belongs to

the *hufelandi* group that consists of 27 species (including *M. marlenae* n. sp.) (PILATO et al. 2003). All species in this group have two macroplacoids, a microplacoid and usually lay eggs with processes in the shape of truncated cones terminated with a disc (these processes are also sometimes described as mushroom-shaped or being in the shape of inverted/upside-down goblet/chalice).

MATERIALS AND METHODS

Moss samples collected by Mrs Barbara KACZMAREK in the Greek part of Cyprus in May 2001 contained 357 specimens and 55 eggs of tardigrades. 329 adults and 41 eggs were mounted on microscopic slides in Hoyer's medium, 19 adults and 13 eggs were prepared for SEM.

All measurements are given in micrometers [μm]. Structures were measured only if their orientation was suitable. Body length was measured from the mouth to the end of the body, not including the hind legs. Buccal tube length and the level of the stylet support insertion point were measured according to PILATO (1981). Buccal tube widths were measured as the external diameters at the level of the stylet support insertion point. Only external claws were measured. Lengths of the claw branches were measured from the base of the claw to the top of the branch, including accessory points. The *pt* ratio is the ratio of the length of a given structure to the length of the buccal tube expressed as a percentage (PILATO 1981). In the description of the holotype, the *pt* is given after μm value [in square brackets and in *italics*]. Terminology describing the oral cavity armature is given according to MICHALCZYK & KACZMAREK (2003).

All measurements of *M. persimilis*, *M. patagonicus* and *M. polonicus* are given according to the original descriptions (BINDA & PILATO 1972, MAUCCI 1988, PILATO et al. 2003).

Photomicrographs were made using Light Microscopes (LM): Phase Contrast Microscope (PCM), Nomarski Differential Interference Contrast Microscope (DIC) and Scanning Electron Microscope (SEM). All drawings were made using a *camera lucida* associated with PCM.

LIST OF TARDIGRADA SPECIES NEW TO CYPRUS

Echiniscus testudo (DOYÈRE, 1840)

MATERIAL EXAMINED

77 specimens (moss from rock), 4 specimens (moss from soil), Greek part of Cyprus, surroundings of the Kykko monastery, 1450 m asl, southern slope, 27.09.2002; leg. B. Kaczmarek; 57 specimens (moss from tree), Greek part of Cyprus, Troodos Mts. near the top of Mt. Olympus (about 1700 m asl), southern slope, 27.09.2002; leg. B. Kaczmarek.

All specimens mounted in Hoyer's medium.

DISTRIBUTION

Very common species, widely distributed throughout the world.

***Macrobiotus marlenae* n. sp.**

TYPE MATERIAL EXAMINED

Holotype: moss from rock, Greek part of Cyprus, surroundings of the Kykko monastery, 1450 m asl., 27.09.2002; leg. B. Kaczmarek; 61 paratypes and 1 egg (62 individuals and 1 egg mounted in Hoyer's medium, 7 individuals prepared for SEM), the same data. The holotype, 18 paratypes and 1 egg are preserved at the Zoological Museum of the Jagiellonian University, ul. Ingardena 6, 30-060 Kraków, Poland; 23 paratypes are preserved at the Department of Animal Taxonomy and Ecology, A. Mickiewicz University, Poznań; 20 paratypes are preserved in the collection of Ł. MICHALCZYK (Jagiellonian University, Poland).

ETYMOLOGY

The name is dedicated in honour of the Polish ecologist and botanist Dr. Marlena LEMBICZ of the A. Mickiewicz University (Poznań, Poland).

DIFFERENTIAL DIAGNOSIS

The greatest inter-specific variability within the *hufelandi* group is observed in the features of the egg and that is why it is commonly used in the species determination. The eggs may differ between species in the size, shape and number of processes (especially of the terminal discs) and in the type of surface between processes. In some species the surface is reticulated, or pitted/dotted when the parts of reticule merge. Other species do not have the reticule and the surface of their eggs is granulated or 'smooth' (it may appear smooth in LM but wrinkled in SEM).

Four species within the *hufelandi* group have a 'smooth' egg surface between the processes. These are: *M. persimilis* BINDA & PILATO, 1972, *M. patagonicus* MAUCCI, 1988, *M. polonicus* PILATO et al., 2003 and *M. marlenae* n. sp.

The new species differs from *M. persimilis* in the following features: smaller cuticular pores, shorter placoids (*M. marlenae* n. sp. in specimen 399.0 long; first: 6.7, second: 5.7 and *M. persimilis* in specimen 400.0 long; 11.7, second: 7.9), lunules of hind legs less developed and with smaller teeth, eggs with fewer processes (16 on the circumference of egg of *M. marlenae* n. sp. and 24-32 in *M. persimilis*), egg processes longer (about 8.6 in *M. marlenae* n. sp. and about 6.4 in *M. persimilis*) and more spaced. Finally the terminal discs are larger and with more evident indentation.

M. marlenae n. sp. differs from *M. patagonicus* in the following features: smaller body size (*M. marlenae* mean 431.5 and *M. patagonicus* mean 524.5), hind legs with large dentate lunules (small and smooth in *M. patagonicus*), presence of the first band of teeth in the oral cavity, the first macroplacoid has a

central constriction, shorter placoids (specimen 502.6 long; first: 8.6, second: 6.7 in *M. marlenae* n. sp. and specimen 528.0 long; 13.2, second: 7.9 in *M. patagonicus*), eggs with a smaller number of processes on the circumference (16 in *M. marlenae* n. sp. and 20-36 in *M. patagonicus*).

The new species differs also from *M. polonicus* by absence of the lateral gibbositities on the hind legs, wider buccal tube (5.7-7.6 in *M. marlenae* n. sp. and 4.6-5.9 in *M. polonicus*), generally slightly longer buccal tube (33.3-47.5 in *M. marlenae* n. sp. and 34.4-41.4 in *M. polonicus*), shorter first macroplacoid (6.7-10.0 in *M. marlenae* n. sp. and 9.4-11.9 in *M. polonicus*) and longer second macroplacoid (5.7-9.0 in *M. marlenae* n. sp. and 6.2-7.9 in *M. polonicus*), eggs with smaller number of processes (16 in *M. marlenae* n. sp. and 20-26 in *M. polonicus*), larger terminal disc on the processes (9.5-11.4 in *M. marlenae* n. sp. and 4.9-6.3 in *M. polonicus*), higher processes (about 8.6 in *M. marlenae* n. sp. and 5.1-6.1 in *M. polonicus*).

DESCRIPTION

Holotype: Body length 489.3, transparent/white (Fig. 1). Eyes present. Cuticle smooth, with very fine granulation on the hind legs (Fig. 13). Small, circular or elliptical pores (about 1.0 in diameter) present on whole cuticle (Fig. 15).

Bucco-pharyngeal apparatus of *Macrobiotus* type (Figs 2-5). Buccal tube 47.5 long and 5.7 [12.0] wide with well-developed bend at anterior part. Stylet supports inserted on buccal tube walls at 38.0 [80.0]. Mouth is antero-ventral with 10 peribuccal lamellae.

Oral cavity armature of *hufelandi*-type, with three bands of teeth (Figs 6-10). Teeth of first band are smaller than those of other two bands and are in shape of small cones (SEM) or granules (LM). First band is present in the anterior portion of the oral cavity just behind, and sometimes on the bases of the peribuccal lamellae. This band, usually consisting of 3-4 irregular rows of teeth is continuous and appears the same on all oral cavity walls, but is not always clearly visible in LM. Teeth of second band are intermediate in size between those of first and third band of teeth. In shape they form larger and higher cones (than those of the first band) (SEM) or granules (LM), and are positioned to rear of oral cavity just behind ring fold and in front of third band of teeth. This second band of teeth is also continuous though wider with 3-5 irregular rows of teeth on both ventral and dorsal walls of the oral cavity but usually only one row present on lateral walls. These teeth are also more variable in size and shape than those of first band. Teeth of third band are larger than those of other two bands and are usually six in number, and in shape of flat and irregularly serrated transverse plates/partitions (SEM) or transverse ridges/baffles (LM). This third band of teeth lies to the rear of oral cavity, behind second band of teeth and just in front of buccal tube opening. The individual teeth 2-3 times higher than largest teeth of the second band (SEM). This band is divided into two series: ventral and dorsal. Both series consist of one median and two lateral teeth, but occasionally ventro-lateral teeth

become joined with dorso-lateral ones and thus this third band becomes continuous (SEM) (Fig. 8). Dorsal teeth higher (SEM), thinner and longer than ventral ones (SEM and LM). The medio-ventral tooth usually very short, and it may also

Table 1. Measurements [in μm] of selected morphological structures of specimens of *Macrobiotus marlenae* n. sp. mounted in Hoyer's medium (min and max refer to the smallest and the largest structure found among all measured specimens).

CHARACTER	MIN	MAX	MEAN	SD	N
Body length	306.9	532.0	424.49	54.92	29
Buccal tube length	38.0	47.5	43.19	3.66	15
Level of the stylet support insertion point	30.4	38.0	34.46	3.06	15
Buccal tube external width	5.7	7.6	6.78	0.68	15
Macroplacoid 1 length	6.7	10.0	8.55	1.00	15
Macroplacoid 2 length	5.7	9.0	7.38	0.96	15
Microplacoid length	2.9	3.8	3.52	0.30	15
Macroplacoid row length	13.3	20.0	17.04	1.99	15
Placoid row length	18.1	25.2	21.91	2.13	15
Primary branch of claw 1 length	10.5	14.3	11.62	1.04	13
Secondary branch of claw 1 length	6.7	10.5	8.37	0.90	13
Primary branch of claw 2 length	11.4	14.3	12.43	1.11	12
Secondary branch of claw 2 length	8.6	10.5	9.10	0.75	12
Primary branch of claw 3 length	11.4	14.3	12.26	0.90	11
Secondary branch of claw 3 length	8.6	10.5	8.98	0.65	11
Primary branch of claw 4 length	13.3	17.1	14.44	1.33	10
Secondary branch of claw 4 length	9.5	12.4	10.45	1.00	10

Table 2. The pt values of specimens and selected morphological structures of *Macrobiotus marlenae* n. sp. mounted in Hoyer's medium (min and max refer to the smallest and the largest structure found among all measured specimens).

CHARACTER	MIN	MAX	MEAN	SD	N
Body length	902.2	1058.0	998.84	42.14	15
Level of the stylet support insertion point	78.0	81.6	79.76	0.74	15
Buccal tube external width	13.6	17.5	15.70	1.05	15
Macroplacoid 1 length	17.1	22.2	19.78	1.36	15
Macroplacoid 2 length	14.6	20.0	17.06	1.49	15
Microplacoid length	7.1	10.0	8.11	0.67	15
Macroplacoid row length	34.1	43.8	39.41	2.78	15
Placoid row length	45.9	57.5	50.78	3.33	15
Primary branch of claw 1 length	22.4	30.0	26.95	2.30	13
Secondary branch of claw 1 length	16.0	23.8	19.41	2.13	13
Primary branch of claw 2 length	26.1	32.5	29.02	2.01	12
Secondary branch of claw 2 length	19.6	27.5	21.31	2.16	12
Primary branch of claw 3 length	24.5	32.5	28.69	2.26	11
Secondary branch of claw 3 length	18.0	27.5	21.08	2.58	11
Primary branch of claw 4 length	30.4	37.5	33.65	2.56	10
Secondary branch of claw 4 length	21.7	27.5	24.35	1.99	10

be broken into two (rarely more) smaller teeth in large specimens, thus there may be up to seven (occasionally more) teeth in this band (SEM and LM).

Large triangular apophyses present at the end of buccal tube. Pharyngeal bulb almost spherical with two rod-shaped macroplacoids clearly different in length from each other. First macroplacoid 9.5 [20.0] long with central constriction, second 8.6 [18.0] long without constriction, tear-shaped microplacoid 3.8 [8.0] long. Placoid row 24.2 [50.9] long, macroplacoid row 19.0 [40.0] long.

Claws of *hufelandi* type (Y-shaped), well developed (Figs 11-14). Primary branches with distinct accessory points. Lunules on first three pairs of legs smooth, on fourth pair distinctly dentate (Fig. 11). External claws on I pair of leg: primary branch (pb.) 11.4 [24.0] long, secondary branch (sb.) 8.5 [17.9] long; II: pb. 14.3 [30.1] long, sb. 9.5 [20.0] long; III: pb. 14.3 [30.1] long, sb. 9.5 [20.0] long; IV pb. 16.2 [34.1] long, sb. 11.4 [24.0] long. Bars and other cuticular structures on legs absent. Lateral gibbosities on hind legs absent.

Egg: White, spherical, laid freely (Figs 16-21). Diameter of egg without processes 75.0 and 91.2 including them. Processes (16 on circumference of egg) in shape of truncated cones terminated with a disc, inter-cone distant 4.5-6.5. Processes 8.4-8.8 high, basal diameter 6.5-6.9. Terminal disc with large, strongly indented edge (6-11 teeth on circumference); diameter of terminal disc (including teeth) 9.5-11.4. Lengths of teeth about 1.0. Under terminal disc 2-3 undulations present. Egg surface between processes almost smooth (with very poorly visible wrinkles).

Table 3. Measurements [in μm] and pt of selected morphological structures of the smallest and the largest measured specimen of *Macrobiotus marlenae* n. sp. mounted in Hoyer's medium.

CHARACTER	μm	pt	μm	pt
Body length	361.0	926.8	502.6	1058.0
Buccal tube length	39.0	-	47.5	-
Level of the stylet support insertion point	31.0	79.6	38.0	80.0
Buccal tube external width	5.7	14.6	7.6	16.0
Macroplacoid 1 length	7.6	19.5	10.0	21.0
Macroplacoid 2 length	6.7	17.1	9.0	19.0
Microplacoid length	3.0	7.7	3.8	8.0
Macroplacoid row length	15.7	40.2	20.0	42.0
Placoid row length	20.4	52.4	25.2	53.0
Primary branch of claw 1 length	?	?	14.3	30.0
Secondary branch of claw 1 length	?	?	10.5	22.0
Primary branch of claw 2 length	?	?	14.3	30.0
Secondary branch of claw 2 length	?	?	10.5	22.0
Primary branch of claw 3 length	?	?	?	?
Secondary branch of claw 3 length	?	?	?	?
Primary branch of claw 4 length	?	?	17.1	36.0
Secondary branch of claw 4 length	?	?	12.4	26.0

REMARKS

Paratypes are similar to the holotype. The results of simple statistical analysis of measurements and *pt* values of selected morphological structures for 15 randomly chosen specimens are given in Tables 1-2. Table 3 shows measurements and *pt* values of the smallest and largest measured specimens.

Among 29 mounted randomly chosen specimens, 15 had eyes (52%).

Macrobiotus marlenae n. sp. was used as a model of the *hufelandi*-type oral cavity and named *Macrobiotus* sp. in MICHALCZYK & KACZMAREK (2003).

THE KEY TO THE SPECIES OF THE *HUFELANDI* GROUP WITH A 'SMOOTH' SURFACE
BETWEEN EGG PROCESSES:

1. Dentate lunules on IV pair of legs present, first macroplacoid with a constriction, three bands of teeth present in the oral cavity 2.
- Lunules on IV pair of legs with smooth edges, first macroplacoid without a constriction, the first band of teeth in the oral cavity absent
..... *M. patagonicus* MAUCCI, 1988
2. Number of processes on the circumference of egg less than 20
..... *M. marlenae* n. sp.
- Number of processes on the circumference of egg 20 or more 3.
3. Lateral gibbosities on IV pair of legs present, number of processes on the circumference of egg 20-26 *M. polonicus* PILATO et. al., 2003
- Lateral gibbosities on IV pair of legs absent, number of processes on the circumference of egg 24-32 *M. persimilis* BINDA & PILATO, 1972

***Macrobiotus* cf. *richtersi* MURRAY, 1911**

MATERIAL EXAMINED

28 specimens (moss from rock), 80 specimens and 22 eggs (moss from soil), Greek part of Cyprus, surroundings of the Kykko monastery, 1450 m asl., 27.09.2002; leg. B. Kaczmarek. 96 specimens and 9 eggs mounted in Hoyer's medium, 12 specimens and 13 eggs prepared for SEM.

DISTRIBUTION

Very common species, widely distributed throughout the world.

***Ramazzottius* cf. *oberhaeuseri* (DOYÈRE, 1840)**

MATERIAL EXAMINED

7 specimens (moss from soil), 2 specimens (moss from rock), Greek part of Cyprus, surroundings of the Kykko monastery, 1450 m asl., 27.09.2002; leg. B. Kaczmarek; 2 specimens (moss from tree), Greek part of Cyprus, Troodos Mts.,

near the top of Mt. Olympus (about 1700 m asl), southern slope, 27.09.2002; leg. B. Kaczmarek. All specimens mounted in Hoyer's medium.

DISTRIBUTION

Common species, widely distributed throughout the world.

Milnesium tardigradum (DOYÈRE, 1840)

MATERIAL EXAMINED

1 specimen mounted in Hoyer's medium (moss from soil), Greek part of Cyprus, surroundings of the Kykko monastery, 1450 m asl., 27.09.2002; leg. B. Kaczmarek.

DISTRIBUTION

Probably common species, widely distributed throughout the world.

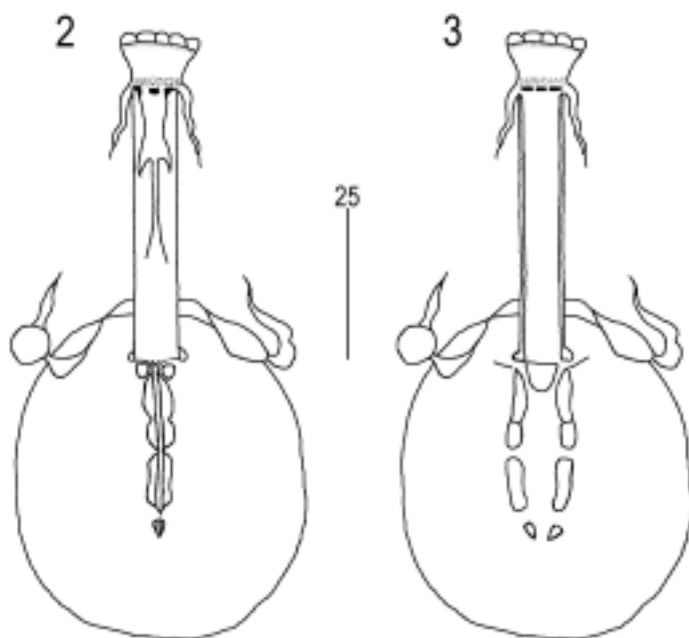
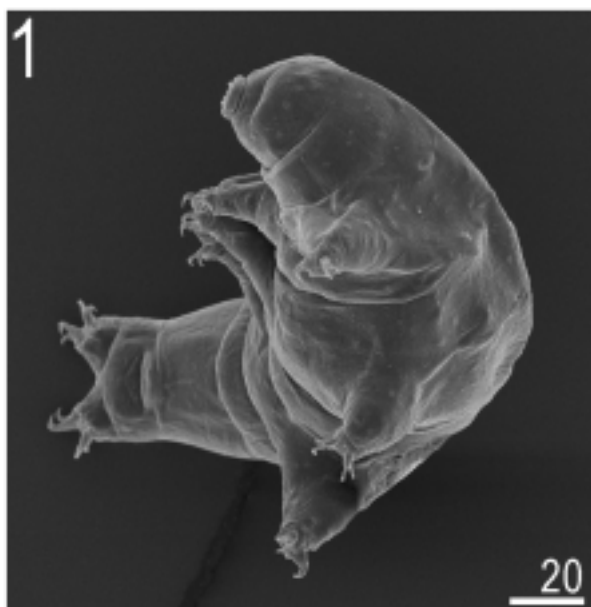
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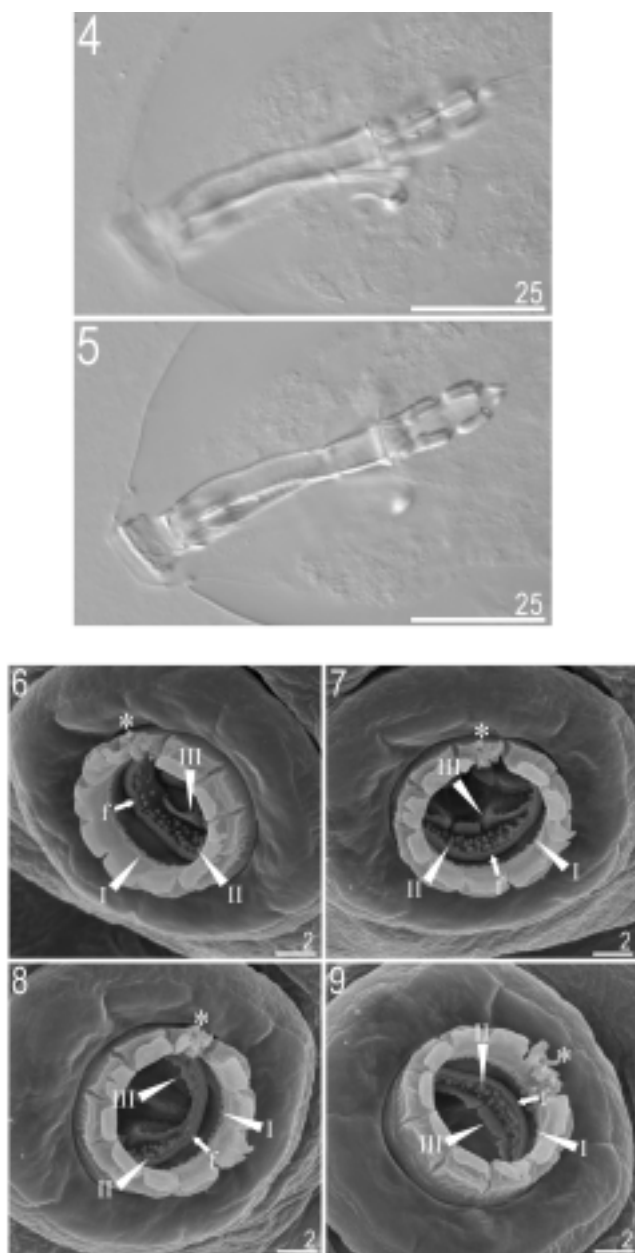
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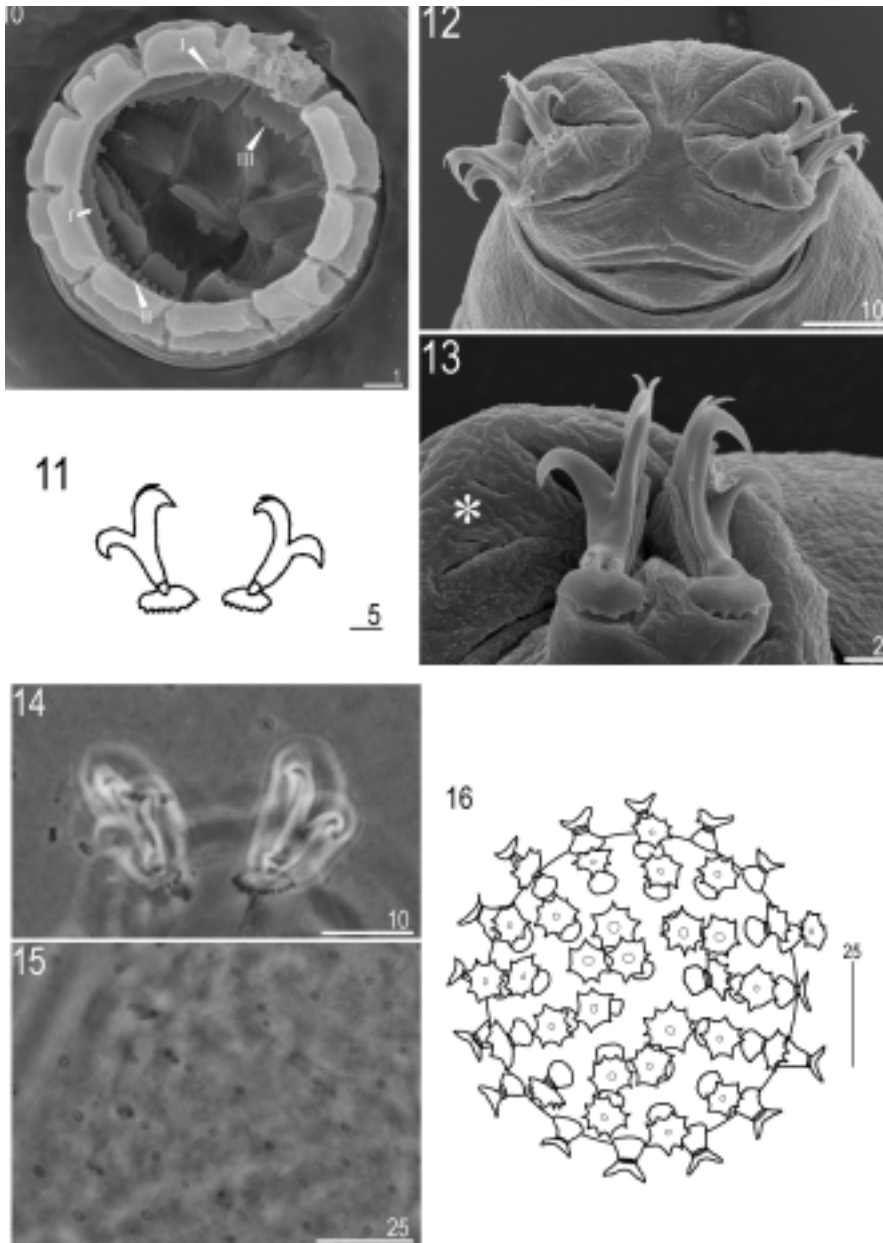
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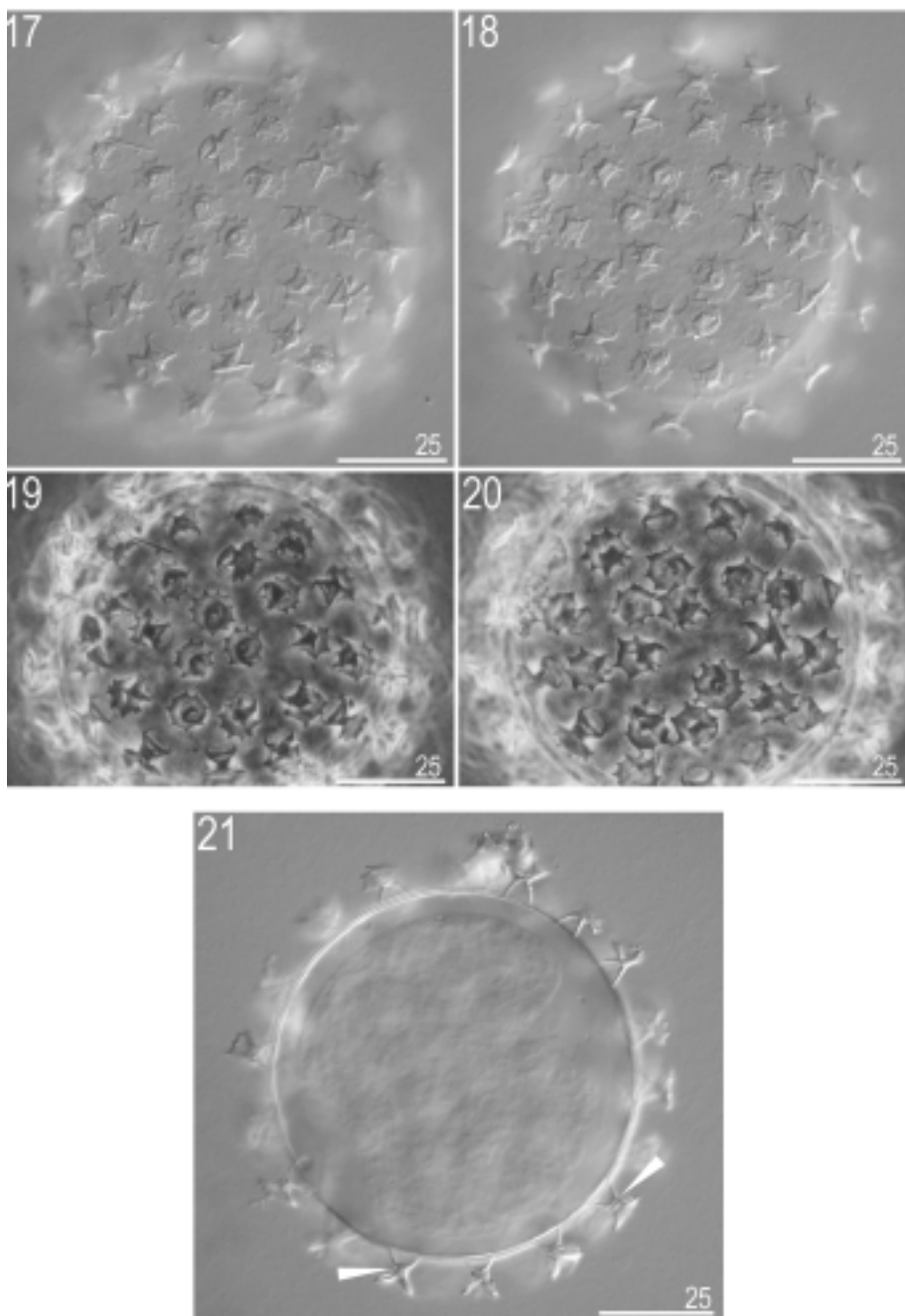
1-3. *Macrobiotus marlenae* n. sp.: 1 – habitus (paratype) (SEM). 2-3. Buccal apparatus: 2 – ventral view; 3 – middle section (paratype)



4-9. *Macrobiotus marlenae* n. sp.: 4-5. Buccal apparatus. 4 – lateral view; 5 – middle section, note the bend in the anterior portion of the buccal tube (paratype) (DIC); 6-9. Oral cavity: armature; arrows indicate three bands of teeth (I-III) and the oral cavity fold (f); the asterisk (*) indicates the most dorsal lamella (paratype) (SEM) (6-9 from MICHALCZYK & KACZMAREK (2003) and with permission of Zootaxa)



10-16. *Macrobiotus marlenae* n. sp.: 10 – oral cavity armature; arrows indicate three bands of teeth (I-III) and the oral cavity fold (f); the asterisk (*) indicates the most dorsal lamella (paratype) (SEM), 11 – claws of IV pair of legs, left internal, right external (paratype), 12 – hind legs (ventral view), 13 – claws of IV pair of legs, the asterisk (*) indicates the granulated area (paratype) (SEM), 14 – claws of IV pair of legs, 15 – pores on the dorsal cuticle (paratype) (PCM), 16 - egg (10 from MICHALCZYK & KACZMAREK (2003) and with permission of Zootaxa)



17-21. *Macrobiotus marlenae* n. sp., egg: 17 to 20, and 18 and 20 show the same side of the egg (17-18 DIC; 19-20 PCM), 21 – egg, processes on circumference, arrows indicate the undulations under the terminal discs (DIC)