

Macrobiotus ramoli sp. nov., a new tardigrade species
from the nival zone of the Ötztal Alps, Austria
(Tardigrada)*

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ABSTRACT. – *Macrobiotus ramoli* sp. nov., a new high alpine tardigrade with bisexual reproduction mode is described from the nival zone of the Ötztal Alps (Nordtirol, Austria). The species belongs to the *hufelandi*-complex and differs from similar taxa mainly in the large, tooth-like dorso-median ridge in the mouth cavity, the large dentate lunulae on the claws of the fourth pair of legs and some characters of the eggshell.

KEYWORDS: Tardigrada, taxonomy, *Macrobiotus ramoli* sp. nov., chromatin bodies, nival zone, the Ötztal Alps, Austria.

Introduction

There is very little data about tardigrades from the Ötztal Alps, the most glaciated part of the Eastern Alps. The first information on the region is given by MARCUS (1930) who described *Hypsibius callimerus* (= *Hypsibius scabropygus* CUËNOT, 1929) from an unspecified site at the Mt. (Ötztal) Wildspitze, the second highest summit (3772 m) in Austria ("Gebiet der Wildspitze, 2800 m"). MIHELČIČ (1959) described a unique glacier-dwelling tardigrade *Hypsibius klebelsbergi* from two glaciers in the Ventertal (= Vent Valley), i.e., Niederjochferner and Marzellferner, based on material collected by H. JANETSCHKE (see also MIHELČIČ 1963). The species has also been found on other glaciers in the Ötztal Alps, i.e., Rotmoosferner, Gaißbergferner and Gurglerferner (KRAUS 1977: unpublished PhD thesis; see DASTYCH *et al.* 2003). Furthermore, DASTYCH *et al.* (l.c.) found eggs of two species, *Macrobiotus richtersi* MURRAY, 1911 and a member of *M. harmsworthi*-group in the remains of macerated grass tussock on the surface of the Rotmoosferner Glacier. Moreover, the authors (l.c.) also reported an accidentally transported individual of *Ramazzottius oberhaeuseri*-group from a cryoconite hole on the same glacier. Finally, DASTYCH & THALER (2002) recorded an arctic-alpine *Hebesuncus conjungens* (THULIN, 1911) from the sub-nival zone of Mt. Festkogel. So far, only six tardigrade species are known from the vast area of the Ötztal Alps.

During a recent survey of the tardigrade fauna of the Ötztal Alps, numerous specimens and eggs of a new species described in the present paper were encountered.

* The paper is dedicated to recently deceased colleague and arachnologist, Univ. Prof. Dr. KONRAD THALER, Innsbruck.

Material and methods

The bryophytes, lichens and accompanying soil for this study come from several localities in the nival zone of the Ramolkamm Range and the summit of Mt. Hangerer in the Ötztal Alps near Obergurgl (Nordtirol, the Austrian Central Alps). The sites are listed at the description of the new species. The plants and soil were collected by the author, unless otherwise indicated, into paper envelopes and air-dried. The tardigrades and the eggs were extracted by the method described by DASTYCH (1985). The animals were examined for morphological or cytological characters either with phase- and differential interference contrast microscopy (ZEISS "Photomikroskop III") or (six individuals, four eggs: the type locality) by scanning electron microscope (LEO 1525).

Most of the material is mounted permanently on microslides in FAURE's or polyvinyl-lactophenol medium and deposited at the Zoologisches Museum Hamburg, unless otherwise indicated. Selected specimens (16) from the type-series were fixed in a mixture of methanol and acetic acid (3:1) and stained with aceto-lactic orcein. Squash technique was employed for detailed anatomical and cytological observations (e.g., BERTOLANI 1971). For SEM examination specimens were washed, transferred to hot BOUIN's medium, dehydrated in ethanol, critical-point-dried and carbon-coated. Measurements are taken as described in DASTYCH (2002), the morphometric indices and coefficients employed are explained in DASTYCH (2004a, b).

For comparative analysis specimens of *Macrobiotus serratus* BERTOLANI, GUIDI & REBECCHI, 1996, were borrowed from the R. BERTOLANI Collection at Modena University (Italy). From the original 4 individuals and 4 eggs of the type-series (see BERTOLANI *et al.* 1996) three specimens (including the holotype and one paratype) and one egg were examined. The paratype was not originally labelled as a such, but as it was from the same sample as the holotype it should be considered as belonging to the type series (Dr. R. GUIDETTI, pers. comm.).

Abbreviations used are: *DIC*- differential interference contrast, *dlr* - dorso-lateral ridges, *dmr*- dorso-median ridge, *ECI*- external claws= index, *hi*- hind (posterior) leg IV, *ml*- mouth lamella, *MPLI*- macropiloids= index, *n* = sample size, *PHC*- phase contrast, *PVL*- polyvinyl-lactophenol, *PT*- the buccal tube index (see PILATO 1981), r^2 = coefficient of determination, *SD* - standard deviation, *V* - coefficient of variation, *vlr*- ventro-lateral ridges, *vmr*- ventro-median ridge, *ZMH*- Zoologisches Museum Hamburg, \bar{x} - (arithmetic) mean.

Description

Macrobiotus ramoli sp. nov.

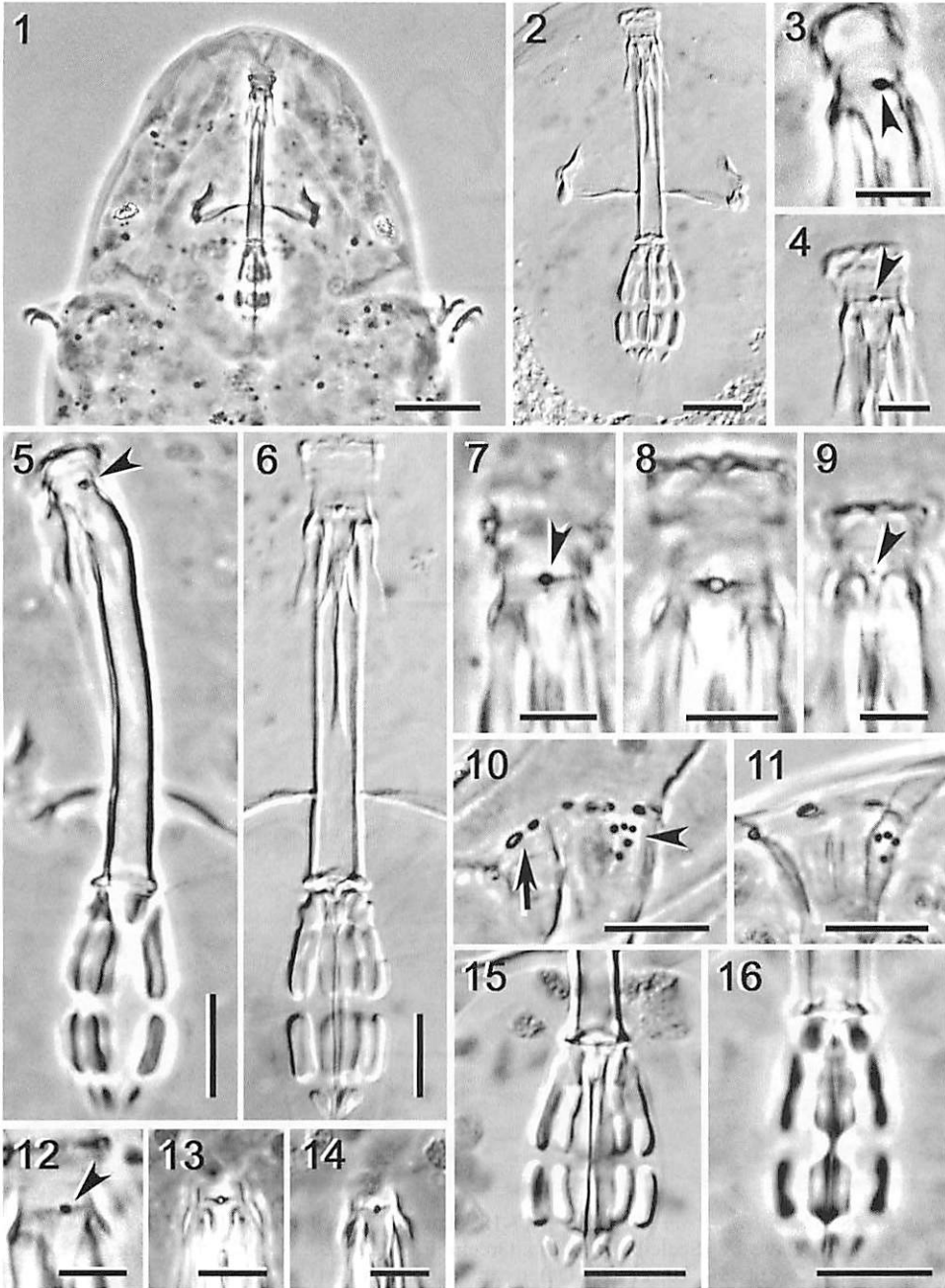
(Figs 1-64, 66-69)

H o l o t y p e . – (Figs 2, 4): sex unknown, 423 μ m long (coll. H. DASTYCH, 7 September 2004); mounted on a microslide in FAURE's medium, together with 11 paratypic animals and two such eggs. The holotype is mounted dorso-ventrally, near a latero-ventrally oriented paratype: both specimens are located near a piece of small cellulose fuzz. The microslide (No. T599) has been deposited in the Zoologisches Museum Hamburg (ZMH Acc. No. A26/05).

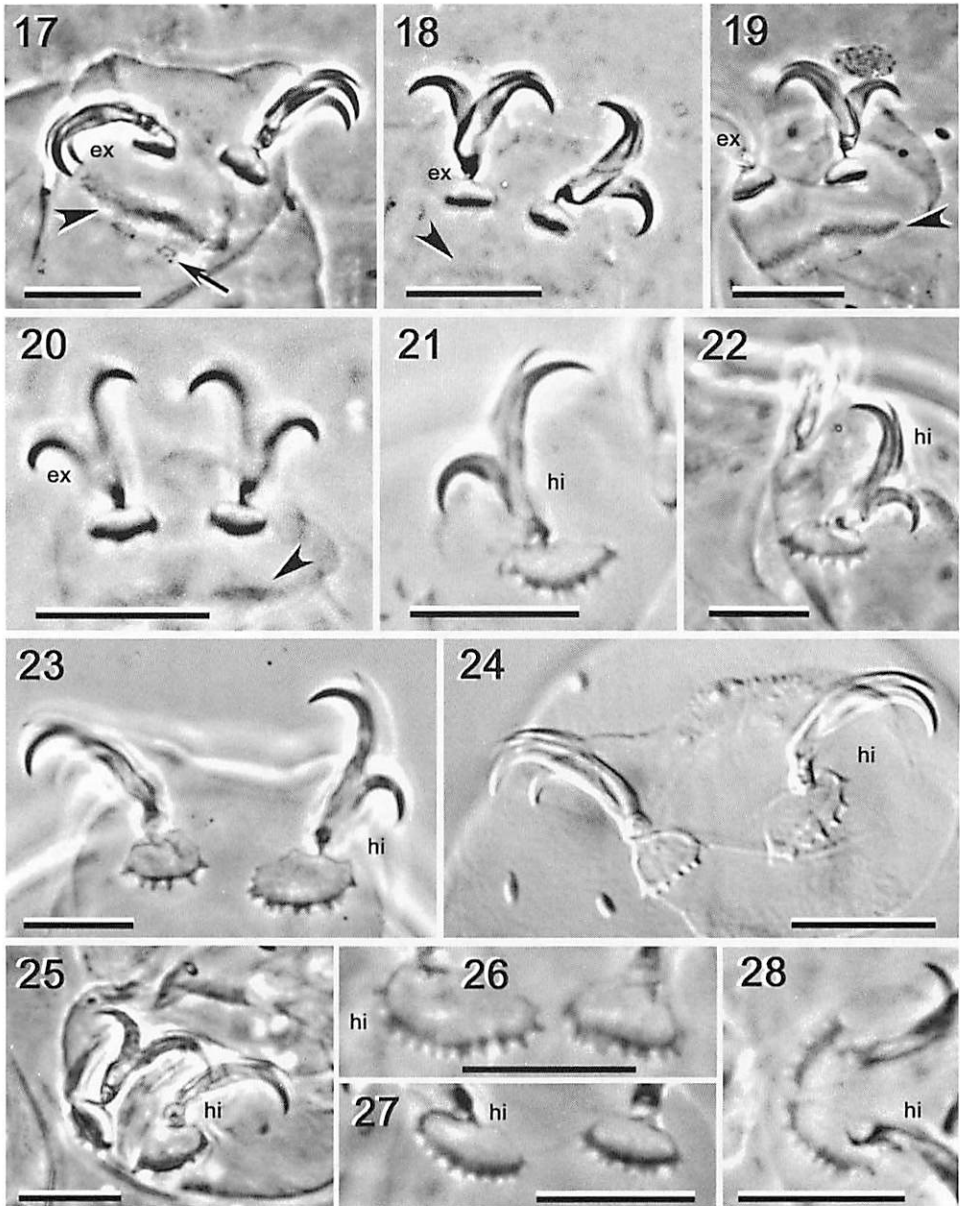
T y p e l o c a l i t y . – (Fig. 65): The Ötztal Alps, the Ramolkamm Range, Mt. Hinterer Spiegelkogel (10° 57' 31" E, 46° 49' 45" N), c. 3420 m a.s.l. (summit area). Sparsely distributed, small cushions of mosses and lichens on underdeveloped thin mineral soil between silicate stones.

P a r a t y p e s . – All locality data as above (two samples): 42 animals and 15 eggs on five numbered microslides (Nos 1-5: ZMH Acc. No A27/05), mounted in FAURE's medium (4 slides) or *PVL* (1). Two slides in the collection of the Museum d'Histoire Naturelle (Department of Arthropods and Entomology I), Geneva, Switzerland [slide No. 1 (T588) and No. 4 (T598) with seven specimens and three eggs, respectively]. The remaining paratypes and other material in the Zoologisches Museum Hamburg.

A d d i t i o n a l m a t e r i a l e x a m i n e d (A-C: the Ramolkamm Range, D: the range from Mt. Hinterer Seelenkogel, at the Rotmoostal Valley):



Figs 1-16. *Macrobiotus ramoli* sp. nov.: 1, anterior part of the body; 2, bucco-pharyngeal apparatus; 3-4, mouth cavity with dorsal ridge(s); 5-6, bucco-pharyngeal apparatus; 7-8, dorsal ridge(s); 9, ventral ridges; 10-11, pore-like structures at the external mouth ring; 12-14, placoids (Scale bars: Figs 3, 4, 7-9 and 12-14: 5 μ m; others: 10 μ m. Orcein: Figs 10-11, 13-14. DIC: Figs 2, 4, 6, 14, 15; others: PHC. Holotype: Figs 2, 4; paratypes: Figs 1, 5, 6, 9. Other explanations in text).



Figs 17-28. *Macrobiotus ramoli* sp. nov.: 17-18, claws III; 19-20, claws II; 21-25, claws IV; 26-28, lunulae of claws IV (Scale bars: 10 μ m. Orcein: Figs 17, 19, 22, 24, 25. DIC: Fig. 24; others: PHC. Paratypes Figs 18, 20, 23, 26. Other explanations in text).

- A). Mt Mittlerer Ramolkogel ($10^{\circ} 57' 46''$ E, $46^{\circ} 50' 45''$ N: summit area), 3518 m a.s.l. Bryophytes and soil from and between silicate rocks, 6 September 2004, coll. M. STOCKMEIER & W. MAURER (one individual).
- B). The Ramoljoch Pass ($10^{\circ} 57' 54''$ E, $46^{\circ} 50' 07''$ N), 3200 m a.s.l., east exposure. Small moss cushions from underdeveloped mineral soil between silicate rocks, 7 September 2004 (one sample, 2 specimens + egg).

- C). Small rocky wall near the base of the eastern crest of Mt. Vorderer Spiegelkogel (10° 58' 11" E, 46° 49' 47" N), ca. 70 m from the alpine hut Ramolhaus, 3020 m a.s.l., 30 August 2004. Sparsely distributed, small moss and lichen cushions on underdeveloped, thin layer of mineral soil, on or between silicate rocks (2 samples: 3 specimens + egg).
- D). Mt. Hangerer (11° 01' 05" E, 46° 50' 07" N), 3005-3010 m a.s.l., summit scree, NW exposure, 31 August 2004. Lichens from thick layer of mineral soil between silicate rocks (one sample, 7 specimens).

E t y m o l o g y. – Named for a mountain range in the Ötztal Alps, the Ramolkamm (= Ramol Range), where the type locality of the new species is located.

D i a g n o s i s. – Medium sized *Macrobotus* of the *hufelandi*-group with eye-spots. Cuticle smooth, “pores“ present. Dorso-median ridge in the mouth cavity shaped like a distinct tooth and discernible as a large granule. Pharynx with two macroplocoids and microplocoid. Claws of *hufelandi*-type, of moderate size, lunules smooth on legs I-III, large and strongly dentated on legs IV. Eggs with more or less flask-like processes, the base of each process wide, the processes' surface smooth. The processes' apices (discs) variably formed but small. The eggshell interprocess area with tiny, pore-like structures (pits), arranged either in an irregular single row (ring) around each process or variably scattered between processes and then more abundant.

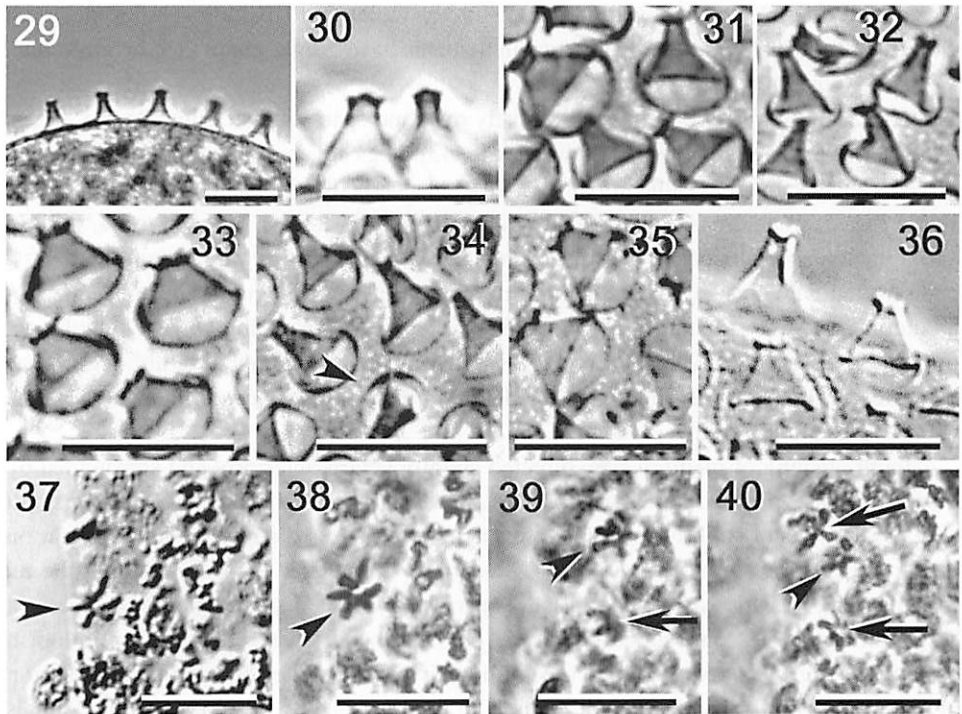
Description. – The body, 246-560 µm long (holotype 423 µm), is whitish or whitish-gray; larger specimens often being light- or distinctly violet and (including holotype) with clumps of brownish pigment. Numerous individuals have a light-green or green intestine. Eye-spots posterior and medium-sized.

Cuticle smooth in *LM*, with small, mostly oval and sparsely distributed “pores“ (Figs 19, 24). In *SEM*, cuticle micro-sculptured (Fig. 43). Legs covered with minute and closely placed granules, hardly visible in *LM* (Fig. 24), but clearly discernible in *SEM* (Figs 47-49). Furthermore, small areas of a different granulation on the dorsum of legs I to III, slightly above the claws. This peculiar form of granulation was distinctly larger than the others, and covered with sparsely distributed, short minute teeth (cones) giving the appearance of a “thornapple“-like structure (Fig. 50). This characteristic morphology of the granulation, discernible only in *SEM*, has not previously been reported in tardigrades, although it has been observed also in *M. crenulatus* RICHTERS, 1904 and *M. spectabilis* THULIN, 1928 (DASTYCH, unpublished).

Mouth opening of medium size, directed slightly ventro-anteriorly and surrounded by a ring of weakly formed lamellae (Fig. 42). Bucco-pharyngeal apparatus relatively small (Fig. 1) and, when slightly retracted, creates a more or less distinct six-lobe pattern on the broad external mouth ring (Fig. 41, asterisk). Just under the edge of the lobe an irregular ring of variously shaped, usually sparsely distributed small “pores“ was present (Figs 10, arrow; 11) and dorsally, slightly below, a small area with 4-6 tiny, roundish structures (Fig. 10, arrowhead; 11). Mouth cavity small. Anterior band of granulation absent. The posterior band, when discernible in *LM*, occurs only in some large individuals as barely visible, sparse points. The band (i.e., its teeth) is more distinct in *SEM* (Fig. 45, arrowhead). Transverse ridges (crests) small, indistinct. Dorso-lateral ridges short, poorly discernible in *LM* (Figs 4, 6), clearer in *SEM* (Fig. 45, *dlr*). The ridges are very closely

placed to the dorso-median ridge which forms a tooth-like structure (Figs 42, 44, 45, *dmr*), visible in *LM* as a characteristic large granule (Figs 3-7, 12, 14; arrowheads). The ridge is discernible either as a separate round structure in its apical focal plane (Figs 3, 5, 12, 14) or, when microscope is focused at the base of the ridge, as a broad roundish thickening in the middle of a short transverse bar (Figs 8, 13). Ventro-lateral ridges weakly formed, indistinct in *LM* (Fig. 9), and sharply terminated dorso-apically when observed in *SEM* (Fig. 46, *vlr*). The ventro-median ridge in *LM* a tiny granule placed slightly anteriorly to the ventro-lateral ridges (Fig. 9, arrowhead) or absent. The ridge is discernible in *SEM* as a minute tooth (Fig. 46, *vmr*).

Length of bucco-pharyngeal apparatus of holotype 80.1 μm . Buccal tube slightly bent (Fig. 5), moderately long and rather narrow, with distinct terminal posterior apophyses. Buccal lamina moderately long. The length ratio between anterior edge of the stylet sheaths and stylet supports ("PT ss" index: see PILATO 1981) between 71.9 and 78.5 %, in the holotype 75.3 %. Pharynx usually spherical, often slightly broader than long, with evident pharyngeal apophyses, two macroplacoids and a microplacoid (Figs 1, 2, 5, 6, 15, 16). The first macroplacoid moderately long, usually indistinctly constricted in its middle and longer than the second which is either smooth (Figs 15, 16) or with a poorly, sometimes hardly marked lateral (external) subterminal incision. Microplacoid elongated and distinct.



Figs 29-40. *Macrobiotus ramoli* sp. nov.: 29, fragment of egg; 30-36, egg processes; 37-40, chromatin bodies in a testis (Scale bars: 10 μm . *DIC*: Figs 36, 37; others: *PHC*. Orcein: Figs 37-40. Paratypes: Figs 29-31, 33-36. Other explanations in text).

The legs relatively short. The claws' peduncles and partly the claws' bases easily pliable, especially on legs IV (Figs 53, 55). Claws of *hufelandi*-type, slender and moderately sized. The lunulae are smooth on legs I-III (Figs 17-20, 47-49), large and dentate on legs IV (Figs 21-28, 51-55). Bases of accessory spines well developed, wide (Figs 17, 24), with distinct spines and relatively thin at their tips. The accessory spines run close to the claw main branch on legs I-III (Figs 17, 19, 47, 48), but directed markedly upwards on legs IV (Figs 21, 23, 53, 54). The lunulae of external and internal claw on legs I-III relatively wide, often almost right-angled (Figs 17, 18, 47-49), their posterior edges markedly broad (Figs 17-20, 47-49). The edges are usually straight, sometimes slightly concave or convex (e.g., Figs 20; 47, asterisk). Lunulae on legs IV much larger than those on other legs, with a wide, strongly formed posterior edge, usually provided with large teeth (spines). The lunula on hind claw distinctly larger than that on fore claw. The teeth of variable size, shape and number, even on two sides of the same specimen. There are 7-15 teeth on hind claw (usually 10-11) and 6-10 (mostly 6) on the fore claw ($n = 15$: Figs 21-28, 51-55). The holotype has 11 and at least 8 teeth on hind claws and 6 teeth on one fore claw (the positioning of the specimen is not optimal and makes the exact count of teeth on all claws impossible). The ventrum of the bases of legs I to III (feet) with a pair of more or less visible bar-like muscle attachments, usually fused in a transverse bar of slightly variable shape (Figs 17-20, arrowheads).

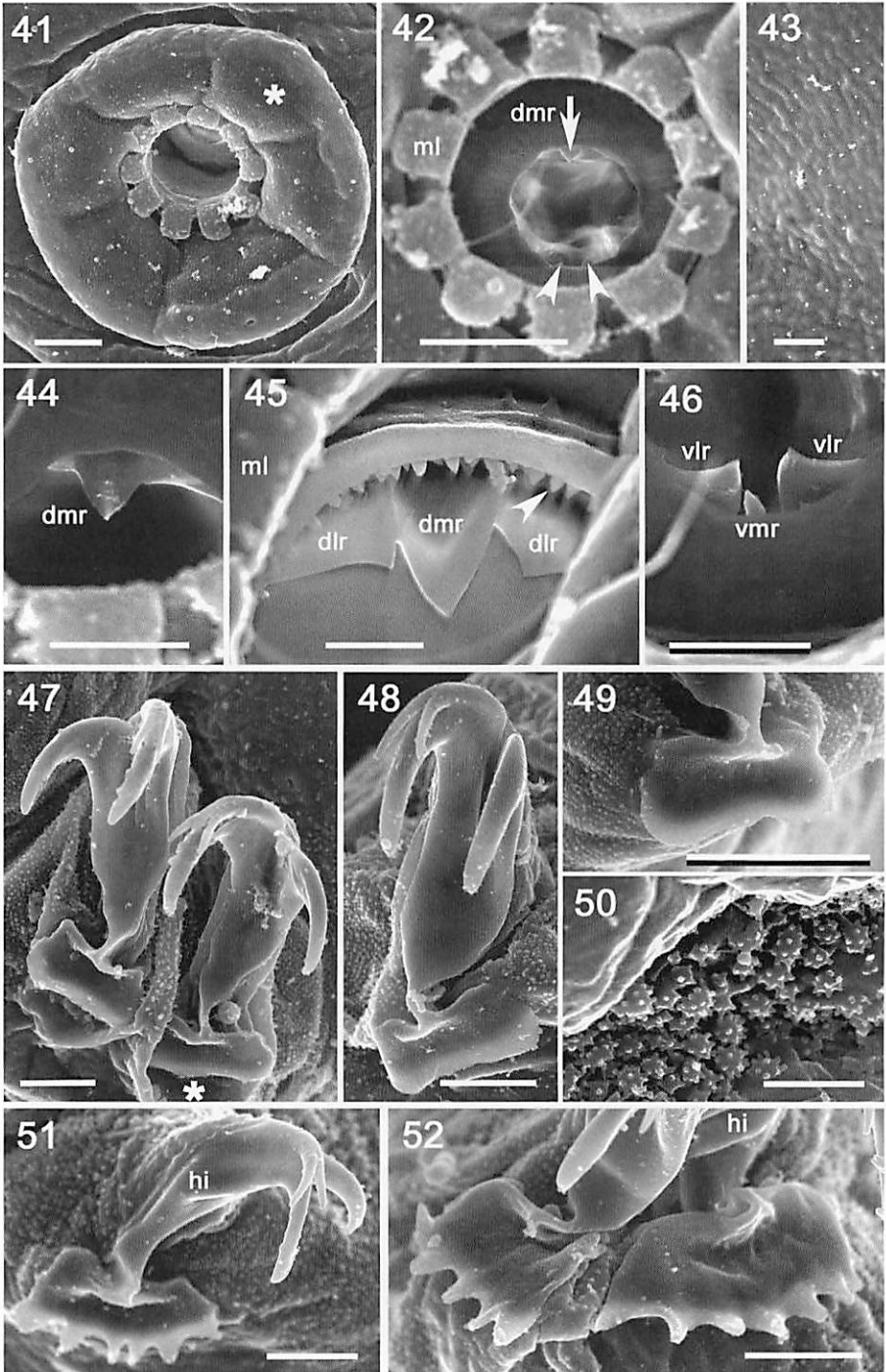
Eggs spherical, medium sized and whitish. Egg processes with their wide bases and narrow distal parts resemble more or less conical flasks (Figs 29-36, 56-63). The neck-like distal portion of each process is terminated apically in an irregular crown-shaped disc (ring) provided with tiny, irregular projections (Figs 33, 56-63). The internal surface of the crown is either slightly concave (e.g., Figs 60, 64) or flat (Fig. 63, arrow). The remaining surface of the egg processes smooth. Some processes aberrant, either doubly formed, or (rarely) fused together apically (Fig. 63, arrowhead). The apices' projections of various shapes and sizes, appearing dentate at lower magnifications (Figs 30, 33, 58). The projections occur often irregularly on the whole disk surface (Figs 56, 62, 64) and greatly resemble the sclerites of gorgonians (Octocorallia, Gorgonidae) (e.g., Figs 60, 64). Irregularly formed, "gorgonian"-like projections prevail among the examined eggs.

Interprocess area with minute, roundish, irregularly and sparsely distributed pore-like structures (pits) (e.g., Figs 32, 34, 59, 62) which are arranged either as a single, irregularly shaped ring (row) around each process (Figs 59, 63) or, when more numerous, in an irregular and variable pattern in the interprocess area (Figs 32, 62). However, the latter pattern is never net-like, as in many members of the *hufelandi*-group.

Morphometric data

Measurement values are in μm , indices and coefficients (*PT*, *V*, r^2 , *MPLI*, *ECI*) in %. For abbreviations see "Material and methods". The morphometric indices and coefficients used are explained in DASTYCH (2004a). The morphometrics of the holotype (423 μm long) is separated from other data by a dot (●). The size of the pharynx of the holotype is 42.3 x 45.9 μm (length : width), internal width of the buccal tube 3.6 μm and its microplacoids 2.7/2.3 μm long. For the presentation of measurements and indices the following convention has been used:

$$\bar{x} \pm SD \text{ (min-max) } [n] * V / r^2$$



Figs 41-42. *Macrobiotus ramoli* sp. nov.: 41, mouth region; 42, details of mouth opening; 43, cuticular surface (dorsum, at legs III); 44, dorso-median ridge; 45, dorsal ridges; 46, ventral ridges; 47, claws I; 48, internal claw III; 49, lunula of internal claw III, 50, “thornapple”-like granulation on leg I; 51, hind claw (IV); 52, lunulae of claws IV (Scale bars: Fig. 45: 0.5 μ m; Figs 44, 46, 50: 1 μ m; others: 2 μ m. Other explanations in text).

Individuals

A) Measurements:

| | |
|-------------------------------|---|
| Body length | 388.50 ± 63.77 (246-550) [29] * 16.4 ● 423 |
| Buccal tube length | 36.18 ± 3.04 (27.9-41.4) [30] * 8.4 ● 40.1 |
| Stylet supports attachments | 27.14 ± 2.40 (21.2-31.7) [30] * 8.9 ● 30.2 |
| Buccal tube width (external) | 4.23 ± 0.61 (2.7-5.4) [24] * 14.4 ● 5.0 |
| Macroplacoid 1 length | 8.66 ± 1.23 (5.9-10.8) [30] * 14.2 ● 10.8 |
| Macroplacoid 2 length | 5.38 ± 0.90 (3.6-8.1) [30] * 16.8 ● 6.3 |
| Macroplacoid row length | 15.27 ± 2.10 (10.8-20.7) [30] * 13.8 ● 18.9 |
| Claw external 1 length | 9.19 ± 1.37 (6.3-13.5) [28] * 14.9 ● 9.9 |
| Hind claw (4) (= ext.) length | 11.81 ± 1.72 (9.0-16.2) [29] * 14.6 ● 12.6 |

B) Indices (including / r squared):

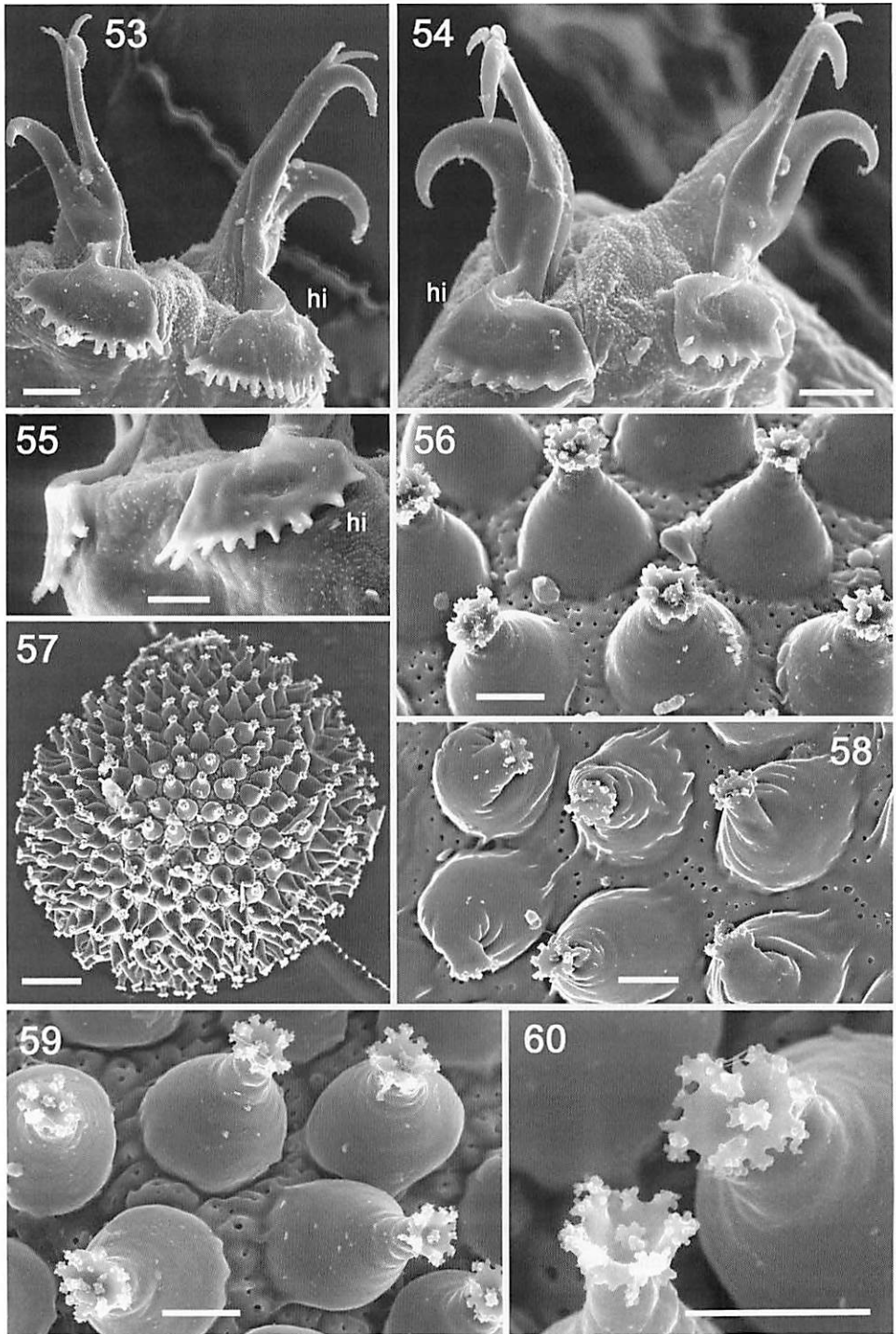
| | |
|------------------------------------|--|
| <i>Pt</i> stylet supports | 74.98 ± 1.39 (71.9-78.5) [30] * 1.9 / 95.4 ● 75.3 |
| <i>Pt</i> buccal tube width (ext.) | 11.72 ± 0.85 (9.7-13.1) [24] * 7.2 / 86.2 ● 12.4 |
| <i>Pt</i> macroplacoid 1 length | 23.83 ± 1.84 (20.3-27.0) [30] * 7.7 / 80.7 ● 27.0 |
| <i>Pt</i> macroplacoid 2 length | 14.79 ± 1.52 (11.0-19.6) [30] * 10.3 / 75.8 ● 15.7 |
| <i>Pt</i> macroplacoid row length | 42.07 ± 3.04 (35.3-50.00) [30] * 7.2 / 80.5 ● 47.2 |
| <i>Pt</i> claw 1 (ext.) length | 25.37 ± 2.52 (21.2-31.5) [28] * 9.9 / 55.9 ● 24.8 |
| <i>Pt</i> hind claw (4) length | 32.32 ± 3.45 (27.0-41.7) [29] * 10.7 / 46.8 ● 31.5 |
| <i>MPLI</i> index | 62.10 ± 4.78 (50.0-75.0) [30] * 7.7 / 77.3 ● 58.3 |
| <i>ECI</i> index | 78.62 ± 4.28 (68.6-88.0) [27] * 5.4 / 84.5 ● 78.6 |

Eggs ($n = 17$); 15 paratypic and two other eggs.

Egg diameter with processes 96.8-113.3 μm , without processes 88.0-103.4 μm . Width of process base 4.0-7.2 μm , length (height) of each process 3.6-7.2 μm . The processes' apical structure, i.e., its distal disc or irregular crown 0.9-3.2 μm in diameter or of that length. Distance between processes 0.9-4.5 μm , usually 1.0-2.0 μm . Diameter of pores (pits) in the interprocess area *c.* 0.2-0.5 μm . Circumference of eggs with 35-41 processes. Equatorial half of egg (i.e., a row in its visible frontal part) with 18-23 processes.

Reproduction mode and ploidy. – A sub-sample of 16 orcein-stained individuals from the type locality was examined for gender and chromosome number. Altogether five males and three females of the new species were identified. The remaining specimens had either immature gonads either or had suffered too much damage (squashing) for interpretation. The gonads are small or medium sized, with germ cells distinctly smaller in males. Mature males have testes filled with early stages of spermatogenesis, spermatogonia (Fig. 67, arrow), spermatids (Fig. 67, arrowhead) and spermatozoa (Fig. 68, arrow), indicating continuous maturation and zonal arrangement as described for *Macrobiotus* by REBECCHI & BERTOLANI (1994). Thus, *M. ramoli* sp. nov. characterizes a bisexual (amphimictic) mode of reproduction.

The testis of one individual had six regular bar-shaped bodies of condensed chromatin in a star-like arrangement (Figs 37-40, arrowheads, Fig. 69, arrow) which can be interpreted, according to the shape and positioning, as three metacentric chromosomes. Since meiosis in Tardigrada is achiasmatic (ALTIERO & REBECCHI 2003), they may represent three bivalents. If this were true, then the new species would represent a bisexual diploid with the chromosome number $2n = 6$ ($n = 3$). (The usual chromosome number in *Macrobiotus* is $n = 6$; $2n = 12$). However, metacentric chromosomes are not known in *Macrobiotus* and in other tardigrades have been reported partly only in *Isohypsibius granulifer* THULIN,



Figs 53-60. *Macrobiotus ramoli* sp. nov.: 53-54, claws IV; 55, lunulae of claws IV; 56, fragment of egg; 57, egg, 58-59, fragment of egg; 60, apical parts of egg processes (Scale bars: Fig. 57: 10 μ m, others: 2 μ m).

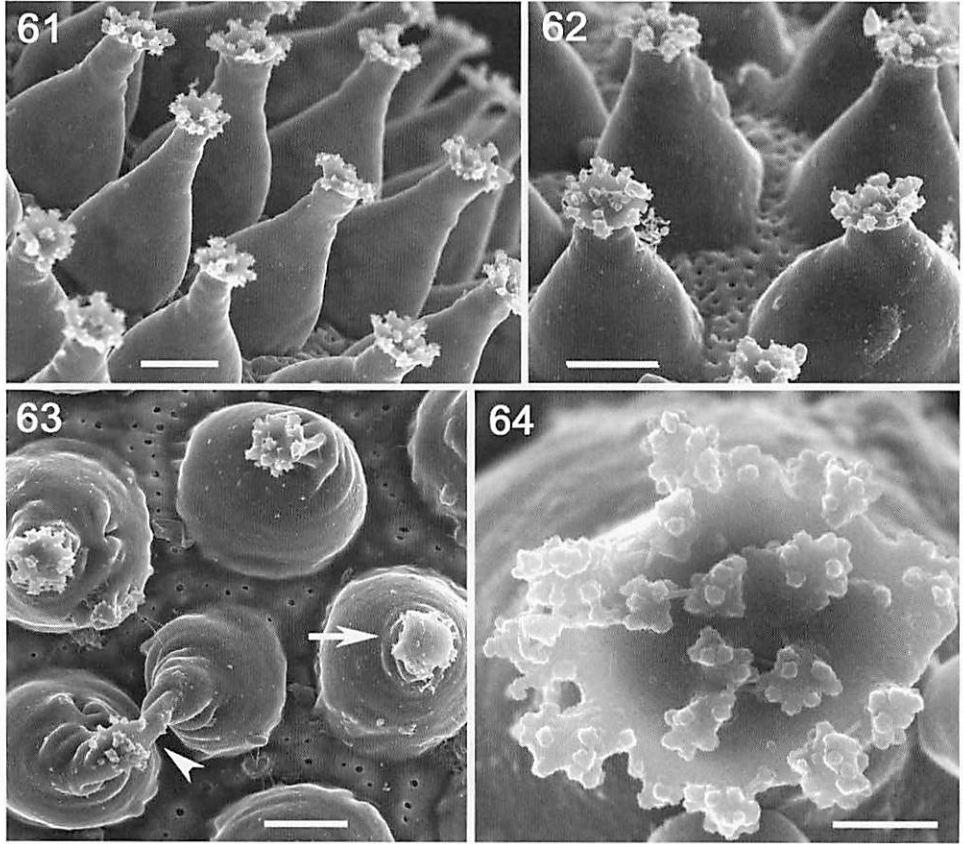
1928 (see BERTOLANI 1975, 1982). In the latter species two different cytotype populations are known, i.e., bisexual with 12 acrocentric chromosomes ($2n = 12$, $n = 6$) and hermaphroditic cytotype with eight chromosomes ($2n = 8$, $n = 4$), from which four are acrocentric and four metacentric. The V-shaped, metacentric chromosomes evolved there by a centric fusion of two chromosome pairs of the population $2n = 12$ (BERTOLANI 1975, 1982). However, the chromatin bodies in *M. ramoli* sp. nov. can also represent mitotic metaphase with six closely V-like placed bivalents ($n = 6$). It should be noted that such bar-shaped bodies occurred also in two other cells in the same testis (different focal planes: see Figs 39, 40, arrows). More material has to be examined before any conclusion can be drawn.

V a r i a b i l i t y. – Generally, individuals of *M. ramoli* sp. nov. show moderate intra-specific variability. However, it is markedly high with respect to shape and number of teeth on the lunulae of legs IV and, to a lesser degree, in size and shape of the dorso-median ridge. This variability was also discernible in the morphometric data. The variation coefficient (V), except for the buccal tube, was relatively low but still marked (~ 13 - 14 %) for all measured characters, the highest (~ 17 %) being for the length of the second macroplocoid. This is confirmed by a wide range of *MPLI* index (50-75 %). The *PT* index for the stylet support (*ss*) is characterized by a very high degree of correlation (association) between both *PT ss* variables (i.e., the length of the whole tube and that of its anterior unit), with the values of *r squared* equaling 95.4 %. Very strong correlation for *PT ss* has also been found in other eutardigrades examined in this respect, i.e., *Macrobotus denticulus* DASTYCH, 2002 ($r^2 = 98.6$ %), *Hypsibius klebelsbergi* ($r^2 = 92.6$ %), *H. janetscheki* ($r^2 = 89.5$ %) or *H. thaleri* ($r^2 = 94.4$ %) (DASTYCH 2002, 2004a, b, DASTYCH et al. 2003). However, the values of *PT ss* index in *M. ramoli* sp. nov. are notable for their wide range (between *c.* 72 and 79 %), an unfavourable character for diagnostic purposes due to the overlap with corresponding values of many other taxa of this species-complex.

Distinct variability characterizes the eggshell structures of the new species. The shape and size of the whole process is quite variable (e.g., Figs 32, 33, 58, 61, 63), with their apex (crowns or discs) varying even more, particularly in their minute apical projections (Figs 32, 34, 56-64). The sculpturing pattern of interprocess areas also varies markedly in the distribution of pores (pits). Although the presence of a more or less regular row of pits between processes prevailed, the pits were often more abundant and irregularly distributed within the whole interprocess area (Figs 58, 63).

D i f f e r e n t i a l d i a g n o s i s. – The shape, size and details of buccal tube, claws, the egg's processes and the presence of two macroplocoids clearly place *M. ramoli* sp. nov. within the assemblage of very similar species known as the *hufelandi*-complex. This grouping most recently comprises *c.* 30 taxa.

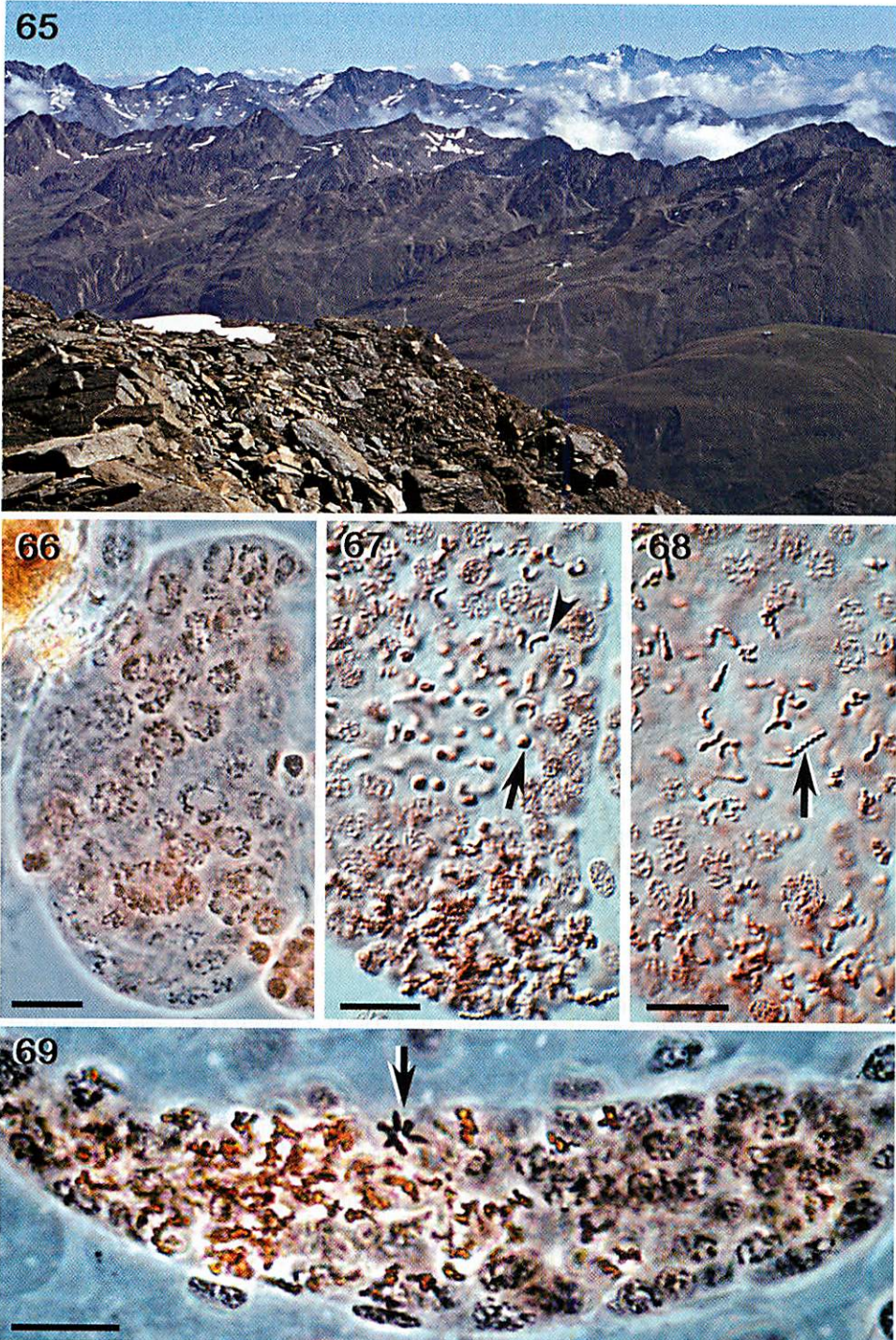
The new species can be easily separated from all other members of the *hufelandi*-group on the basis of a combination of several features. To these belong 1). The characteristically tooth-shaped dorsal median ridge (discernible in dorsal view as a large granule), 2). The



Figs 61-64. *Macrobiotus ramoli* sp. nov.: **61-63**, fragment of egg; **64**, apical part of the egg process (Scale bars: Fig. 64: 0.5 μ m; others: 2 μ m).

markedly long and wide lunulae, those on legs IV additionally with large, numerous teeth, and 3). the conical flask-like processes of the egg with an irregularly formed crown/disk-like apices that has tiny, variable projections.

In the morphology of claws, particularly of legs IV, the new species resembles most closely *M. serratus*. However, the dorsal ridge (*dmr*) in *M. serratus* is shaped as an elongated bar, compared with that of *M. ramoli* sp. nov. Moreover, the new species has on average more (smaller) teeth on lunulae IV and the tips of accessory spines are more splayed, especially on claws IV, than in *M. serratus*. Furthermore, the pore-like cuticular structures and granulation on legs IV are generally larger in *M. serratus* than in the new taxon. The basically similar eggs of the two species differ in the lack of tiny projections on the processes' apices (discs) in *M. serratus*, their larger, less numerous egg processes, the distinctly larger pores in the interprocess area and the resulting more net-like structure, as compared to those in the new species. Also *M. ramoli* sp. nov. has been found exclusively on silicate bedrock, and *M. serratus* only on calciferous bedrock.



Figs 65-69. *Macrobiotus ramoli* sp. nov.: 65, type locality (Mt Spiegelkogel, summit area, 3420 m); 66, ovary; 67-68, fragment of testis with various stages of spermatogenesis; 69, testis with bodies of condensed chromatin (Scale bars: 10 μ m. Fig. 65: Phot. K. THALER, Sept 2004. Figs 66-69: orcein stained. Other explanations in text).

The presence of a granular (tooth-like?) ridge (*dmr*) in the *hufelandi*-group has only been reported in *M. denticulus* DASTYCH, 2002. In this taxon all dorsal ridges are extremely variable in shape and size (DASTYCH 2002: Figs 14-23), but the *dmr* (rarely) resembles in *LM* that seen in the new species (l.c.; Fig. 18; no *SEM* data available). However, the presence of a unique asymmetric minute tooth in the buccal tube and smooth lunulae on legs IV in *M. denticulus* clearly differentiate the species from *M. ramoli* sp. nov. The somewhat similar eggs of these two species can be separated by the presence of markedly larger, less numerous processes in *M. denticulus*, which has differently shaped apical projections and (usually) regular pattern in the distribution of the pits, in comparison to the new species.

The eggs of *M. diversus* BISEROV, 1990 and those of the new species are also similar, particularly in the distribution of pits in the interprocess area. However, in *M. ramoli* sp. nov. the pits are usually less numerous and slightly larger, while the processes' apices are also differently formed. Moreover, individuals of *M. diversus* have their dorsal ridges fused into a uniform, elongated bar, while the claws on legs IV are more stumpy and the lunules have distinctly smaller teeth as those of the new species (comp. BISEROV 1990).

M. hyperboreus BISEROV, 1990 and *M. ramoli* sp. nov. also have similarly shaped eggs' processes. Nevertheless, in the former taxon the pits in the interprocess area are absent and the shape of apical parts of the processes is also different (BISEROV 1990). Moreover, in *M. hyperboreus* the lunulae on legs IV are smooth and its *dmr* is shaped as an uniform, elongated bar.

Biology and distribution. – The information on the biology and distribution of the new species is scanty. All records come from sites above 3000 m a.s.l. (3005-3518 m), indicating a high alpine character for this taxon. The majority of specimens were found in small bryophyte cushions on organic poor mineral soil between/or on rocks or stones, a characteristic habitat in the nival zone, while a few were collected from soil or lichens, but always on silicate (i.e., non calciferous) bedrock.

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