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**Research article**

**Integrative description of *Mesobiotus anastasiae* sp. nov. (Eutardigrada, Macrobiotoidea) and first record of *Lobohalacarus* (Chelicerata, Trombidiformes) from the Republic of South Africa**

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**Abstract.** A new species of the genus *Mesobiotus* is described from the Republic of South Africa using a traditional morphological approach (light and scanning electron microscopy) combined with molecular analysis (18S rRNA, 28S rRNA, ITS-2 and COI markers). *Mesobiotus anastasiae* sp. nov. differs from all known *Mesobiotus* species by having a unique combination of characters of the adult animals and the eggs. Adults of the new species have an oral cavity armature without elongate teeth in the second band, while the processes of the egg chorion have a basal collar and distinct rows of large pores. An updated key to the species of the genus *Mesobiotus*, including 66 of 70 currently described species, is given. An aquatic mite species from the *Lobohalacarus weberi* complex (freshwater Halacaridae) co-occurs with *M. anastasiae* sp. nov., suggesting that the newly described tardigrade inhabits constantly wet moss cushion habitats.

**Keywords.** Tardigrada, Macrobiotidae, molecular taxonomy, new species, key to species, freshwater Halacaridae, new record.

The Tardigrada Doyère, 1840 is a phylum of microscopic multicellular animals widely distributed in nature. Currently there are more than 1300 species described (Degma et al. 2009–2020), but this is very likely an underrepresentation of the actual number of taxa, as the global diversity of tardigrades is poorly investigated (Bartels et al. 2016).

The tardigrade fauna of the Republic of South Africa is the most investigated on the African continent (Middleton 2003; McIness et al. 2017). However, the current state of knowledge is far from complete. Among 46 tardigrade species reported from the Republic of South Africa (McIness et al. 2017;
Meyer et al. 2018; Stec et al. 2020), 22 records (48%) belong to poorly described species, or members of (semi-)cryptic species complexes, whose attribution needs verification. To date, only one species of South African tardigrade (Minibiotus ioculator Stec, Kristensen & Michalczyk, 2020) was described using an integrative approach, including light and scanning electron microscopy investigation, as well as sequencing of molecular markers (Stec et al. 2020).

In 2008 I received a few moss samples collected in the Republic of South Africa. One of these samples contained a new species of the genus Mesobiotus Vecchi, Cesari, Bertolani, Jönsson & Guidetti, 2016. Currently this genus includes 69 species (Kaczmarek et al. 2020; see also Tumanov 2018b and Tumanov & Pilato 2019), being the second-largest genus in the family Macrobiotidae Thulin, 1928. A recent integrative redescription of M. harmsworthi (Murray, 1907), the type species of the genus (Kaczmarek et al. 2018), gave a powerful impetus for further research on the real diversity of Mesobiotus. To date, however, only a small fraction of its species have been genetically characterized, so phylogenetic relationships within the genus remains poorly resolved (Kaczmarek et al. 2020).

In this paper, I describe a new Mesobiotus species using integrative taxonomy. The detailed morphological description is supplemented by DNA sequences of four standard genes used in tardigrade taxonomy and phylogenetics (the nuclear 18S rRNA, 28S rRNA, ITS-2, and the mitochondrial COI).

An aquatic mite species of the genus Lobohalacarus Viets, 1939 (freshwater Halacaridae Murray, 1877), belonging to the Lobohalacarus weberi complex was also found in the sample, being a new record for this genus in the south African region.

**Material and methods**

**Sampling**

The moss sample was collected at the top of Table Mountain (approx. 33°57′43.9″ S, 18°24′38.0″ E, ≈ 1000 m a.s.l.), in the Republic of South Africa, by Irina Nikolaeva, on January 10, 2008. Material was stored within paper envelopes at room temperature. Tardigrade specimens were extracted from rehydrated samples using the standard technique of washing them through two sieves (first with ≈ 1 mm mesh size and second with 35 μm mesh size; Tumanov 2018a). The contents of the finer sieve were examined under a Leica M205C stereo microscope.

**Microscopy and imaging**

The tardigrades found in moss samples were fixed with acetic acid (a few drops of the concentrated acetic acid were added to the glass staining block containing tardigrade specimens in ddH$_2$O) and mounted on slides in Hoyer’s medium. The mite specimen was directly mounted on a slide in Hoyer’s medium without fixation. Light microscopy (LM): resulting permanent slides were examined under a Leica DM2500 microscope equipped with phase contrast (PhC) and differential interference contrast (DIC). Photographs were made using a Nikon DS-Fi3 digital camera with NIS software.

For scanning electron microscopy (SEM), specimens were dehydrated in an ascending ethyl alcohol series (10%, 20%, 30%, 50%, 70%, 96%) and acetone, critical-point dried in CO$_2$, mounted on stubs and coated with gold. A Tescan MIRA3 LMU scanning electron microscope was used for observations (Centre for Molecular and Cell Technologies, St. Petersburg State University).

**Morphometrics**

The sample size for morphometrics was chosen following the recommendations of Stec et al. (2016). Structures were measured only if their orientations were suitable. Body length was measured from the anterior end of the body to the posterior end, excluding the hind legs. The bucco-pharyngeal tube
was measured from the dorsal crests of the oral cavity armature (OCA) to the caudal end of the buccal tube, not including the buccal apophyses. Terminology for the structures within the bucco-pharyngeal apparatus and for the claws follows those of Pilato & Binda (2010) and Michalczyk & Kaczmarek (2003). Elements of the buccal apparatus, claws and eggs were measured according to Kaczmarek & Michalczyk (2017). The macroplacoid length sequence is given according to Kaczmarek et al. (2014). All measurements are given in micrometres (μm). The \( pt \) index used is the percentage ratio between the length of a structure and the length of the buccal tube (Pilato 1981), and is presented here in italics. Morphometric data were handled using ver. 1.6 of the “Parachela” template, which is available from the Tardigrada Register (Michalczyk & Kaczmarek 2013).

**Genotyping**

DNA was extracted from two individual animals using QuickExtract™ DNA Extraction Solution (Lucigen Corporation, USA) using the protocol kindly provided by Torbjørn Ekrem, Norwegian University of Science and Technology (see Supplementary file 1). Preserved exoskeletons were recovered, mounted on a microscope slide in Hoyer’s medium and retained as the hologenophore (Pleijel et al. 2008).

Four genes were sequenced from one specimen: a small ribosome subunit (18S rRNA) gene, a large ribosome subunit (28S rRNA) gene, internal transcribed spacer (ITS-2), and the cytochrome oxidase subunit I (COI) gene. The primers and PCR programs used are provided in Table 1.

The PCR products were visualized in 1.5% agarose gel stained with ethidium bromide. All amplicons were sequenced directly using the ABI PRISM Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) using an ABI Prism 310 Genetic Analyzer in the Core Facilities Center ‘Centre for Molecular and Cell Technologies’ of St. Petersburg State University. Sequences were edited and assembled using ChromasPro software (Technelysium, USA). The COI sequences were translated to amino acids using the invertebrate mitochondrial code, implemented in MEGA7 (Kumar et al. 2016), in order to check for the presence of stop codons and therefore of pseudogenes.

All sequences of the genus *Mesobiotes* available in GenBank at the time of the analysis were downloaded, and those originating from published works with reliable attribution of the investigated taxa were selected (see Appendix 1). Sequences of the 28S gene published by Vecchi et al. (2016) (KT226079–KT226087) and Guil et al. (2019) (MH079488, MH079489) were excluded from comparison as they do not overlap with the sequences obtained in this study. Sequences were aligned using the Muscle algorithm (Edgar 2004) with default settings, as implemented in SeaView ver. 4.0 (Gouy et al. 2010). Uncorrected pairwise distances were calculated using MEGA7 (Kumar et al. 2016) with gaps/missing data treatment set to “complete deletion”. All obtained sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

**Mite identification**

A review paper (Bartsch 1989) was used for the preliminary identification of the mite specimen found. Other publications devoted to the descriptions of *Lobohalacarus* species and subspecies, and zoogeographical records for this genus (Harvey 1988; Bartsch 1995a, 1995b, 2008, 2018), were used for the final identification.

**Institutional acronyms**

Specimens from the following institutions and collections were examined (curator in parentheses).

SPbU = St. Petersburg University, Russia, Faculty of Biology, Department of Invertebrate Zoology (Denis Tumanov)

UNICT = Università degli Studi di Catania, Italy, Museum of the Department of Animal Biology ‘Marcello La Greca’, Binda and Pilato collection (Giovanni Pilato)
Results

Phylum Tardigrada Doyère, 1840
Class Eutardigrada Richters, 1926
Superfamily Macrobiotoidea Thulin, 1928
Family Macrobiotidae Thulin, 1928
Genus Mesobiotus Vecchi, Cesari, Bertolani, Jönsson, Rebecchi & Guidetti, 2016

Mesobiotus anastasiae sp. nov.
urn:lsid:zoobank.org:act:8651D164-4CBC-41DD-A5C4-FF9E584F8088
Figs 1–6, Tables 2–3

Etymology
I dedicate this new species to my daughter, Anastasia.

Material examined

Holotype
REPUBLIC OF SOUTH AFRICA • ♀; Cape Town, top of Table Mountain; approx. 33°57′43.9″ S, 18°24′38.0″ E; ≈ 1000 m a.s.l.; 10 Jan. 2008; I. Nikolaeva leg.; moss on soil, wet depression with a small temporary pond; SPbU 256(8).

Paratypes
REPUBLIC OF SOUTH AFRICA • 5 ♀♀, 3 ♂♂, 22 eggs; same collection data as for holotype; SPbU 256(1) to 256(6) and 256(9) to 256(18) • 2 adults, 7 eggs; same collection data as for holotype; SEM stub; SPbU_Tar16.

Comparative material
PEOPLE’S REPUBLIC OF CHINA • 7 eggs, paratypes of Mesobiotus mauccii (Pilato, 1974); Canton; moss on tree bark; UNICT 2132.

Morphological description
Body elongated (Fig. 1A–B) (morphometrics in Table 2, raw morphometric data are provided in Supplementary file 2). Fresh specimens uncolored or whitish with slightly greenish gut content,
transparent after fixation in Hoyer’s medium. Four specimens with eyes (Fig. 1A), usually well-distinguishable after slide mounting, three specimens without eyes; in two specimens processed for DNA extraction the presence or absence of eyes was not registered. Dorsal cuticle in LM with poorly visible network-like pattern (Fig. 2A). SEM investigation revealed presence of flat tubercles all over the body surface (Fig. 2B–C). Additionally, the body surface is covered with regularly distributed small granules (invisible in LM; Fig. 2B). In the dorso-lateral position, above the bases of the hind legs, zones of concentrated granules are present (Fig. 2C). All legs with granulated areas. Legs I–III with small granulated areas on the external surfaces, near the claw bases (Fig. 4A), invisible or extremely poorly visible in LM (Fig. 4D, black arrowhead), the internal leg surfaces without granulation, with indistinct pulvinus (Fig. 4B, white asterisk). Legs IV with better-developed granulation dorsally (Fig. 2D) and around the claw bases (Fig. 4G).

Buccal-pharyngeal apparatus of *Macrobiotus* type (Fig. 3A) with ventral lamina and ten peribuccal lamellae. Oral cavity armature (OCA) of modified *krynauwi* type (according to Kaczmarek et al. 2020) with three bands of teeth visible in LM. Evident first (anterior) band consists of a single uneven line of relatively large and slightly longitudinally elongated teeth (Fig. 3F, J–K, O, black arrowheads). Second band consists of a wide zone of small dot-like teeth (Fig. 3G, I, L, N), teeth of the anterior-most and

Fig. 1. *Mesobiotus anastasiae* sp. nov., total view. A. Holotype, ♀ (SPbU 256(8)). Dorso-ventral view, PhC. B. Paratype (SPbU_Tar16). Lateral view in SEM. Scale bars = 50 μm.
Table 2. Summary of morphometric data for *Mesobiotus anastasiae* sp. nov. Measurements are given in \( \mu m \), *pt* values in % (the *pt* index is the percentage ratio between the length of a structure and the length of the buccal tube).

| CHARACTER | N | RANGE | MEAN | SD | Holotype |
|-----------|---|-------|------|----|----------|
| Body length | 7 | 284–426 | 366 | 44 | 426 |
| Buccopharyngeal tube | | | | | |
| Buccal tube length | 9 | 35.1–45.5 | 40.8 | 3.5 | 42.6 |
| Stylet support insertion point | 9 | 26.5–35.8 | 31.5 | 3.1 | 33.1 |
| Buccal tube external width | 7 | 4.1–6.0 | 5.1 | 0.6 | 5.4 |
| Buccal tube internal width | 7 | 2.8–4.0 | 3.4 | 0.4 | 3.6 |
| Ventral lamina length | 9 | 21.9–30.7 | 26.2 | 2.7 | 27.1 |
| Placoid lengths | | | | | |
| Macroplacoid 1 | 9 | 3.7–6.1 | 4.8 | 0.9 | 5.2 |
| Macroplacoid 2 | 9 | 2.8–4.4 | 3.6 | 0.5 | 4.4 |
| Macroplacoid 3 | 9 | 3.2–6.4 | 4.7 | 1.1 | 5.4 |
| Microplacoid | 9 | 2.0–3.3 | 2.6 | 0.5 | 3.3 |
| Macroplacoid row | 9 | 11.7–18.3 | 15.1 | 2.5 | 16.6 |
| Placoid row | 9 | 13.9–21.5 | 18.2 | 2.9 | 19.7 |
| Claw 1 lengths | | | | | |
| External primary branch | 5 | 7.6–10.8 | 9.4 | 1.2 | 9.9 |
| External secondary branch | 5 | 5.9–8.0 | 7.2 | 0.8 | 7.4 |
| Internal primary branch | 7 | 7.3–10.5 | 9.0 | 1.0 | 9.4 |
| Internal secondary branch | 7 | 5.9–8.3 | 7.0 | 0.9 | 6.8 |
| Claw 2 lengths | | | | | |
| External primary branch | 6 | 8.0–10.8 | 9.4 | 1.0 | 10.2 |
| External secondary branch | 6 | 6.6–8.4 | 7.4 | 0.7 | 8.0 |
| Internal primary branch | 6 | 7.4–10.1 | 9.0 | 1.1 | 10.1 |
| Internal secondary branch | 6 | 6.0–7.7 | 7.0 | 1.1 | 6.8 |
| Claw 3 lengths | | | | | |
| External primary branch | 6 | 7.8–10.9 | 9.5 | 1.1 | 10.3 |
| External secondary branch | 6 | 6.1–8.8 | 7.5 | 0.9 | 8.0 |
| Internal primary branch | 7 | 7.1–10.7 | 9.4 | 1.3 | 10.1 |
| Internal secondary branch | 7 | 5.8–8.2 | 7.2 | 0.9 | 7.8 |
| Claw 4 lengths | | | | | |
| Anterior primary branch | 6 | 9.8–12.3 | 11.1 | 1.0 | 11.4 |
| Anterior secondary branch | 6 | 7.0–8.6 | 7.9 | 0.6 | 8.6 |
| Posterior primary branch | 5 | 11.7–12.9 | 12.3 | 0.6 | 12.9 |
| Posterior secondary branch | 5 | 8.0–9.4 | 8.6 | 0.7 | 8.7 |

(rarely) posterior-most rows of the second band are slightly larger than others, sometimes teeth of the anterior-most row are very slightly longitudinally elongated. Third band comprises three dorsal and three ventral transverse ridges (Fig. 3H, J, M, O). Medio-ventral ridge is usually more or less clearly divided into two separate parts (Fig. 3J, O). Pharyngeal bulb with apophyses, three macroplacoids and a large microplacoid (Fig. 3A). Macroplacoid length sequence is \( 2 < 3 = 1 \). First macroplacoid is anteriorly
narrowed, third macroplacoid without distinct subterminal constriction, but with strong terminal protrusion, directed towards pharynx lumen (Fig. 3B–E, black arrowheads).

Claws of *Mesobiotus* type with minute stalk, distinct distal part of the basal portion, short common tract and developed internal septum, defining a distal part. Primary and secondary branches diverge at a point near half the claw height, main branches with long accessory points, which at a large distance from the main claw (Fig. 4C, E–F, H). Claws of fourth pair of legs slightly longer than claws of first three pairs of legs (Fig. 4H). All claws with smooth lunules (Fig. 4C, E–F, H). Anterior (internal) and posterior (external) claws of legs IV are similar in shape, with equally sized lunules. Poorly developed bar-like cuticular thickenings are present below claw bases of the first three pairs of legs (Fig. 4C, black arrowhead). Claws of legs IV are connected with a wide horseshoe-like structure (Fig. 4F, black arrowhead).

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**Fig. 2.** *Mesobiotus anastasiae* sp. nov., cuticular sculpture. **A.** Paratype (SPbU 256(18)). Sculpture of the dorsal body surface, PhC. **B–D.** Paratype (SPbU_Tar16). **B.** High magnification of the sculpture of the dorsal body surface, SEM. **C.** Dorso-lateral zone of concentrated cuticular dots, SEM. **D.** Dot-like sculpture on the dorsal side of hind legs, SEM. Scale bars A, C, D = 5 µm; B = 2 µm.
TUMANOV D.V., Description of *Mesobiotus anastasiae* sp. nov.

**Fig. 3.** *Mesobiotus anastasiae* sp. nov., bucco-pharyngeal apparatus. **A**, **E–O**. Paratype, ♀ (SPbU 256(9)). **B–D**. Holotype, ♀ (SPbU 256(8)). **A**. Total dorso-ventral view of the bucco-pharyngeal apparatus, DIC. **B**. Ventral row of macroplacoids, focused on their outer surface, DIC. **C**. Ventral row of macroplacoids, focal plane shifted deeper into the parynx, black arrowheads indicate appendices of the third macroplacoid bent inward, DIC. **D**. Lateral rows of macroplacoids, black arrowheads indicate appendices of the third macroplacoid bent inward, DIC. **E**. Ventral row of macroplacoids, focused on their outer surface; note the absence of the preterminal constriction of the third macroplacoid, DIC. **F–O**. Oral cavity armature, dorsal (F–H, K–M) and ventral view (I–J, N–O), black arrowheads indicate teeth of the first band, F–J = PhC, K–O = DIC. Scale bars: A = 10 µm; B–O = 5 µm.
Fig. 4. *Mesobiotus anastasiae* sp. nov., paratype (SPbU_Tar16). Legs. A. External surface of leg III with developed dot-like sculpture, SEM. B. Internal surface of leg II without dot-like sculpture, white asterisk – pulvinus on the inner side of the leg, SEM. C. Claws of leg I, black arrowhead indicates bar-like cuticular thickening (holotype), PhC. D. Leg II, black arrowhead indicates dot-like sculpture on the external surface (SPbU 256(1), paratype), PhC. E. Claws of leg II, black arrowhead indicates dot-like sculpture on the external surface of the leg, SEM. F. Claws of leg IV, black arrowhead indicates horseshoe-like structure (SPbU 256(13), paratype), PhC. G. Leg IV, dot-like sculpture on the ventral surface (holotype), PhC. H. Claws of leg IV, SEM. Scale bars = 5 µm.
Fig. 5. *Mesobiotus anastasiae* sp. nov., eggs. A–F. Paratype (SPbU 256(15)). G–I. Paratype (SPbU 256(4)). A. Total view of the egg surface, PhC. B. Total view of the optical section of the embryonated egg, PhC. C–D. Details of the egg surface, DIC, PhC. E. Optical section of the egg process, black arrowhead indicates pore, DIC. F–I. Optical sections of different egg processes, black arrowheads indicate collar, PhC. Scale bars: A–B = 20 µm; C–D = 10 µm; E–I = 5 µm.
Fig. 6. *Mesobiotus anastasiae* sp. nov., paratype (SPbU_Tar16). Eggs. A–C. Total view of the egg, SEM. D–F. Details of the egg surface, white arrowheads indicate pores above the collar, white arrows indicate pores below the collar, SEM. Scale bars: A–C = 20 µm; D–F = 5 µm.
Eggs spherical, white, ornamented and laid freely (Figs 5A–B, 6A–C; morphometrics in Table 3). Chorion with conical processes that can be attributed to the “sharp wide cones with collars” and “reticular design with “bubbles”” morphotypes (according to Kaczmarek et al. 2020). Egg processes with wide bases and thinned and flexible apices (Figs 5C–I, 6D–F). Basal parts of the processes with bilayered walls, with a net of trabecular structures between the internal and external layers, forming irregular rounded meshes of different size, so the processes seem to be reticulated in LM (Fig. 6C–I). Apical parts of the processes with bubble-like internal structure (Fig. 5E–I), often branching (Figs 5E–F, 6E–F). The SEM observations revealed circular wrinkles on the outer surface of the processes (Fig. 6D–F). Basal parts of the processes with well-developed collar elevated above the egg surface (Figs 5G–I, black arrowheads; 6D–F). Large pores (1–3 µm in diameter), visible in LM and in SEM, are present on the surface of all processes, near the collar, in two rows: one row of larger pores above the collar and second row of smaller pores below the collar (Figs 5C–E, black arrowhead; 6D–F). Process bases are smooth, without a crown of granules or teeth. Egg surface between the processes without areolation, with a system of

| CHARACTER                        | N   | RANGE     | MEAN  | SD  |
|----------------------------------|-----|-----------|-------|-----|
| Egg bare diameter                | 13  | 61.7–74.7 | 66.7  | 4.0 |
| Egg full diameter                | 13  | 84.6–108.5| 95.9  | 7.1 |
| Process height                    | 39  | 14.6–21.3 | 17.7  | 1.8 |
| Process base width                | 39  | 11.8–18.6 | 15.5  | 1.6 |
| Process base/height ratio        | 39  | 63%–117%  | 88%   | 9%  |
| Inter-process distance            | 39  | 0–3.8     | 1.3   | 0.9 |
| Number of processes on egg circumference | 13  | 12–15    | 13.4  | 0.9 |

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Fig. 7. *Mesobiotus mauccii* (Pilato, 1974). Paratype egg (UNICT 2132). A–B. Egg processes, white arrowhead indicates polygonate mesh-like pattern, black arrowheads indicate the collar, black arrows indicate pores, DIC. Scale bars: 10 µm.
irregularly distributed ridges and small pores between them (Fig. 6D–F). In some eggs underdeveloped small processes are present among normal processes (Fig. 6B, E).

Reproduction
The new species is dioecious. Adult males were identified by having testis filled with spermatozoa, visible under PhC on mounted slides. Males of *M. anastasiae* sp. nov. exhibit no secondary sexual dimorphism.

DNA sequences
Sequences of good quality for the four aforementioned molecular markers were obtained from one specimen (voucher slide SpbU 256(11)):

- COI sequence (GenBank: MT904513), 658 bp long;
- 18S rRNA sequence (GenBank: MT903468), 1030 bp long;
- 28S rRNA sequence (GenBank: MT903612), 740 bp long;
- ITS-2 sequence (GenBank: MT903470), 419 bp long.

Phylum Arthropoda von Siebold, 1848
Subphylum Chelicerata Heymons, 1901
Class Arachnida Lamarck, 1801
Superorder Acariformes Zakhvatkin, 1952
Family Halacaridae Murray, 1877

*Lobohalacarus* cf. *weberi* (Romijn & Viets, 1924)

Material examined
REPUBLIC OF SOUTH AFRICA • 1 ♀; Cape Town, top of Table Mountain; approx. 33°57′43.9″ S, 18°24′38.0″ E; ≈ 1000 m a.s.l.; 10 Jan. 2008; I. Nikolaeva leg.; moss on soil, wet depression with a small temporary pond; SpbU 256(20).

During sample processing, a single mite specimen from the family Halacaridae (Chelicerata, Trombidiformes) was found (Fig. 8A). In having an undivided ventral shield (Fig. 8B) and developed frontal spine (Fig. 8C) this specimen undoubtedly belongs to the *Lobohalacarus weberi* complex of species, and is the first record of this genus from southern Africa (Bartsch 2018). Apart from its nominative variable species *Lobohalacarus weberi* (Romijn & Viets, 1924), this complex includes several similar species and subspecies with unclear taxonomic status (Bartsch 1995a, 2018). The studied specimen is similar to *Lobohalacarus weberi tristanensis* Bartsch, 1995 (known from Tristan da Cunha Islands only) by having genu I with two ventral spines (Fig. 8A, black arrows) and genital sclerites with two pairs of genital acetabula (Fig. 8D, white arrowheads), but differs from this form by having five pairs of perigenital setae (Fig. 8B, white arrowheads).

Discussion
Phenotypic differential diagnosis of *Mesobiotus anastasiae* sp. nov.

Adult animals
Within the genus *Mesobiotus*, only two species have OCA of the *krynauwi* type – without the row of the longitudinally elongated teeth in the second band and with a developed first row of teeth: *M. krynauwi* (Dastych & Harris, 1995) and *M. tehuelchensis* (Rossi, Claps & Ardoihain, 2009).
Fig. 8. *Lobohalacarus* cf. *weberi*, ♀ (SPbU 256(20)). A. Total view, black arrows indicate spines on genua I, PhC. B. Ventral shield, white arrowheads indicate bases of perigenital setae, PhC. C. Frontal spine, DIC. D. Genital opening, white arrowheads indicate genital acetabula, DIC. Scale bars: A = 100 µm; B = 50 µm; C = 10 µm; D = 5 µm.
**Mesobiotus anastasiae** sp. nov. differs from *M. krynauwi* (Dastych & Harris, 1995) (known from several locations in Antarctica only, Dastych & Harris 1995) by having cuticle with thin reticulate pattern visible in PhC (smooth cuticle with small pores in *M. krynauwi*), by having the first band of teeth of OCA consisting of a single line of elongated teeth (several lines of minute dot-like teeth in *M. krynauwi*), by having lunules of the hind legs without teeth (usually with sharp teeth in *M. krynauwi*), by having eggs with a smaller bare egg diameter (61.7–74.7 µm in *M. anastasiae* sp. nov. vs 98–115 µm in *M. krynauwi*), larger egg processes (processes height 14.6–21.3 µm in *M. anastasiae* sp. nov. vs 9–14 µm in *M. krynauwi*) with large pores and collar (absent in *M. krynauwi*), and by having the egg surface between processes with ridges and pores (smooth in *M. krynauwi*).

**Mesobiotus anastasiae** sp. nov. differs from *M. tehuelchensis* (Rossi, Claps & Ardohain, 2009) (known only from the type locality in Agentina, Rossi et al. 2009) by having cuticle with thin reticulate pattern visible in PhC (smooth cuticle in *M. tehuelchensis*), by having the first band of teeth of OCA consisting of a single line of elongated teeth (several lines of minute dot-like teeth in *M. tehuelchensis*), by having a thinner buccal tube (external buccal tube width is 4.1–6.0 µm vs 9.9–14.9 µm in *M. tehuelchensis*), by having eggs with a smaller bare egg diameter (61.7–74.7 µm in *M. anastasiae* sp. nov. vs 77 µm in *M. tehuelchensis*), and smaller egg processes (processes height 14.6–21.3 µm in *M. anastasiae* sp. nov. vs ≈ 50 µm in *M. tehuelchensis*) with large pores and a collar (absent in *M. tehuelchensis*).

**Eggs**

Based on the morphology of the processes, eggs of *M. anastasiae* sp. nov. should be attributed to the “sharp wide cones with collar” morphotype (Kaczmarek et al. 2020). Within the genus *Mesobiotus* only two species have this type of egg processes: *M. joenssoni* Guidetti et al., 2019 and *M. mauccii* (Pilato, 1974) (the former species’ eggs were erroneously attributed to the “sharp narrow cones” morphotype in Kaczmarek et al. (2020)). In all three species, the conical processes have a wide base with a developed collar (or “circular thickness” (Guidetti et al. 2019)), situated on the process wall above the egg surface. Additionally, the egg processes of all three species bear large pores on their side walls.

**Mesobiotus anastasiae** sp. nov. differs from *M. joenssoni* (known only from the type locality on Elba Island, Italy, Guidetti et al. 2019) by having cuticle with thin reticulate pattern visible in PhC (cuticle with large (visible in LM) granules in the caudal region of the dorsal side in *M. joenssoni*), by having OCA without elongated teeth in the second band (developed in *M. joenssoni*), by having the first band of teeth of OCA consisting of a single line of elongated teeth (several lines of minute dot-like teeth in *M. joenssoni*), by having eggs with smaller egg processes (processes height 14.6–21.3 µm in *M. anastasiae* sp. nov. vs 27–36 µm in *M. joenssoni*) with larger and more numerous pores above the collar, and with a second line of pores below the collar (absent in *M. joenssoni*).

**Mesobiotus anastasiae** sp. nov. differs from *M. mauccii* (known from several Asian locations: China (Pilato 1974; Beasley & Miller 2007, 2012), South Andaman Island (Maucci & Durante Pasa 1980), Japan (Utsugi 1988; Abe & Takeda 2000, 2005)) by having cuticle with thin reticulate pattern visible in PhC (cuticle smooth in *M. mauccii*), by having OCA without elongated teeth in the second band (developed in *M. mauccii*), by having the first band of teeth of OCA consisting of a single line of elongated teeth (several lines of minute dot-like teeth in *M. mauccii*), by having a thinner buccal tube (external buccal tube width is 4.1–6.0 µm vs ca 11 µm in *M. mauccii* type material), by having egg processes with numerous pores arranged in lines above and below the collar (rare single pores are distributed all over the process surface in *M. mauccii*), and in lacking ridges on the egg surface between the processes, forming a mesh-like pattern with polygonate cells circling each process (Fig. 7A–B).
Genotypic differential diagnosis of *Mesobiotus anastasiae* sp. nov.

The ranges of uncorrected genetic $p$-distances between the studied population of *Mesobiotus anastasiae* sp. nov. and other species of the genus *Mesobiotus*, for which sequences are available from GenBank (see Appendix 1), are as follows:

COI: 19.15%–26.60% (mean 21.66%), with the most similar being *M. gr. furciger* from Norway (MH195153, Kaczmarek *et al.* 2018), and the least similar being *M. furciger* (Murray, 1907) from Antarctica (JX865308, Czechowski *et al.* 2012).

18S rRNA: 1.29%–6.01% (mean 3.47%), with the most similar being *M. occultatus* Kaczmarek, Zawierucha, Buda, Stec, Gawlak, Michalczyk & Roszkowska, 2018 from Svalbard (MH197147, Kaczmarek *et al.* 2018), and the least similar being *M. dilimanensis* from the Philippines (MN257048, Itang *et al.* 2020).

28S rRNA: 4.56%–12.11% (mean 7.70%), with the most similar being *M. harmsworthi* (Murray, 1907) from Svalbard (MH197264, Kaczmarek *et al.* 2018), and the least similar being *M. dilimanensis* (MN257049, Itang *et al.* 2020).

ITS-2: 13.03%–29.83% (mean 20.47%), with the most similar being *M. philippinicus* Mapalo, Stec, Mirano-Bascos & Michalczyk, 2016 from the Philippines (KX129795, Mapalo *et al.* 2016), and the least similar being *M. dilimanensis* (MN257050, Itang *et al.* 2020).

Full matrices with $p$-distances are provided in Supplementary file 3.

Ecological notes

The location where the investigated moss sample was taken was characterized by the collector as a wet depression with a small temporary pond. But the genus *Lobohalacarus*, which was also found in the sample, is known to inhabit constant water bodies, primarily subterranean waters and areas where hypogean waters meet the surface (Bartsch 1995a), so the presence of this mite in the sample likely indicates that the type location for *Mesobiotus anastasiae* sp. nov. can be a permanently wet moss cushion. The presence of the single mite specimen can also be the result of an accidental introduction, but this seems to be less probable, because of the absence of permanent bodies of water in close proximity to the sampling site (I. Nikolaeva pers. com.) and the limited abilities of Halacaridae to tolerate desiccation.

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**Supplementary material**

**Supplementary file 1.** DNA extraction protocol. [https://doi.org/10.5852/ejt.2020.726.1179.3271](https://doi.org/10.5852/ejt.2020.726.1179.3271)

**Supplementary file 2.** Raw morphometric data for *Mesobiotus anastasiae* sp. nov. [https://doi.org/10.5852/ejt.2020.726.1179.3273](https://doi.org/10.5852/ejt.2020.726.1179.3273)

**Supplementary file 3.** Matrices of $p$-distances for species of *Mesobiotus* Vecchi, Cesari, Bertolani, Jönsson & Guidetti, 2016. [https://doi.org/10.5852/ejt.2020.726.1179.3275](https://doi.org/10.5852/ejt.2020.726.1179.3275)
Appendix 1 (continued on next page). Complete list of sequences used for molecular comparisons between the new *Mesobiotus* species described in this study and all other species of the genus *Mesobiotus* for which homologous DNA sequences are currently available.

| Gene      | Species                                   | Accession number | Source                          |
|-----------|-------------------------------------------|------------------|---------------------------------|
| COI       | *Mesobiotus anastasiae* sp. nov.           | MT904513         | this study                      |
|           | *Mesobiotus cf. barabanovi* (Tumanov, 2005)| MN313170         | Kaczmarek et al. 2020          |
|           | *Mesobiotus datanlanicus* Stec, 2019       | MK578905         | Stec 2019                      |
|           | *Mesobiotus dilimanensis* Itang et al., 2020 | MN257047         | Itang et al. 2020              |
|           | *Mesobiotus ethiopicus* Stec & Kristensen, 2017 | MF678794         | Stec & Kristensen 2017         |
|           | *Mesobiotus fiedleri* Kaczmarek et al., 2020 | MH676056         | Kaczmarek et al. 2020          |
|           | *Mesobiotus furciger* (Murray, 1907)       | JX865306, JX865308, JX865314 | Czechowski et al. 2012         |
|           | *Mesobiotus harmsworthi* (Murray, 1907)    | MH195150, MH195151 | Kaczmarek et al. 2018          |
|           | *Mesobiotus hilariae* Vecchi et al., 2016 | KT226108         | Vecchi et al. 2016             |
|           | *Mesobiotus insans* Mapalo et al., 2017    | MF441491         | Mapalo et al. 2017             |
|           | *Mesobiotus occultatus* Kaczmarek et al., 2018 | MH195152         | Kaczmarek et al. 2018          |
|           | *Mesobiotus philippinicus* Mapalo et al., 2016 | KK129796         | Mapalo et al. 2016             |
|           | *Mesobiotus radiatus* (Pilato et al., 1991) | MH195148         | Stec et al. 2018b              |
|           | *Mesobiotus romanii* Roszkowska et al., 2018 | MH195149         | Roszkowska et al. 2018         |
|           | *Mesobiotus gr. furciger*                 | MH195153         | Kaczmarek et al. 2018          |
|           | *Mesobiotus gr. harmsworthi*              | MH195154         | Kaczmarek et al. 2018          |
|           | *Mesobiotus cf. mottai* (Binda & Pilato, 1994) | KT226072         | Vecchi et al. 2016             |
|           | *Mesobiotus occultatus* Kaczmarek et al., 2018 | MH197146         | Kaczmarek et al. 2018          |
|           | *Mesobiotus philippinicus* Mapalo et al., 2016 | KK129793         | Mapalo et al. 2016             |
|           | *Mesobiotus polaris* (Murray, 1910)       | EU266927, EU266928, EU266929 | Czechowski et al. 2012         |
|           | *Mesobiotus harmsworthi* (Murray, 1907)    | MH197146         | Kaczmarek et al. 2018          |
|           | *Mesobiotus hilariae* Vecchi et al., 2016 | KT226068, KT226069, KT226070, KT226071 | Czechowski et al. 2012         |
|           | *Mesobiotus insans* Mapalo et al., 2017    | MK584659         | Stec 2019                      |
|           | *Mesobiotus dilimanensis* Itang et al., 2020 | MN257048         | Itang et al. 2020              |
|           | *Mesobiotus ethiopicus* Stec & Kristensen, 2017 | MF678793         | Stec & Kristensen 2017         |
|           | *Mesobiotus fiedleri* Kaczmarek et al., 2020 | MH676056         | Kaczmarek et al. 2020          |
|           | *Mesobiotus furciger* (Murray, 1907)       | JX865306, JX865308, JX865314 | Czechowski et al. 2012         |
|           | *Mesobiotus harmsworthi* (Murray, 1907)    | MH195150, MH195151 | Kaczmarek et al. 2018          |
|           | *Mesobiotus hilariae* Vecchi et al., 2016 | KT226108         | Vecchi et al. 2016             |
|           | *Mesobiotus insans* Mapalo et al., 2017    | MF441488         | Mapalo et al. 2017             |
|           | *Mesobiotus cf. mottai* (Binda & Pilato, 1994) | KT226072         | Vecchi et al. 2016             |
|           | *Mesobiotus occultatus* Kaczmarek et al., 2018 | MH197147         | Kaczmarek et al. 2018          |
|           | *Mesobiotus philippinicus* Mapalo et al., 2016 | KK129793         | Mapalo et al. 2016             |
|           | *Mesobiotus polaris* (Murray, 1910)       | KT226075, KT226076, KT226077, KT226078 | Czechowski et al. 2012         |
|           | *Mesobiotus radiatus* (Pilato et al., 1991) | MH197153         | Stec et al. 2018b              |
### Appendix 1 (continued).

| Gene         | Species                              | Accession number                  | Source                        |
|--------------|--------------------------------------|-----------------------------------|-------------------------------|
| 28S rRNA     | **Mesobiotus anastasiae sp. nov.**    | **MT903612**                      | this study                    |
|              | Mesobiotus cf. barabanovi (Tumanov, 2005) | MN310388                          | Kaczmarek et al. 2020         |
|              | Mesobiotus datanlanicus Stec, 2019    | MK584658                          | Stec 2019                     |
|              | Mesobiotus dilimanensis Itang et al., 2020 | MN257049                          | Itang et al. 2020             |
|              | Mesobiotus ethiopicus Stec & Kristensen, 2017 | MF678792                          | Stc & Kristensen 2017         |
|              | Mesobiotus fielederi Kaczmarek et al., 2020 | MH681693                          | Kaczmarek et al. 2020         |
|              | Mesobiotus harmsworthi (Murray, 1907) | MH197264                          | Kaczmarek et al. 2018         |
|              | Mesobiotus insanis Mapalo et al., 2017 | MF441489                          | Mapalo et al. 2017            |
|              | Mesobiotus philippinicus Mapalo et al., 2016 | KX129794                          | Mapalo et al. 2016            |
|              | Mesobiotus radiatus (Pilato et al., 1991) | MH197152                          | Stc et al. 2018b              |
|              | Mesobiotus romani Roszkowska et al., 2018 | MH197151                          | Roszkowska et al. 2018        |
|              | Mesobiotus gr. furciger               | MH197265                          | Kaczmarek et al. 2018         |
|              | Mesobiotus gr. harmsworthi.           | MH197266                          | Kaczmarek et al. 2018         |
| ITS-2        | **Mesobiotus anastasiae sp. nov.**    | **MT903470**                      | this study                    |
|              | Mesobiotus cf. barabanovi             | MN310390                          | Kaczmarek et al. 2020         |
|              | Mesobiotus datanlanicus Stec, 2019    | MK584657                          | Stec 2019                     |
|              | Mesobiotus dilimanensis Itang et al., 2020 | MN257050                          | Itang et al. 2020             |
|              | Mesobiotus ethiopicus Stec & Kristensen, 2017 | MN122776                          | Stc & Kristensen 2017         |
|              | Mesobiotus fielederi Kaczmarek et al., 2020 | MH681724                          | Kaczmarek et al. 2020         |
|              | Mesobiotus harmsworthi (Murray, 1907) | MH197154                          | Kaczmarek et al. 2018         |
|              | Mesobiotus insanis Mapalo et al., 2017 | MF441490                          | Mapalo et al. 2017            |
|              | Mesobiotus occultatus Kaczmarek et al., 2018 | MH197155                          | Kaczmarek et al. 2018         |
|              | Mesobiotus philippinicus Mapalo et al., 2016 | KX129795                          | Mapalo et al. 2016            |
|              | Mesobiotus radiatus (Pilato et al., 1991) | MH197267, MH197268                | Stc et al. 2018b              |
|              | Mesobiotus romani Roszkowska et al., 2018 | MH197150                          | Roszkowska et al. 2018        |
|              | Mesobiotus gr. furciger               | MH197156                          | Kaczmarek et al. 2018         |
|              | Mesobiotus gr. harmsworthi.           | MH197157                          | Kaczmarek et al. 2018         |
Appendix 2. Key to the species of the genus *Mesobiotus* Vecchi, Cesari, Bertolani, Jönsson & Guidetti, 2016.

A key to the *Mesobiotus* species was published recently (Kaczmarek et al. 2020). Unfortunately, two species (*M. nikolaevae* Tumanov, 2018 and *M. helenae* Tumanov & Pilato, 2019) were not included in this work, and three additional species (*M. datanlanicus* Stee, 2019, *M. dilimanensis* Itang, Stee, Mapalo, Mirano-Bascos & Michalczyk, 2020 and *M. joenssoni* Guidetti, Gneuß, Cesari, Altiero & Schill, 2019) are not present because the key predates their descriptions. Further, some minor inaccuracies in the key were revealed. Here I present an updated key to the species of the genus *Mesobiotus*, including all above-mentioned omitted species, as well as the new species described here. The key is based on the version published by Kaczmarek et al. (2020). *Mesobiotus meridionalis* (Richters, 1909) nom. inq., *M. polaris* (Murray, 1910) nom. inq., *M. stellaris* (du Bois-Reymond Marcus, 1944) nom. inq. and *M. armatus* (Pilato & Binda, 1996) nom. inq. were excluded from the key, following Kaczmarek et al. (2020).

1. Dorsal cuticle with sculptured surface (visible in PhC as granulation or thin reticulate pattern) or with pores or stripes of pigmentation ................................................................. 2
   - Cuticle smooth (except for minute regular granulation visible only in SEM) ......................... 11
2. Stripes of pigmentation present .......................................................................................... 3
   - Stripes of pigmentation absent .......................................................................................... 4
3. First band in oral cavity present .......................................................................................... 5
   - First band in oral cavity absent or not visible in PCM ......................................................... 6
4. Cuticle with pores without granulation on the body surface or on legs, oral cavity armature without longitudinally elongated teeth in the second band, egg processes in shape of cones with long slender endings, egg processes with “bubbles”, egg processes bases without crown of thickenings .................. *M. krynauwi* (Dastych & Harris, 1995)
   - Cuticle with sculpture, visible in PhC as granulation or dot-like sculpture, or with thin reticulate pattern, without pores .................................................................................................................... 7
5. Cuticular sculpture consists of relatively large granules, well-visible in PhC (granules size ≥ 1 µm) in the caudal region of the dorsal body surface ................................................................................................................................. 8
   - Cuticular sculpture without large granules, with fine dot-like sculpture poorly visible in LM or with thin reticulate pattern only ................................................................................................................................. 9
6. Granules of the cuticular sculpture are present from the level of the third legs to the posterior end of the animal, *pt* of stylet supports 72.41–83.64, egg processes 27–36 µm high, with collar and large pores above it ................................................................. *M. joenssoni* Guidetti, Gneuß, Cesari, Altiero & Schill, 2019
   - Granules of the cuticular sculpture are present only on the caudal extremity of the body, *pt* of stylet supports ca 85.4, maximal height of egg processes is 19 µm ......................................................................................................................... *M. arguei* (Pilato & Sperlinga, 1975)
7. Cuticular sculpture appears in PhC as thin reticulate pattern, oral cavity armature without longitudinally elongated teeth in the second band, with one row of elongated teeth in the first band, egg processes with collar and large pores above it ................................................................. *M. anastasiae* sp. nov.
   - Cuticular sculpture consists of fine granules or tubercles, poorly visible in PhC, without reticular pattern, oral cavity armature with longitudinally elongated teeth in the second band ........................................ 8
8. Egg shell porous .............................................................................................................. *M. sicheli* (Binda, Pilato & Lisi, 2005)
   - Egg shell different ............................................................................................................. 9
9. Egg shell surface with reticular sculpture, egg processes smooth, number of egg processes on the circumference 22 or more, width of egg processes bases less than 8.9 µm ......................................................... \( M. \ contii \) (Pilato & Lisi, 2006)
  – Egg shell surface smooth, egg processes with reticular design, number of egg processes on the circumference 21 or less, width of egg processes bases 8.9 µm or more ........................................ 10

10. Lunules under claws IV smooth, eyes absent, the egg processes bases elongated into long stripes which forms the areolation (5–7 areoles around each egg process) on the egg shell surface (full areolation) ......................................................... \( M. \ pseudonuragicus \) (Pilato, Binda & Lisi, 2004)
  – Lunules under claws IV with indented margin, eyes present, areolation on the egg surface absent, egg process bases with crown of thickenings …… \( M. \ pseudocoronatus \) (Pilato, Binda & Lisi, 2006)

11. The egg processes in shape of flat hemispherical domes ...................................................... 12
  – The egg processes in shape of cones or “mammillate-like domes” ........................................ 13

12. The egg surface with reticular sculpture, egg processes bases without crown of thickenings ........
  – The egg surface without reticular sculpture, egg processes bases with crown of thickenings and wrinkles ......................................................... \( M. \ montanus \) (Murray, 1910)

13. The egg processes “mammillate-like domes” ............................................................. 14
  – The egg processes in shape of cones ................................................................................. 15

14. The egg processes bases with finger-like projections, egg processes with reticular design and without additional, small hemispherical projections on the top, number of egg processes on the circumference 16 or more, width of egg processes bases more than 11.0 µm …… \( M. \ peterseni \) (Maucci, 1991)
  – The egg processes bases with finger-like projections, egg processes without reticular design and with additional, small hemispherical projections on the top, number of egg processes on the circumference 15 or less, width of egg processes bases less than 7.0 µm ..................................................................................................................................................... \( M. \ lusitanicus \) (Maucci & Durante Pasa, 1984)

15. Egg processes with basal collar, egg shell with polygonal relief …….. \( M. \ mauccii \) (Pilato, 1974)
  – Egg processes without collar, egg shell without polygonal relief ...................................................... 16

16. The egg processes in shape of truncated cones ….. \( M. \ zhejiangensis \) (Yin, Wang & Li, 2011)
  – The egg processes different ......................................................................................... 17

17. The egg processes bases elongated into long stripes that form the areolation on the egg shell surface (full areolation) .................................................................................................................. 18
  – Full areolation on egg shell surface absent ........................................................................ 26

18. First band of teeth in oral cavity absent .............................................................................. 19
  – First band of teeth in oral cavity present ............................................................................ 20

19. Lunules under claws IV smooth, egg processes in shape of cones with long slender endings ……
  – Lunules under claws IV indented, egg processes in shape of sharp wide cones …….. \( M. \ hilariae \) Vecchi, Cesari, Bertolani, Joonsson, Rebecchi & Guidetti, 2016

20. Egg processes with terminal filaments .............................................................................. 21
  – Egg processes without terminal filaments ........................................................................... 22
21. Eyes absent, macroplacoid length sequence (2<1<3), process apices divided into at least 15 filaments ......................................................... \textit{M. insanis} Mapalo, Stec, Mirano-Bascos & Michalczyk, 2017
- Eyes present, macroplacoid length sequence (2<1=3), process apices divided into 2–5 filaments ......................................................... \textit{M. nuragicus} (Pilato & Sperling, 1975)

22. Egg processes are usually terminated by a multifurcated crown of several finger-shaped appendages, which often are terminated by short spines ........................................... \textit{M. datanlanicus} Stec, 2019
- Egg processes without crown of appendages at the top ....................................................... 23

23. Egg processes in shape of cones with long slender endings ................................................. \textit{M. barbarae} (Kaczmarek, Michalczyk & Degma, 2007)
- Egg processes sharp, narrow or wide cones ........................................................................ 24

24. Granulation on legs absent, egg processes in the shape of sharp wide cones, height of egg processes less than 16.0 µm ........................................................ \textit{M. neuquensis} (Rossi, Claps & Ardohain, 2009)
- Granulation on legs present, egg processes in the shape of sharp narrow cones, height of egg processes more than 24.0 µm .............................................................. 25

25. The \textit{pt} of stylet supports less than 75.0, six areoles around each egg process, height of egg processes less than 35.0 µm, width of egg processes bases less than 22.0 µm ..................................................... \textit{M. hieronimi} (Pilato & Claxton, 1988)
- The \textit{pt} of stylet supports more than 77.0, sixteen areoles around each egg process, height of egg processes more than 41.0 µm, width of egg processes bases more than 27.0 µm ................................................ \textit{M. pseudoliviae} (Pilato & Binda, 1996)

26. Egg processes bases elongated into long stripes, which form the semi-areolation (the branches at least in some cases not connected to each other) ................................................................. 27
- Egg processes bases different ........................................................................................... 31

27. Eyes absent, egg processes with filaments ......................................................... \textit{M. ethiopicus} Stec & Kristensen, 2017
- Eyes present, egg processes without filaments ................................................................................ 28

28. Additional teeth in oral cavity armature present, egg processes with “bubbles” ................................. 29
- Additional teeth in oral cavity armature absent, egg processes without “bubbles” ......................... 30

29. Claws IV with large, protruding accessory points, egg processes with reticular design, number of egg processes on the circumference 11–12 ........................................ \textit{M. harmsworthi} (Murray, 1907)
- Large and protruding accessory points on claws IV absent, egg processes without reticular design, number of egg processes on the circumference 15–24 ................ \textit{M. blocki} (Dastych, 1984)

30. Granulation on legs I–III absent, egg processes in shape of cones with long slender endings, number of egg processes on the circumference ca 20 ................................................ \textit{M. barabanovi} (Tumanov, 2005)
- Granulation on legs I–III present, egg processes in shape of sharp wide cones, number of egg processes on the circumference 10–12 ................................................................. \textit{M. skoracki} Kaczmarek, Zawierucha, Buda, Stec, Gawlak, Michalczyk & Roszkowska, 2018

31. Egg processes with finger-like projections, i.e., egg processes bases elongated into long stripes, but never connected to each other ......................................................... 32
- Egg processes without finger-like projections ........................................................................ 39

32. Egg processes in shape of cones with long slender endings ................................................... 33
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- Egg processes in the shape of sharp, narrow or wide cones ................................................................. 35

33. Egg processes with “bubbles” and flexible filaments in apical part, height of egg processes less than 13.0 µm .................................................. M. pseudoblocki Roszkowska, Stec, Ciobanu & Kaczmarek, 2016
- Egg processes without “bubbles” or flexible filaments in apical part, height of egg processes more than 20.0 µm .................................................. 34

34. Eyes present, number of egg processes on the circumference 6–8, height of egg processes 50.0 µm or more .......................................................... M. liviae (Ramazzotti, 1962)
- Eyes absent, number of egg processes on the circumference ca 12, height of egg processes ca 21.0 µm ................................................................. M. snaresensis (Horning, Schuster & Grigarick, 1978)

35. Egg processes with “bubbles”, in shape of sharp narrow cones .................................................. 36
- Egg processes without “bubbles”, in shape of sharp wide cones .................................................. 38

36. Eyes absent, additional teeth in oral cavity absent .................................................................................. M. tehuelchensis (Rossi, Claps & Ardohain, 2009)
- Eyes present, additional teeth in oral cavity present ................................................................................ 37

37. Finger-like projections very small, present only on some processes and irregularly distributed ........ M. reinhardii (Michalczyk & Kaczmarek, 2003)
- Six, large and regularly distributed finger-like projections present on all processes ......................... M. szeptyckii (Kaczmarek & Michalczyk, 2009)

38. Eyes and first band of teeth in oral cavity present, pt of stylet supports 77.0 or more, egg full diameter (with processes) 100.0–116.0 µm, number of egg processes on the circumference 8–9, height of egg processes 12.0–24.0 µm .................................................. M. erminiae (Binda & Pilato, 1999)
- Eyes absent, first band of teeth in oral cavity absent or not visible in PCM, pt of stylet supports 75.5 or less, egg full diameter (with processes) 88.0–92.0 µm, number of egg processes on the circumference 12–15, height of egg processes 9.0–11.0 µm ........ M. diguensis (Pilato & Lisi, 2009)

39. Egg processes with reticular design ........................................................................................................ 40
- Egg processes smooth or with refracting areas ...................................................................................... 56

40. First band of teeth in oral cavity absent or not visible in PCM .......................................................... 41
- First band of teeth in oral cavity present ................................................................................................. 42

41. Eyes present, lunules under claws IV indented, granulation on legs I–III present, number of egg processes on the circumference 15–17 ........................................................................ M. philippinicus Mapalo, Stec, Mirano-Bascos & Michalczyk, 2016
- Eyes absent, lunules under claws IV smooth, granulation on legs I–III absent, number of egg processes on the circumference 11–12 .......... M. pseudopatiens Kaczmarek & Roszkowska, 2016

42. Egg processes with terminal filaments (at least a significant part of them) ........................................ 43
- Egg processes without terminal filaments ............................................................................................. 48

43. Egg processes in the shape of sharp wide cones .................................................................................. 44
- Egg processes in the shape of cones with long, slender endings .............................................................. 45

44. Egg processes with bunch of short filaments, number of egg processes on the circumference 10–12, width of egg processes bases 14.5–22.5 µm .......... M. radiatus (Pilato, Binda & Catanzaro, 1991)
Egg processes with few long filaments, number of egg processes on the circumference 15–17, width of egg processes bases 10.0–14.0 µm .......................................................... M. dimentmani (Pilato, Lisi & Binda, 2010)

45. Lunules under claws IV without indentation ................................................. M. nikolaevae Tumanov, 2018

46. Egg processes bases without distinct crown of thickenings, processes are connected with very thin ridges ..................................................................................................................... M. diffusus (Binda & Pilato, 1987)

47. Eyes present, lunules under claws IV with 4–5 denticles, egg processes tips sometimes trifurcated ................................................................. M. wuzhishanensis (Yin, Wang & Li, 2011)

48. Egg processes in the shape of sharp wide cones .......................................................... M. simulans (Pilato, Binda, Napolitano & Moncada, 2000)

49. Lunules under claws IV indented .............................................. M. simulans (Pilato, Binda, Napolitano & Moncada, 2000)

50. Additional teeth in oral cavity present, egg bare diameter (without processes) 55.0 or less, egg full diameter (with processes) 71.0 µm or less, width of egg processes bases less than 10.5 .......................................................... M. coronatus (De Barros, 1942)

51. The basal tract of posterior and anterior claws IV much longer, primary and secondary branches forming an almost 90° angle (Pilato et al., 2014: fig. 1d) .............................................................................................................. M. insuetus (Pilato, Sabella & Lisi, 2014)

52. Eyes absent, macroplacoid length sequence (2 < 3 ≤ 1), additional teeth in oral cavity absent, height of egg processes 8–16 µm .......................................................... M. patiens (Pilato, Binda, Napolitano & Moncada, 2000)

53. Egg shell porous, egg processes bases without crown of thickenings, height of egg processes 22.0 µm or more, width of egg processes 17.0 µm or more .......................................................... M. altitudinalis (Biserov, 1997–98)

54. Egg processes consists of wide short conical basal part very distinctly separated from the apical part in form of a thin long spine with poorly visible internal structure, number of egg processes on the circumference 27–32 ............................................. M. biniecki (Kaczmarek, Gołdyn, Prokop & Michałczyk, 2011)
55. Number of egg processes on the circumference ca 12, height of egg processes 15.2–16.2 µm, with well visible reticular design of the egg processes, apical parts of egg processes rigid and never subdivided, egg surface with radial ridges .............................................. \textit{M. rigidus} (Pilato & Lisi, 2006)
   - Number of egg processes on the circumference ca 22, height of egg processes 11.0 µm, with faint and almost invisible reticular design of the egg processes, apical parts of egg processes flexible and rarely bifurcated, egg surface smooth .............................................. \textit{M. helenae} Tumanov & Pilato, 2019

56. The egg processes in the shape of rough cones, egg processes bases smooth .............................................. \textit{M. kovalevi} (Tumanov, 2004)
   - The egg processes in the shape of branched or sharp wide cones, egg processes bases with crown of thickenings or wrinkles .......................................................... 57

57. The egg processes in the shape of sharp wide cones, the egg shell surface without pores or reticular sculpture .......................................................... \textit{M. australis} (Pilato & D’Urso, 1976)
   - The egg processes in the shape of branched cones, the egg shell surface porous or with reticular sculpture .......................................................... 58

58. The egg processes with refracting areas ........................................................................................................... 59
   - The egg processes without refracting areas ................................................................................................... 61

59. Bases of egg processes without band of pores, large and numerous refracting areas visible on the apical part of all processes ........................................................................ \textit{M. furciger} (Murray, 1907)
   - Bases of egg processes with band of pores, small and single refracting areas present only on some processes ........................................................................................................... 60

60. Apical parts of egg processes always divided into 2–4 branches, height of egg processes ca 15.0 µm, \textit{pt} of buccal tube width 22.8–25.4 .................................................. \textit{M. pilatoi} (Binda & Rebecchi, 1992)
   - At least some of apical part of egg processes not divided, height of egg processes 8.5–13.1 µm, \textit{pt} of buccal tube width 16.5–18.6 ......................................................................................... \textit{M. fiedleri} Kaczmarek, Bartylak, Stec, Kulpa, M. Kepel, A. Kepel & Roszkowska, 2020

61. The egg shell surface with reticular sculpture or ridges, without pores ............................................... 62
   - The egg shell surface porous ....................................................................................................................... 65

62. The egg processes in the shape of branched cones with long slender endings, the egg shell surface with ridges radiating from process bases, only sometimes resembling reticulate pattern, height of egg processes 10.7–11.8 µm, width of egg processes 7.4–10.0 µm .................................................................................. \textit{M. siamensis} (Tumanov, 2006)
   - The egg processes in the shape of branched cones, the egg shell surface with clearly reticular sculpture, height of egg processes less than 10.0 µm, width of egg processes usually less than 7.0 µm (up to 7.5 in \textit{M. dilimanensis}) .............................................. 63

63. The \textit{pt} of stylet supports less than 76.5, egg processes sparsely distributed over the egg surface, number of egg processes on the circumference ca 17, egg processes with relatively long branches, nearly equal in length to the basal part, with multiple bifurcations, height of egg processes 4.7–4.8 µm ................................................................................ \textit{M. divergens} (Binda, Pilato & Lisi, 2005)
   - The \textit{pt} of stylet supports more than 77.0, egg processes densely distributed over the egg surface, egg processes with relatively short branches, distinctly shorter than the basal part, height of egg processes usually exceeds 4.8 µm .............................................. 64
64. Additional teeth in oral cavity absent, granulation on legs I–III present, \( pt \) of the buccal tube external width 14.0–17.4, number of egg processes on the circumference 18–24 ........................................... \( \textit{M. dilimanensis} \) Itang, Stec, Mapalo, Mirano-Bascos & Michalczyk, 2020

- Additional teeth in oral cavity present, granulation on legs I–III absent, \( pt \) of the buccal tube external width 17.9–19.6, number of egg processes on the circumference 27–30 ........................................... \( \textit{M. creber} \) (Pilato & Lisi, 2009)

65. Egg processes divided close to or at the top, number of egg processes on the circumference ca 25, width of egg processes 7.3–7.8 \( \mu \)m ........................................... \( \textit{M. orcadensis} \) (Murray, 1907)

- Egg processes begin to divide in the middle of their length, number of egg processes on the circumference 21–23, width of egg processes 8.4–9.5 \( \mu \)m ........................................... \( \textit{M. aradasi} \) (Binda, Pilato & Lisi, 2005)