

A new species of the genus *Macrobiotus* SCHULTZE, 1834 from Îles Kerguelen, the sub-Antarctic (Tardigrada)

HIERONYMUS DASTYCH

Universität Hamburg, Zoologisches Institut und Zoologisches Museum, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

ABSTRACT. – *Macrobiotus denticulus* sp. nov., a new tardigrade from the sub-Antarctic archipelago Kerguelen, is described. The species, a bisexual diploid ($2n = 12$), belongs to the *hufelandi*-complex and differs from similar taxa mainly by its unique tooth-like structure in the anterior part of the buccal tube and the characteristics of the egg shell.

KEYWORDS: Tardigrada, *Macrobiotus denticulus* sp. nov., Îles Kerguelen, the sub-Antarctic, chromosomes, $2n = 12$.

Introduction

One of the oldest described and still valid tardigrade species is *Macrobiotus hufelandi* SCHULTZE, 1834, the type species for the ubiquitous genus *Macrobiotus* SCHULTZE, 1834. The species has been recognized for a long time as one of the most common and worldwide distributed tardigrades and has given its name to the assemblage of extremely similar taxa known as the *hufelandi*-complex or -group. The close resemblance and unknown range of morphological variability of the majority of these species make their correct identification very problematic.

Some older and more recent papers have contributed considerably to the revision of the taxa complex (CUÉNOT 1932, GRIGARICK et al. 1973, TOFTNER et al. 1975, PILATO & BINDA 1983, BINDA & PILATO 1984, BERTOLANI 1975, 1982, BERTOLANI & MAMBRINI 1977, BISEROV 1990a, b, BERTOLANI & REBECCHI 1993) and several cryptic species have been separated from *M. hufelandi* and described as new species. With time the originally wide taxonomic criteria for *M. hufelandi* have gradually been narrowed and recently the species has been re-diagnosed (BERTOLANI & REBECCHI 1993). The latter authors (*l.c.*) also discussed and keyed the *hufelandi*-complex. Since then the following new taxa from the group have been described: *Macrobiotus made-gassus* MAUCCI, 1993, *M. drakensbergi* DASTYCH, 1993, *M. seychellensis* BISEROV, 1994, *M. biserovi* BERTOLANI, GUIDI & REBECCHI, 1996 and *M. serratus* BERTOLANI, GUIDI & REBECCHI, 1996. To the species assemblage belong also *M. personatus* BISEROV, 1990 and *M. punctillus* Pilato, BINDA & AZZARO, 1990, both of which were omitted in the discussion by BERTOLANI & REBECCHI (1993). Today the *hufelandi*-

group is a practical but not quite precisely defined grouping, which comprises *c.* 24 phylogenetically more or less related taxa. However, in spite of revisionary progress, this species assemblage still poses a serious taxonomic problem, as our knowledge about the majority of these taxa is very limited.

The past misidentifications of cryptic species such as *M. hufelandi* resulted in widely accepted but incorrect opinions about its cosmopolitan range and omnipresence (e.g., MARCUS 1936, RAMAZZOTTI & MAUCCI 1983). Recent data, however, do not confirm such views. BERTOLANI & REBECCI (1993), who redescribed the species on the basis of material from a type locality (Freiburg, Germany), also implied its occurrence in the Nearctic (Canada). BISEROV (1998) suggested that *M. hufelandi* represents "...boreal-montane species with a Holarctic or even Palearctic (?) distribution" (comp. also DUDICHEV & BISEROV 2001).

During a survey of the tardigrade fauna of the Îles Kerguelen, numerous specimens representing a new species of the *hufelandi*-complex were encountered, of which a description is given below. The present paper is based on material kindly provided by Dr. YVES FRENOT (Université de Rennes, Paimpont) and Dr. JEAN LOUIS CHAPUIS (Museum National d'Histoire Naturelle, Paris).

Material and methods

The new species was found in 16 bryophyte samples from the French sub-Antarctic archipelago of the Îles Kerguelen by Y. FRENOT and J. L. CHAPUIS in 1996. The material was collected into paper envelopes, air-dried and processed in Europe. Animals and their eggs were extracted in Hamburg by the method described by DASTYCH (1985). Two additional eggs of the new species were discovered in the remnants of the RICHTERS' historic tardigrade collection, housed at the Forschungsinstitut Senckenberg, Frankfurt a.M.

The specimens were mounted on microslides in FAURE'S medium or polyvinyl-lactophenol, or in temporary water mounts. All were examined with phase- and interference contrast microscopy. Photomicrographs were taken with ZEISS "Axiomat" and "Photomikroskop III". For karyological observations tardigrades were fixed in CARNOY'S fluid (methanol + acetic acid, 3:1) and stained with aceto-lactic orcein (e.g., BERTOLANI 1971). For SEM (CamScan S4) examination specimens were washed, transferred to hot BOUIN'S medium, dehydrated in ethanol, critical-point-dried and gold-coated. All SEM micrographs and most other photos are based on specimens from the type locality.

Measurements taken from type specimens are based on those mounted either in FAURE'S medium (15 individuals) or in *PVL* (12). The length of the body was measured without legs IV, that of the bucco-pharyngeal apparatus excluded the lamellae. The measurement of the buccal tube length follows that proposed by PILATO (1981) and is the distance between the upper (dorsal) edge of the stylet sheaths (almost at the level of dorsal transversal crests) and the posterior edge of the buccal tube [which includes its terminal apophyses (Fig. 9, arrowhead), but excludes the pharyngeal apophyses (Fig. 9, arrow)]. The (external) diameter of the tube is taken just above the level of the stylet supports. The measurements of placoids are taken from those along the pharyngeal (medial) lumen radius, i.e., located ventrally in the pharynx (Figs 9, 10), to minimize measurement errors. The macroplacoids' width is that of the first macroplacoid at its broadest point. The length of the (external) claw includes accessory spines, main branch and the claw base with pedunculus but excluding the lunula; on the leg IV this is the length of the hind claw, homologous with external claw on legs I-III. Unless otherwise indicated, the measurements provided in the description are those of the holotype.

The following abbreviations are used in text or illustrations: *n* = sample size, *PVL*- polyvinyl-lactophenol, *r*² = coefficient of determination, *SD* - standard deviation, *V* - coefficient of variation, \bar{x} - (arithmetic) mean.

Description

Macrobiotus denticulus sp. nov.

(Figs 1-69)

Macrobiotus hufelandi: RICHTERS 1908, p. 295 (in part?)

H o l o t y p e. – (Figs 8, 20): a male, 466 µm long (coll. Y. FRENOT, 28 Dec 1996), mounted on microslide in FAURE's medium, together with a paratype, a male, 470 µm long. The holotype is mounted dorso-ventrally, the paratype latero-ventrally. The microslide (No 278 y1) has been deposited in the Zoologisches Museum Hamburg (ZMH, Acc. No 52/01).

T y p e l o c a l i t y. – The sub-Antarctic, Îles Kerguelen. Île Mayes (S: 49° 28' 41", E: 69° 53' 50"), 70 m a.s.l., 200 m from the seashore. Fell-field with mineral soil (3 cm thick), from moss. The sample's original reference is: "Ker 07", its working number "99". The following taxa co-occurred in the sample: *Echiniscus arctomys*-group, *Pseudechiniscus suillus*-group, *Macrobiotus harmsworthi*-group.

P a r a t y p e s. – All locality data as above: 67 animals and 74 eggs and numbered on 24 microslides (ZMH, Acc. No 53/01) and mounted in FAURE (19) or PVL medium (5 slides). Three slides (Nos 278c, m, o: three individuals and egg) in PILATO's Collection (University of Catania) and two slides (Nos 278e, g: two individuals and egg) in BERTOLANI's Collection (University of Modena). The remaining paratypes in the Zoologisches Museum Hamburg.

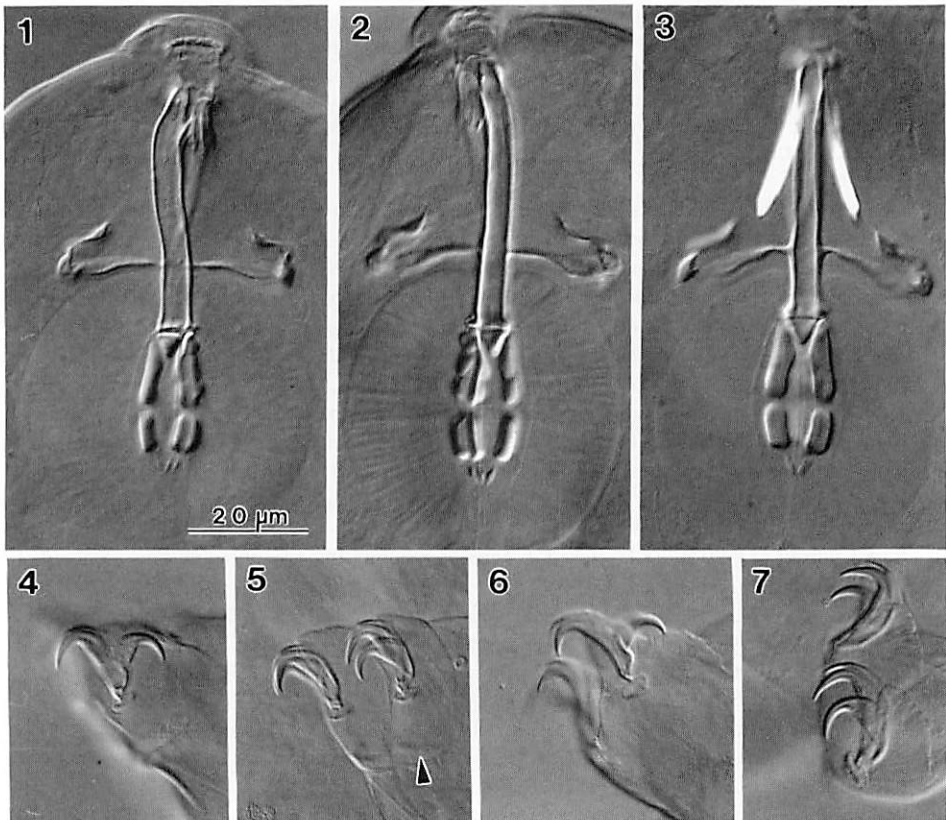
A d d i t i o n a l m a t e r i a l e x a m i n e d (altogether 188 animals, 85 eggs). – The bulk of material originates from Grande Terre, the archipelago's main island, the remaining four samples came from Île Guillou and Île Mayes. Two eggs from the RICHTERS' Collection can be placed on the Îles Kerguelen in general only. The number of extracted animals and their eggs is given in square brackets; the original sample reference number being quoted in rounded brackets (JLC = coll. J. L. CHAPUIS, YF = coll. Y. FRENOT).

- 1) Mortadelle (Site J), Lac (lake) David-Néel. Kame terrace; loamy soil with a desert pavement in a surface. Age of deglaciation 20 years. Small moss cushions growing on loam between pebbles, at 220 m above sea level (a.s.l.), collected Feb 1996, YF [49 specimens + 11 eggs].
- 2) Mortadelle (Site F), Lac Ampère. Outwash of fluvioglacial plain. Flat area. Age of deglaciation 35 years. Moss on pebbles of the desert pavement. 25 m a.s.l., 9 Feb 1996, YF [11 + 1].
- 3) Mortadelle (Site A). Outwash of fluvioglacial plain. Age of deglaciation estimated at 200 years. Moss fixed on pebbles of the desert pavement. 25 m a.s.l., 10 Feb 1996, YF [8 + 2].
- 4) Mortadelle near the huts, on rocks, 150 m. a.s.l. Moss, Feb 1996, YF [9 + 4].
- 5) Baie de la Dauphine/cabane (Ker 01), 50 m a.s.l., 300 m from the seashore, a rocky plateau with mineral soil, moss on stones and gravel, 15 Dec 1996, YF [1 + 1].
- 6) Baie Clemenceau (Ker 06), 50 m from the seashore, 20 m a.s.l., fell-field, moss from stones and gravel, 15 Dec 1996, YF [20 + 9].
- 7) Baie Charrier (1), 20 m a.s.l., closed *Acaena* community, moss with some grass, on soil, 17 July 1996, JLC [7 + 1].
- 8) Baie Charrier (2), wetlands, 20 m a.s.l., moss on soil, 17 July 1996, JLC [1+ 1].
- 9) Grande Muraille (5), 250 m a.s.l., moss on rock, 14 July 1996, JLC [5 + 2].
- 10) Grande Muraille (7), 250 m a.s.l., moss on rock, 15 July 1996, JLC [6 + 1].
- 11) Plaine Ampère (25), 1 m a.s.l., moss, 10 July 1996, JLC [6 + 2].
- 12) Plateau du Larzac (28), 200 m a.s.l., moss, 16 July 1996 [15 + 12].
- 13) Île Mayes (8), moss, 29 Jan 1996, YF [12 + 3].
- 14) Île Guillou (12), moss, 5 Feb 1992, YF [11 + 11 eggs].
- 15) Île Guillou (12 and 14: two samples), wetlands, 60 m a.s.l., moss, 3 June 1996, JLC [20 + 21 and 7 + 1, respectively].
- 16) Two eggs (Figs 53, 54) mounted on two microslides labelled "Kerguelen / Stationsbg / Ei / *Macrobiotus Hufelandi* / 106" and "Kerguelen /22 / Ei von *Macrobiotus Huf*", respectively. The latter egg with a well-developed embryo. The material belongs to RICHTERS' Collection (the Forschungsinstitut Senckenberg, Frankfurt a.M.).

E t y m o l o g y. – The species name is derived from “*denticulus*” (= small tooth, Latin), and stands for characteristic asymmetric tooth-like structure in the anterior part of the buccal tube.

D i a g n o s i s. – Medium or large *Macrobotus* of the *hufelandi*-group *s. lato* with eye-spots. Cuticle smooth, pores present. Anterior part of buccal tube internally with a small asymmetric cuticular tooth; pharyngeal bulb with two macroplacoids and microplacoid. Moderately sized claws of *hufelandi*-type, with smooth lunules. Eggs with flask-shaped processes that are wide based, with smooth surface and variably formed terminal disc (apex). The egg shell interprocess area with a characteristic pattern formed by peribasal ring of mostly large pores around each process.

D e s c r i p t i o n. – The body 264-702 μm long (holotype 466 μm), is mostly whitish; larger specimens often being light- or also distinctly violet and with clumps of brownish pigment. Numerous individuals have a green or green-brownish intestine. Eye-spots posterior and medium-sized.



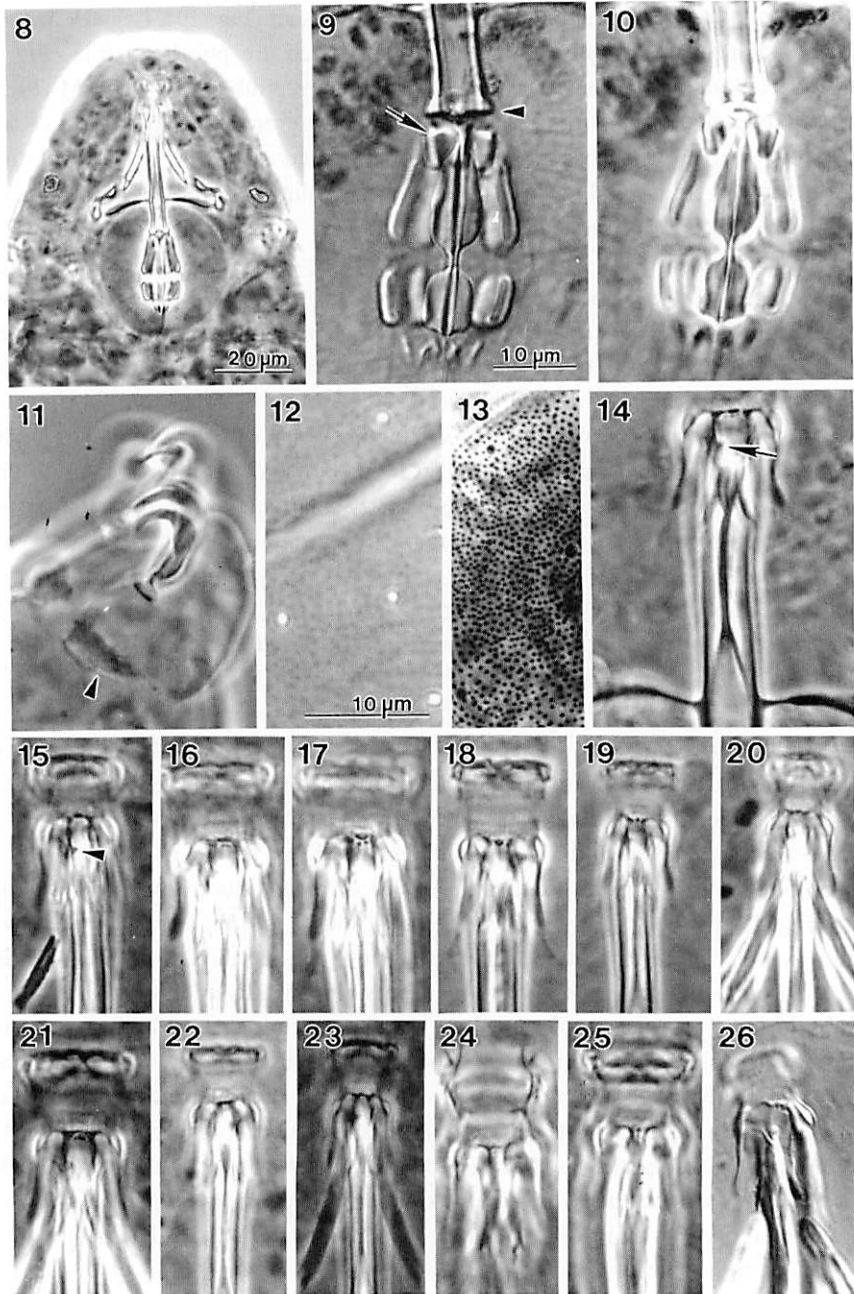
Figs 1-7. *Macrobotus denticulus* sp. nov., 1-3: buccopharyngeal apparatus (1, 2: dorso-lateral, 3: dorsal view); 4 - internal claw I; 5 - claws I; 6 - claws of leg II; 7 - claws of leg IV (Figs 1, 2, 6: PVL; the other: FAURE'S medium. All to same scale as in Fig. 1).

Cuticle smooth in light microscope, small pores present (Fig. 29). Legs IV with tiny, irregularly shaped and closely placed granules, which are poorly visible (Fig. 28). Slightly shrunk cuticle on legs I-III, when present, discernible only in SEM (Figs 27, 30). Most specimens with tiny, regularly distributed granules dispersed over whole body, hardly visible or not discernable in water-mounted animals (Fig. 12) and distinct only after orcein staining (Fig. 13). The latter granulation completely disappears in mounting (PVL or FAURE'S) media.

Mouth opening directed slightly ventro-anteriorly and surrounded by a ring of weakly formed lamelles. Bucco-pharyngeal apparatus moderately sized. Mouth cavity medium-sized, oriented distinctly ventrally, so that its obliquely positioned armature is difficult to observe. Anterior band of granulation absent, the posterior band, when present, weak and relatively narrow, better developed only in larger individuals. Dorsal part of the band composed mostly of 2-4 irregular rows of tiny granules, which are slightly larger in the proximal (posterior) part of the band. Bottom of mouth cavity relatively narrow. Transverse ridges (crests) mostly small, indistinct, difficult to discern and, like the posterior band of granules, markedly variable in shape and size. Dorsolateral ridges short, rounded, and usually with very closely placed dorso-median ridge between and often hardly discernible edges (Figs 19, 20). The dorso-median ridge is rounded (Figs 18, 19) or slightly elongated (Figs 16, 17). Occasionally the ridges are fused on one side, i.e., the dorso-lateral with the dorso-median ridge (Fig. 14). All dorsal ridges often completely merged, particularly in small specimens. In larger individuals 1-2 granule-like structures often occur just under such a fused ridge (Fig. 17). Ventro-lateral ridges weakly formed, mostly indistinct and short, the ventro-median ridge shaped either as a small granule or absent, or in its place occur two or more tiny granule-like structures, often of different size (Figs 24, 25).

Bucco-pharyngeal apparatus in holotype 77.4 μm long. Buccal tube indistinctly S-shaped (Fig. 1), distally slightly bent, moderately long (38.7 μm) and rather narrow (the external diameter just above stylet supports 4.5 μm , the internal 2.7 μm). Buccal lamina long. Distance between anterior edge of the stylet sheaths and stylet supports 28.4 μm , thus in holotype the "pt ss" index (= the length ratio: see below) for the latter structure being 75.1%, this ratio ranges in paratypes between 71.2 and 76.7 %. Anterior part of buccal tube internally on one side with a tiny asymmetric cuticular tooth of unknown function (e.g., Figs 14 and 15: arrow and arrowhead, respectively). This tooth is placed more or less at the level of the anterior part of a stylet sheath, slightly under the mouth cavity ridges and it lies almost on the same plane as the dorsal ridges. The tooth in the holotype is c. 1 μm long and c. 1.3 μm wide at its base. The position of the tooth (left or right side of the buccal tube) dependent on orientation of the specimen on the microslide, being on left when the specimen is mounted with its dorsum up. The tooth is clearly visible at magnification of 1250 times in dorsally or ventrally oriented animals, and is better seen in PVL than FAURE'S slide preparations. The tooth occurs also in smallest individuals. This structure appears to be unique to the species as it has not been reported in any other tardigrade.

Pharynx ovoid, almost spherical (38.7 x 37.8 μm), with distinct apophyses, two macroplacoids and a microplacoid (Figs 1-3, 8-10). First macroplacoid moderately long



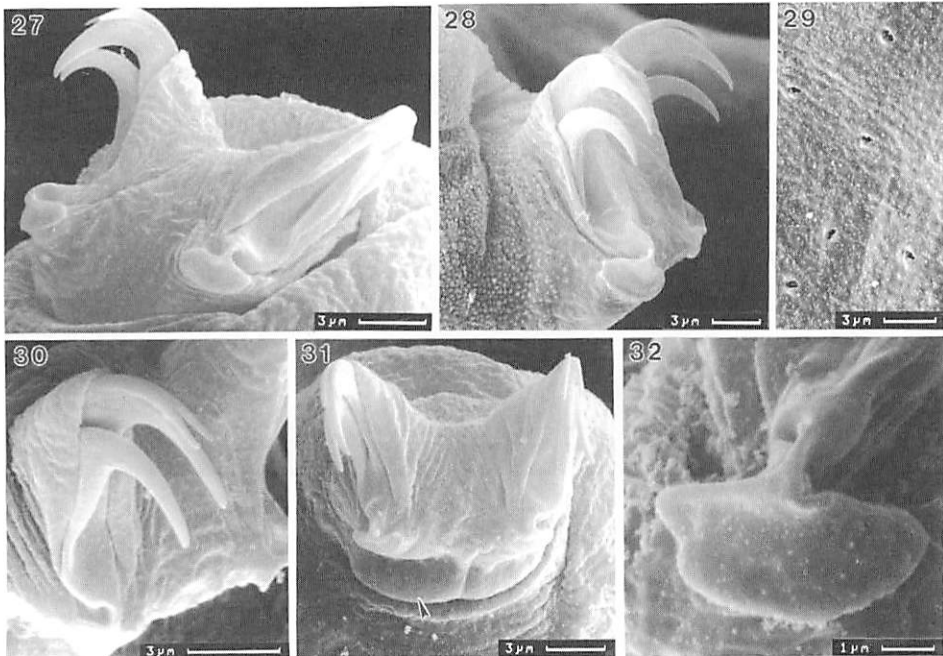
Figs 8-26. *Macrobiotus denticulus* sp. nov.: 8 - front of the body and bucco-pharyngeal apparatus; 9 + 10: fragment of pharynx with placoids and a part of buccal tube; 11- claws of leg III; 12 +13: cuticular granulation; 14-26: variability of dorsal (14-23, 26) and ventral (24, 25) transversal ridges [(Figs 10, 11, 13-26: same scale as in Fig. 9). Figs 9, 26: interference contrast, the others: phase contrast. Figs 9, 10, 13: aceto-lactic orcein stain. Fig. 12: water-mounted specimen, Figs 17, 18, 19 - PVL; the others: FAURE'S medium. Holotype: Figs 8, 20; paratypes: Figs 14-19, 21-25. Juvenile: Fig. 23. Dorsal: Figs 15-23, ventral: Figs 24, 25, dorso-lateral view: Fig. 26. Other explanations in text].

(10.8 μm), usually distinctly constricted in its middle and longer than the second (5.8 μm), the latter usually with a moderate or poorly marked lateral (external) subterminal incision, which is absent in smaller specimens. Microplacoid elongated (2.4 μm long) and thin. Macroplacoids in the holotype 2.7 μm wide, the macroplacoid row length 18.0 μm .

Claws of *hufelandi*-type, moderately sized, strong, with smooth lunules (Figs 4-7, 27, 28, 30-32). Bases of accessory spines well developed, wide (Figs 6, 7), with distinct spines and relatively thin at their tips. The accessory spines lie close to the claw main branch and are not markedly directed upwards (Figs 5-7, 27, 30). Base of legs usually with clearly visible pair of bar-like muscle attachments (Figs 5, 11, 31: arrowheads). External claw I, 10.8 μm long, that on leg IV, 13.5 μm .

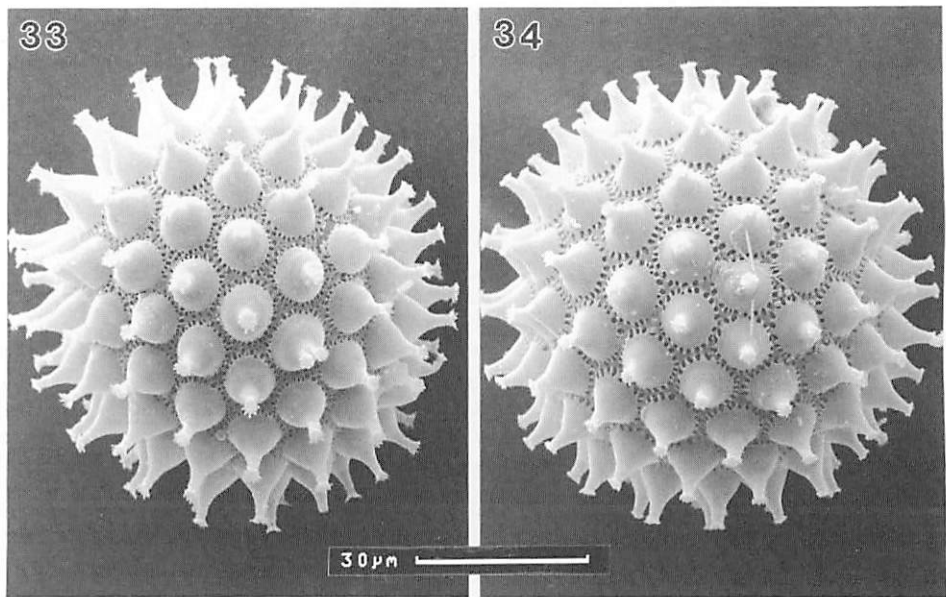
Eggs sub-spherical (Figs 33, 34), rarely ovoid, median sized, whitish, rarely slightly pinkish. Egg processes with their wide bases and narrow distal parts mostly resemble conical (ERLENMEYER's) flasks with slightly widened apices. Some processes double (aberrantly formed) (Figs 40, 45), other (rarely) with a small internal transversal septum separating the process' basal part from its "neck-like" portion (Figs 62, 66).

The apical parts of the processes very variable in shape, either as a more or less regular disc or as an elongated and irregularly shaped asymmetrical cuticular structure (Figs 35-66). When the processes' apices are disc-like, then their surface never concave (e.g., as in *M. hufelandi*) but slightly convex or flat.



Figs 27-32. *Macrobotus denticulus* sp. nov.: 27- claws III; 28 - claws IV; 29 - dorsal cuticle (between II and III pair of legs); 30 - external claw III, latero-dorsal view; 31 - claws II, frontal view; 32 - lunula of hind claw IV.

The apices of the egg processes have a ring of tiny projections, which are of variable shape (mostly teeth, spines, finger-like structures, tubercles or granules, e.g., Figs 33, 37, 42, 43, 49, 64) and size, may frequently be blunt, rounded and either without any projection (e.g., Figs 39, 58, 59) or with a poorly formed, more or less regular ring-like structure (e.g., Figs 39, 58, 59, 62) and can be almost or even completely reduced (e.g., Figs 37, 43, 57, 64). Irregularly formed apical structures prevail among the eggs examined. The remaining surface of the process is smooth. Each process is surrounded by a peribasal ring of numerous (*c.* 20-30), mostly large pits (pores), which are roundish or elongated and 0.8-1.8 μm in diameter. Surface between processes usually with two such rows (rings), forming a species-specific pattern (Figs 35, 45). Length and width of cuticular projections between neighbouring pits slightly variable; the projections mostly forming a distinct "collar" around the base of each process (e.g., Fig. 35, 42, 58, 66). Additional pores between peribasal rings, when present, are usually small. In a few eggs the pits of the interprocess area were relatively small, of similar size and uniformly distributed (e.g., Figs 46, 51, 64), and a few others had pits of intermediate shape and location (e.g., Figs 48, 49, 56, 62), which emphasised the marked variability of the eggs' shell sculpture.



Figs 33-34. *Macrobiotus denticulus* sp. nov., eggs.

Morphometric data

A) Individuals – [based on type material; $n = 27$, unless otherwise indicated. Mean (\bar{x}) \pm standard deviation (SD), (minimum-maximum), variation coefficient (V , in %), respectively]:

1) Measurements (in μm): Body length: $\bar{x} = 495.95 \pm SD 85.0$ (*min-max* 264.0-650.0), $V = 17.0\%$; buccal tube length: 44.00 ± 5.7 (32.8-51.1), $V = 13.0$; buccal tube (external) width: 5.81 ± 1.1 (3.8-8.1), $V = 19.0$; macroplacoid I length: 13.00 ± 2.7 (7.2-18.0), $V = 21.0$; macroplacoid II

length: 7.27 ± 1.6 (4.0-9.9), $V = 22.8$; macroplacoids row length: 21.60 ± 4.5 (12.6-28.3), $V = 20.7$; external claw I length I: 11.69 ± 1.5 (7.2-14.4), $V = 13.2$, $n = 26$; hind claw IV length: 14.59 ± 2.0 (9.0-18.4), $V = 13.8$, $n = 26$.

2) "pt indices" [= a size ratio between length of buccal tube and that of considered structure (comp. Pilato 1981); in %]: Pt index for stylet supports (= pt ss): 73.52 ± 1.1 (71.2-76.7), $V = 1.5$; pt buccal tube width: 13.45 ± 1.3 (11.1-16.1), $V = 10.2$; pt macroplacoid row: 48.67 ± 4.6 (38.3-57.2), $V = 9.4$; pt macroplacoid I: 28.97 ± 3.4 (21.9-36.4), $V = 11.7$; pt macroplacoid II: 16.48 ± 1.8 (12.3-19.5), $V = 11.2$; pt external claw I: 26.45 ± 2.4 (21.9-31.1), $V = 9.0$, $n = 26$; pt external claw IV: 33.45 ± 3.1 (27.4 - 41.8), $V = 9.2$, $n = 26$. The ratio between the body length and that of the bucco-pharyngeal apparatus (in %) equals 18.56 ± 1.5 (16.3 - 21.9), $V = 8.1$, the length ratio (in %) between 1st and 2nd macroplacoid is 57.00 ± 4.5 (50.0-68.8), $V = 8.0$.

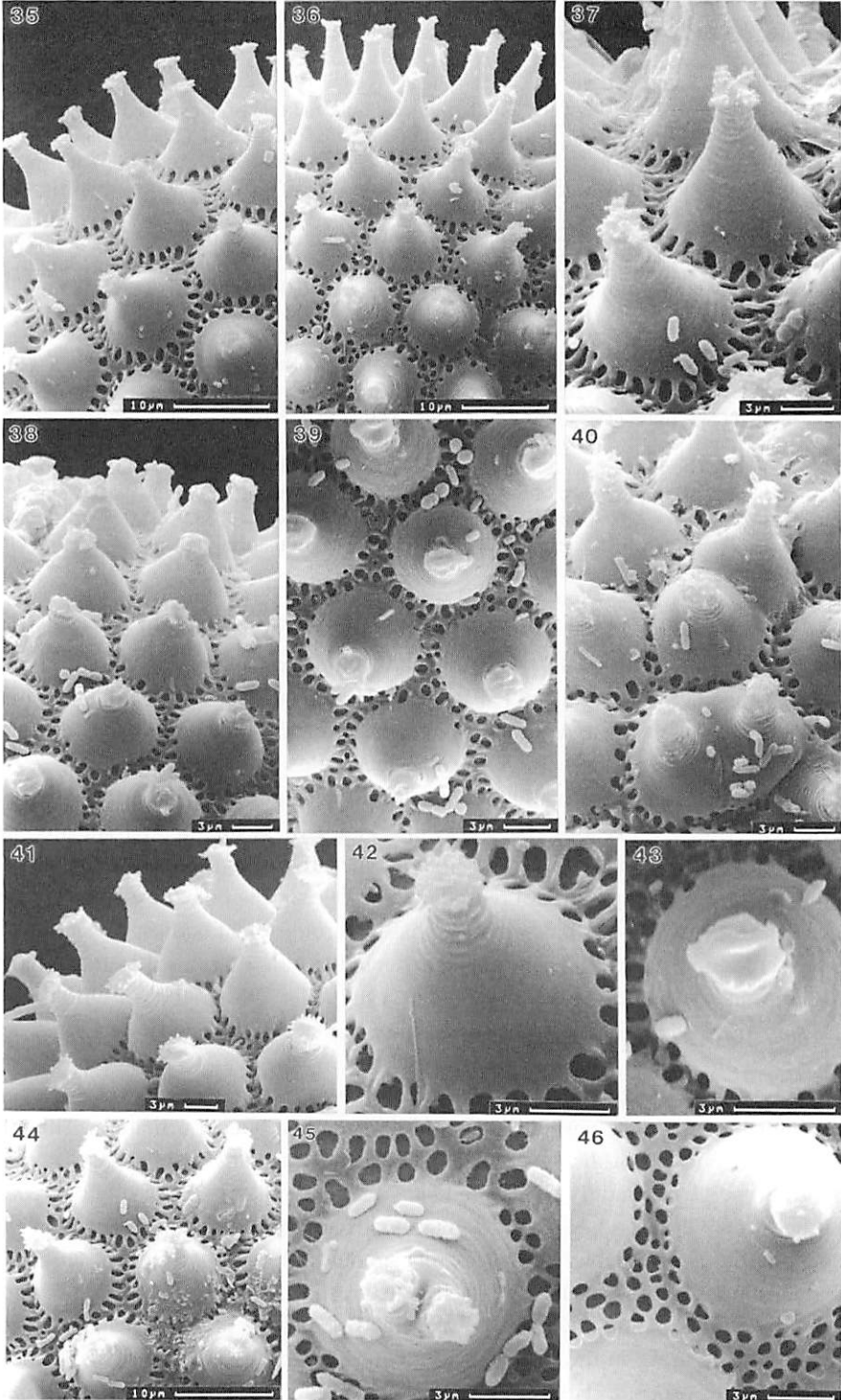
B) Eggs – (paratype and other eggs, $n = 85$. Measurements of two eggs from RICHTERS' Collection in parentheses):

Egg diameter with processes 94.6-127.0 μm (102.0, 134.0), without processes 81.4-114.4 μm (92.0-121.0). Width of process base 8.1-11.7 (8.0-10.0, 12.0), length (height) of each process 8.1-12.6 (11.0-12.0). The processes' apical structure, i.e., its distal disc or its reduced remnants are 1.8-5.4 μm in diameter or they are of that length. Diameter of pores (pits) in the peribasal ring 0.8-1.8 μm (0.8-1.5), with c. 20-30 such pores around each process ($\bar{x} = 23.75$, $SD = 4.4$, *min-max*: 18-32, $n = 8$). Circumference of eggs with 22-29 processes. Equatorial half of egg (i.e. its visible frontal part) with 9-12 processes.

Reproduction mode and ploidy. – A sub-sample of the new species population from the type locality was screened with respect to gender and chromosome number. Altogether 33 orcein-stained individuals were examined, revealing 16 males, 10 females and 7 specimens with gonads not fully matured (differentiated). In mature males a single testis is filled with germ cells, spermatids and spermatozoa (Fig. 69). (A zonal arrangement and continuous maturation of a testis in *Macrobiotus* have been described by REBECCI & BERTOLANI 1994). Males of *M. denticulus* sp. nov. were found relatively often in samples throughout the archipelago, confirming a widely distributed bisexual (amphimictic) reproduction mode. In the oocytes of three of seven females of *M. denticulus* sp. nov. six metaphase I chromosomal bodies (bivalents) (Figs 67, 68) were found, indicating a chromosomal number of $n = 6$ ($2n = 12$), thus implying the diploid bisexual cytotype (comp. BERTOLANI 1982). The number of chromosomes in the somatic tissues of *M. denticulus* sp. nov. was not determined.

Variability. – Individuals of *M. denticulus* sp. nov. are characterized by a relatively high intra-specific variability, particularly of the buccal armature. The variability is mainly found in the shape of dorsal and ventral transversal ridges (= crests). The former merge commonly into a single bar and then separate to varying degree. The variation of the ventral ridges includes mainly difference in shape of the median ridge, its substitution by a variable number of tiny granules or even its frequent absence.

The striking variability of the egg shell structures is another characteristic feature of *M. denticulus* sp. nov. and includes the shape and size of the processes' apices, various patterns of sculpturing in the interprocess areas and, to a lesser degree, the shape and size of a whole process. The latter concerns mainly various length of the process' tapering portion (e.g., Figs 33, 36, 50, 55, 60 vs. 39, 51, 54, 62), sometimes different even in the same egg (Figs 40, 45, 59, 64).



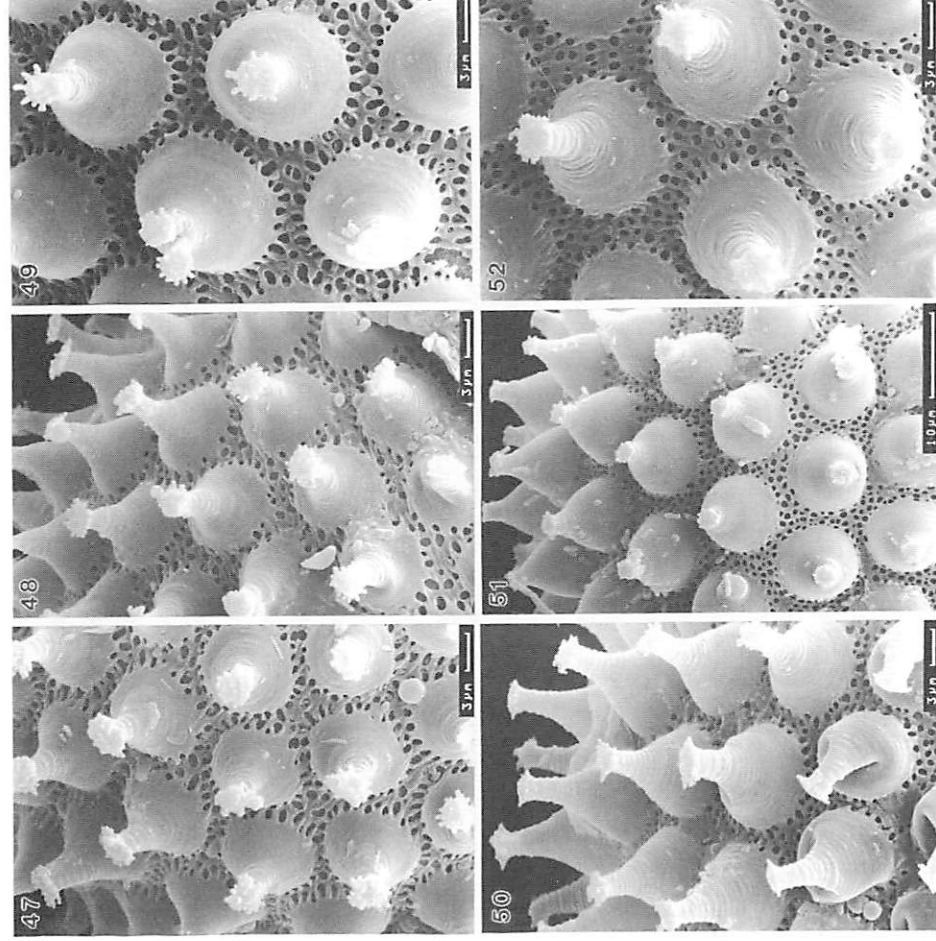
Figs 35-46. *Macrobiotus denticulus* sp. nov.: variability of egg processes and interprocess area.

The sculpturing of interprocess areas also varies considerably, with the prevailing, "typical" pattern of two rows of relatively large and similarly sized pits (pores) between neighbouring processes (e.g. Figs 34, 35, 45, 53, 58, 65), i.e., as the result of the presence of one peribasal ring around each process. In some eggs the size of pits varies markedly, forming an intermediate type of sculpture, either with large and small pits or only small pits in each peribasal ring (e.g., Figs 48, 50, 52, 56). The most diverging arrangement is however one of numerous, small and almost uniformly sized pits between the processes (e.g., 46, 51, 64). The variation in egg morphology were found in the sample from the type locality but also, occasionally, in samples from other sites on the Îles Kerguelen. It should be noted that when considering the different pit arrangements found in the new species (e.g., Figs 42, 45 vs. 46, 51, 64) whilst knowing the taxonomical criteria recently validated for *Macrobotus*, it would be possible to ascribe two such different patterns into separate taxa. The wide range of variability stresses caution which should be born in mind when describing new morphospecies within the *hufelandi*- and other groups, particularly if the description is based on limited type material.

Another aspect of the variability of *M. denticulus* sp. nov. was shown in the quantitative data (see "Measurements" and "*Pt* indices"). The variation coefficient (V ; = vc , in BERTOLANI & REBECCHI 1993) calculated for some characters indicated a small, but still marked variability in the length of buccal tube ($V = 13.0\%$) and that of claws I and IV ($V = 13.2$ and 13.8% , respectively). The remaining characters were distinctly more varied ($V = 17.0$ - 22.8%), particularly the length of placoids and especially that of the macroplacoid II. In comparison, all the size ratios (see *pt* indices) were distinctly less varied. Strikingly low variability was shown by the index *pt* ss ($V = 1.5\%$), confirming the diagnostic importance of that character, already standardized for eutardigrades (e.g., PILATO 1981, BISEROV 1990a, b, BERTOLANI & REBECCHI 1993). Other *pt* indices of the new species were more variable and less useful, i.e., with their variation coefficients ranging between 9.2 and 11.7 %.

Interestingly, *M. denticulus* sp. n. demonstrates also a very high degree of association between both *pt* ss variables, i.e., the length of the whole tube and that of its anterior unit. This is evidenced by the value of the coefficient of determination (r^2 , r squared), i.e., the square of the Pearson's product-moment correlation coefficient, r (e.g., SOKAL & ROHLF 1981) which describes the proportion of variance in one variable explained by variation in the other variable. The coefficient is very high: $r^2 = 0.9860$ the two-tailed p values is < 0.0001 , considered extremely significant), indicating distinct correlation of compared variables, in contrast to other (*pt*) characters, such as buccal tube width ($r^2 = 0.7471$), macroplacoid I ($r^2 = 0.8433$), macroplacoid II ($r^2 = 0.8750$), external claw I ($r^2 = 0.6002$) and hind claw IV ($r^2 = 0.5833$). Worth of notice in *M. denticulus* sp. nov. is that the coefficient value for *pt* macroplacoids row indicates also such a strong relationship ($r^2 = 0.9146$).

Differential diagnosis. – Individuals of the new species can easily be separated from all members of the perplexing *hufelandi*-complex by the presence of the unique, tiny tooth-like structure in the anterior part of the buccal tube. That until



Figs 47-52. *Macrobiotus denticulus* sp. nov.: variability of egg processes and interprocess area.

now had not been reported from any other tardigrade species. As the tooth occurred in juveniles as well as in adults, it permits a relatively certain separation of even the earliest life stages of the new taxon, although such a practice is rarely reliable within the *hufelandi*-assemblage. The “compact” shape of the closely placed dorsal transverse ridges and the presence of a relatively narrow mouth cavity bottom were also distinguishing characters, though more variable and less definite. The combination of other features, such as the relatively high (anterior) location of stylet supports (comp. other *pt* ss indices: BISEROV 1990a, b, BERTOLANI & REBECCHI 1993), the characteristics of the claws, the other characters of buccal armature, buccal tube morphology and pla-coids can also help in separating this species from remaining taxa of the *hufelandi*-group.

The eggs of *M. denticulus* sp. nov are also easily distinguished. However, the marked variability of their shell structures sometimes makes the species identification problematic, when based only on egg morphology. This is due to the (rare) presence in the

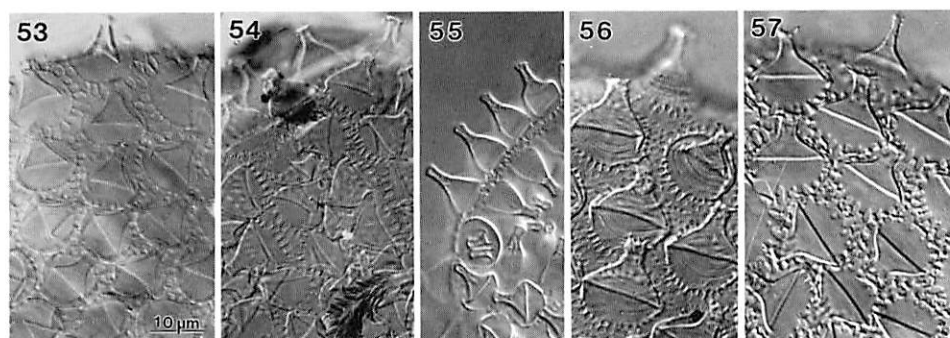
new species of at least two different types of interprocess sculpture (e.g., Figs 37, 63 vs. Figs 51, 64). Thus, as in other taxa of that species assemblage (and also many other species), both eggs and individuals must be available for secure identification.

The relatively anterior insertion of stylet supports and the numerous, small pits in the interprocess sculpture in some eggs of *M. denticulus* sp. nov. (Figs 46, 51, 52, 64) resemble the characters described in the recently reported *M. biserovi* and *M. serratus* (comp. BERTOLANI, GUIDI & REBECCHI 1996). Eggs of *M. biserovi* differ from those of the new species by their distinctly larger and diversely shaped (polygonal) discs (see *l.c.*: Figs 5G, H and 7). While the eggs of *M. serratus* (comp. *l.c.*: Figs 8G, H and 9, 10) are in some aspects more similar to those of *M. denticulus* sp. nov., this taxon can still be easily separated from the new species by its strongly dentated lunules on claws IV.

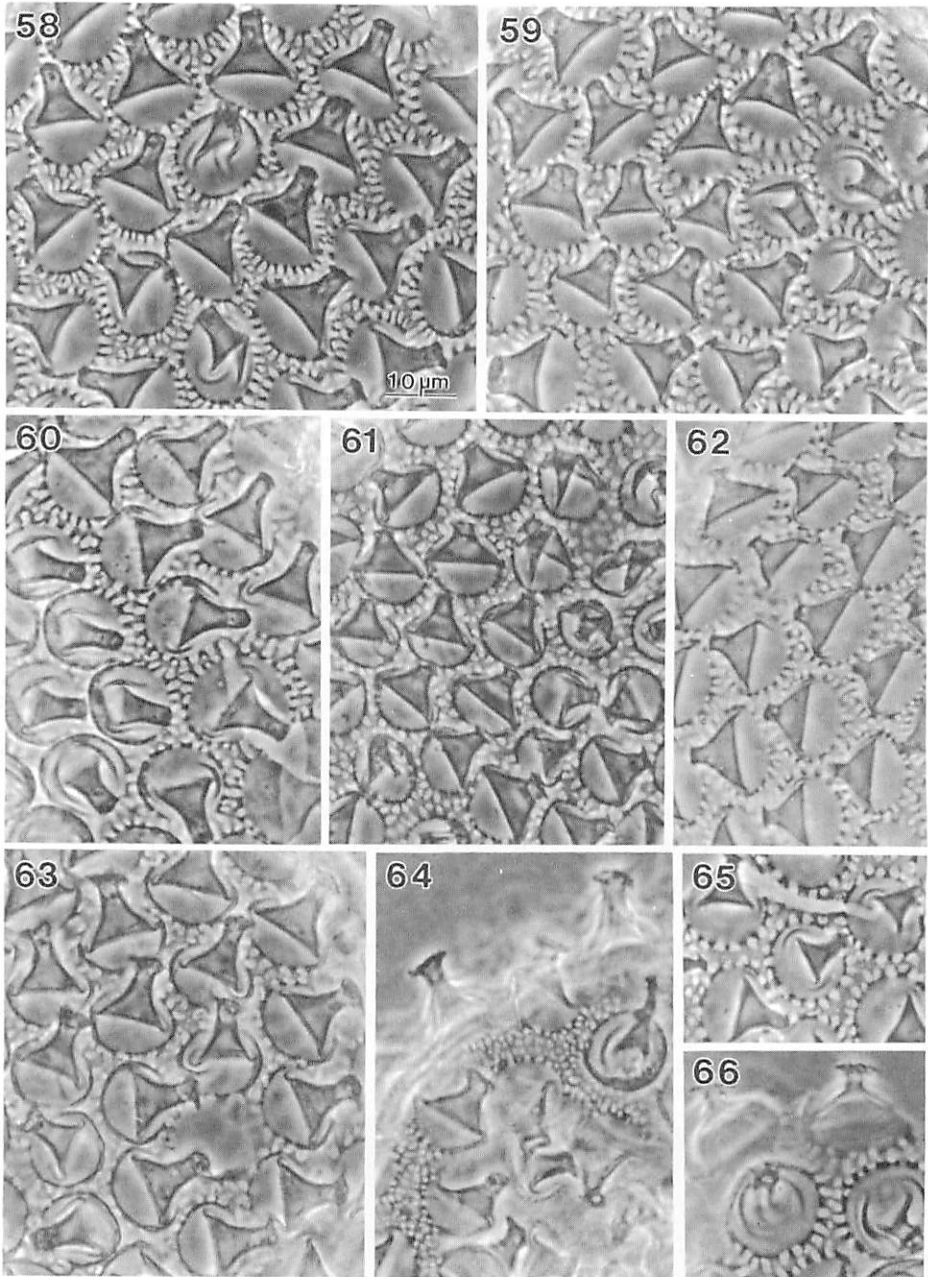
The large pits in the interprocess area on eggs of *M. denticulus* sp. nov. resemble those of *M. rawsoni* HORNING et al., 1978. However, the egg processes are distinctly smaller in *M. rawsoni* and the distal discs and marginal teeth are larger and more regular in shape than in *M. denticulus* sp. nov. Furthermore, *M. rawsoni* has only one row (rarely two) of distinctly larger pits between the processes, compared with the two rows of relatively small pits in *M. denticulus* sp. nov. (GRIGARICK et al., 1973: Fig. 262 and HORNING et al. 1978: Fig 164, upper (!) photos). *M. rawsoni* has 9-11 pits around each process ($\bar{x} = 10.00$, $SD = 0.9$, diameters of pits up to 2.0 μm ; $n = 10$: measurements in a paratypic egg from Rangitira Isl., slide NZ 891), while in *M. denticulus* sp. nov. the pits are smaller and more numerous (18-32 pits; $\bar{x} = 23.75$; up to 1.8 μm in diameter, $n = 8$).

In the eggs of *M. macrocalix* BERTOLANI & REBECCI, 1993 large pits occur in a similar pattern to that of the new species, i.e., two peribasal rings between neighbouring processes (see BERTOLANI & REBECCI 1993: Figs 3G, H, 21, 22). However, the distance between these rings and the size of the apical discs (the latter nearly as wide as the process base) are distinctly larger in *M. macrocalix* than in the new species. The regular dish-like shape of the discs in the former species is also different.

The new species differs distinctly from *M. madegassus* mainly due to the size and shape of the egg processes and sculpturing of the interprocess area (in the latter taxon the processes are small, conical structures with flat apices, while the sculpturing con-



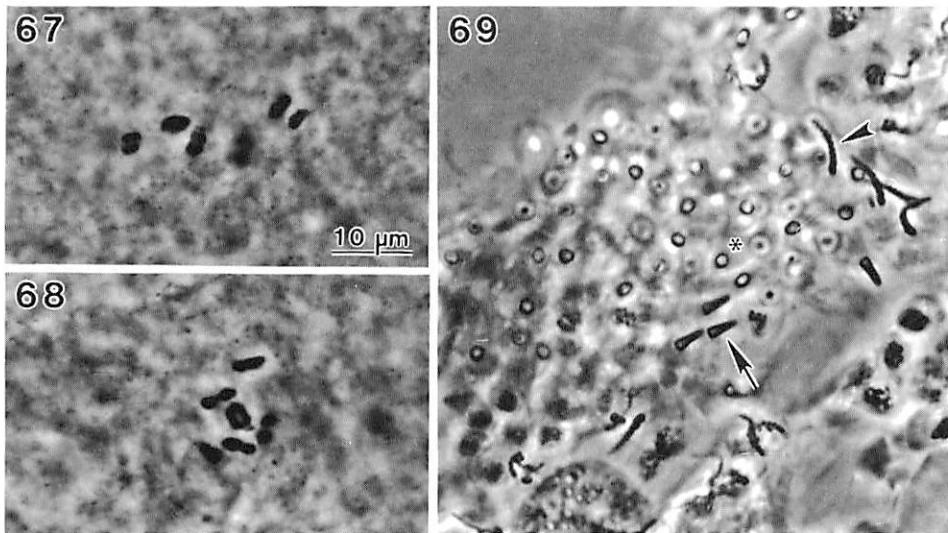
Figs 53-57. *Macrobiotus denticulus* sp. nov.: variability of egg processes and interprocess area (Figs 53, 54: RICHTERS' Collection. Interference contrast; all to same scale as in Fig. 53).



Figs 58-66. *Macrobiotus denticulus* sp. nov.: variability of egg processes and interprocess area (phase contrast; all to same scale as in Fig. 58).

sists of tiny and barely visible pits), longer macroplicoids and the more anterior insertion of stylet supports [the values of *pt ss* in two examined paratypes of *M. madegassus* are 68.3 and 69.8 %, mean (\bar{x}) of that index for $n = 19$ is 68.29 %, according to MAUCCI (1993)].

M. denticulus sp. n. can easily be distinguished from *M. punctillus* by its smooth dorsal and lateral cuticle, narrower mouth cavity and buccal tube, less clearly formed transversal ridges (well separated in *M. punctillus*), smaller accessory spines and distinctly higher (anterior) insertion of the stylet supports (for *M. punctillus* *pt* ss: \bar{x} = 80.1 % in six examined individuals; *pt* ss values 80.72-82.19 %, according to PILATO et al. 1990). Eggs of *M. punctillus* are more similar to those of *M. hufelandi* than to those of the new species with smaller processes (3.6-5.4 μ m in the three eggs examined) and concave and larger distal discs. Furthermore, the disc margin teeth are larger, compared to those in the new species and the interprocess area is more uniform and mesh-like, resembling more that of *M. hufelandi* than the pattern of the new species.



Figs 67-69. *Macrobiotus denticulus* sp. nov.: 67, 68: chromosomal bodies (bivalents) in the oocyte metaphase I (lateral and polar (?) view, respectively); 69 - fragment of testis with germ cells (asterisk), spermatids (arrow) and spermatozoa (arrowhead). (All to same scale as Fig. 67; phase contrast, aceto-lactic orcein).

R e m a r k s. – RICHTERS (1908) reported several species from Îles Kerguelen, including 18 individuals and 31 eggs of *M. hufelandi* and provided photomicrograph of an egg (*l.c.*: Plate 20, Fig. 4). Unfortunately, RICHTERS (1908) does not specify any location for this sampling. Two microslides labelled as *M. hufelandi* are still among the remnants of his tardigrade collection in Frankfurt a. M, but proved to contain only two eggs that *M. denticulus* sp. nov. (Figs 53, 54). From the photomicrograph by RICHTERS (1908: Plate 20, fig. 4) the egg shown is not *M. denticulus* sp. nov. The size and shape of the egg processes in this illustration resembles another species within the *hufelandi*-complex. This second and supposedly new species was also found in the present survey of Îles Kerguelen, though less frequently, and was more similar to *M. hufelandi* (as re-defined by BERTOLANI & REBECCI 1993) than to *M. denticulus* sp.

nov. Sadly, while RICHTERS probably found both species in his survey of Îles Kerguelen almost a century ago, the majority of his microslide collection was destroyed in World War II and thus lost to modern taxonomy.

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