

The first record of *Macrobiotus vladimiri* Bertolani, Biserov, Rebecchi & Cesari, 2011 (Tardigrada: Eutardigrada: Macrobiotidae: *hufelandi* group) from Poland

Bernadeta NOWAK, Daniel STEC*

Department of Entomology, Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland

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Abstract: Tardigrade studies in Poland have been carried out for more than a century and to date, 102 species have been reported from this central European country. This constitutes nearly 9% of all known species within the phylum. Although previous studies have been thorough, a number of taxa now known to belong to species complexes have been treated in only a very general way. One such complex is the *Macrobiotus hufelandi* group, which has a worldwide distribution. To date, only three *hufelandi* group species have been recorded from Poland: *M. hufelandi hufelandi* C.A.S. Schultze, 1834; *M. macrocalix* Bertolani & Rebecchi, 1993; and *M. polonicus* Pilato, Kaczmarek, Michalczyk & Lisi, 2003. Here we first report *M. vladimiri* Bertolani, Biserov, Rebecchi & Cesari, 2011 from Poland. Moreover, we provide new morphometric data for the type series of the species.

Key words: *Macrobiotus hufelandi* group, first report, Poland, species complex

Tardigrada is a phylum of microinvertebrates living in aquatic and terrestrial environments throughout the world (Nelson et al., 2015), with approximately 1200 described species (Guidetti and Bertolani, 2005; Degma and Guidetti, 2007; Degma et al., 2016). Studies on Polish tardigrades have been conducted for more than a century (Minkiewicz, 1914; Jakubski, 1915), and up to now, 102 species have been reported from this country (e.g., Kaczmarek, 2008; Kaczmarek et al., 2010; Zawierucha et al., 2015). However, the most comprehensive account on this subject so far is that of Dastych (1988). Although his monograph is both thorough and extensive, some tardigrade groups or species complexes were treated in very general terms. This was particularly the case for specimens identified as "*Macrobiotus hufelandi* C.A.S. Schultze, 1834". This species was previously thought to be cosmopolitan but now is considered a species group comprising over 40 species. The complex, known as the *Macrobiotus hufelandi* group, is characterized by a porous cuticle, two macroplacoids and a microplacoid in the pharynx, and eggs most often ornamented with mushroom-shaped processes that make them easily distinguishable from those of other tardigrades (Bertolani and Rebecchi, 1993; Guidetti et al., 2013). For many years, specimens exhibiting these morphological traits were commonly and erroneously identified as the nominal species. Following the revision of this group by Bertolani and Rebecchi (1993), researchers began to

recognize differences in morphological details, and as a result, numerous new species within the complex were identified (e.g., Bertolani and Rebecchi, 1993; Guidetti et al., 2013; Stec et al., 2015; Bąkowski et al., 2016).

Although *M. hufelandi* has been reported from Poland by several authors (Pigoń and Węglarska, 1953; Węglarska, 1959, 1973; Pilato and Dastych, 1974; Hęciak, 1976; Węglarska and Korecka, 1983), these records should be considered as dubious because the formal redescription of this species comes from 1993 (Bertolani and Rebecchi, 1993). To date, only three *Macrobiotus hufelandi* group species have been reported from Poland for which the records are considered valid: *Macrobiotus hufelandi hufelandi* by Dastych, (1997), *Macrobiotus macrocalix* Bertolani & Rebecchi, 1993 by Kaczmarek and Michalczyk (2004); and *Macrobiotus polonicus* Pilato, Kaczmarek, Michalczyk & Lisi, 2003. In this report, we record for the first time in Poland the presence of a fourth *hufelandi* group species, *Macrobiotus vladimiri* Bertolani, Biserov, Rebecchi & Cesari, 2011. We also provide additional morphometrics for the type population of the species.

A moss sample containing *M. vladimiri* individuals and eggs was collected from calcareous rock in the Zakrzówek district of Krakow, Poland (50°02'12"N, 19°53'56"E; 206 m a.s.l.) in September 2015 by the first author. The sample was collected and examined for terrestrial tardigrades using standard methods (e.g., as described by Stec et al.,

* Correspondence: daniel_stec@interia.eu

2015). Animals and eggs of *M. vladimiri* were extracted from the sample and mounted on microscope slides following the protocol of Morek et al. (2016). Slides were then dried for 5 days at 60 °C, sealed with transparent nail polish, and examined under a Nikon Eclipse 50i phase contrast light microscope (PCM) equipped with a Nikon Digital Sight DS-L2 digital camera. All slides are deposited in the Department of Entomology, Institute of Zoology and Biomedical Research Jagiellonian University.

All figures were assembled in Corel Photo-Paint X6, ver. 16.4.1.1281. For deep structures that could not be fully focused on in a single photograph, a series of 2–10 images were taken approximately every 0.25 µm and then assembled into a single deep-focus image.

All measurements are given in micrometers. Sample size for morphometry was chosen following recommendations by Stec et al. (2016). Structures were measured only if their orientation was suitable. Body length was measured from the anterior extremity to the end of the body, excluding the hind legs. The terminology used to describe oral cavity armature follows that established by Hansen and Katholm (2003) and adopted by Michalczyk and Kaczmarek (2003). Buccal tube length and the level of the stylet support insertion point were measured according to Pilato (1981). Buccal tube width was measured as the external and internal diameter at the level of the stylet support insertion point. Macroplacoid length sequence is given according to Kaczmarek et al. (2014). Lengths of the claw branches were measured from the base of the claw (i.e. excluding the lunula) to the top of the branch, including accessory points. The *pt* index is the ratio of the length of a given structure to the length of the buccal tube expressed as a percentage (Pilato, 1981). Distance between egg processes was measured as the shortest line connecting the base edges of the 2 closest processes. Morphometric data were handled using the Parachela ver. 1.2 template available from the Tardigrada Register (Michalczyk and Kaczmarek, 2013). Taxonomy for Eutardigrada follows Bertolani et al. (2014).

For exact identification, our specimens were compared with original descriptions of all *Macrobiotus hufelandi* group species. Moreover, they were also compared with the type material of *M. vladimiri* kindly loaned to us by Michele Cesari (University of Modena and Reggio Emilia, Italy).

Phylum: Tardigrada Doyère, 1840

Class: Eutardigrada Richters, 1926

Order: Parachela Schuster, Nelson, Grigarick & Christenberry, 1980

Superfamily: Macrobiotioidea Thulin, 1928 (Sands, McInnes, Marley, Goodall-Copestake, Convey & Linse, 2008)

Family: Macrobiotidae Thulin, 1928

Genus: *Macrobiotus* C.A.S. Schultze, 1834

Macrobiotus vladimiri, Bertolani, Biserov, Rebecchi and Cesari, 2011

(Tables 1–4, Figures 1–4)

Material examined: Twenty-two animals (including 3 simplexes) and 2 eggs from Poland (mounted on microscope slides in Hoyer's medium, slide codes: PL.103.19–25, 29, 30, 32, 35; preserved at the Department of Entomology, Institute of Zoology and Biomedical Research Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland); 3 paratypes and 3 eggs from the type population (mounted on microscopic slides in polyvinyl-lactophenol, slide codes: C475-S2, C475-SU1; collected in Andalo (Italy): 46°10'07"N, 11°00'01"E; 1050 m a.s.l.).

Description of Polish specimens:

Animals (measurements and statistics in Tables 1 and 2): Body white/transparent without any transversal bands of pigmentation, transparent after fixation in Hoyer's medium (Figure 1A). Eyes present (visible also after mounting). Body cuticle smooth with small round (diameter range: 0.5–1.1 µm) and oval (diameter range: 1.0–1.7 µm) pores, situated mostly on the posterior part of the dorsum and poorly visible under a light microscope (LM) (Figure 1B, empty arrowhead). Ventral cuticle smooth. Granulation on the external surface of legs I–IV present, but not very developed (Figure 1C–D).

Mouth anteroventral. Buccopharyngeal apparatus of the *Macrobiotus* type (Figure 2), with 10 small peribuccal lamellae and ventral lamina. The oral cavity armature is composed of two bands of teeth (the first band is not visible under LM; Figure 2). The second band of teeth is situated between the ring fold and the third band of teeth and comprises several rows of small, barely visible dots (Figure 2, lower insert, empty indented arrowhead). The teeth of the third band are located within the posterior portion of the oral cavity, between the second band of teeth and the buccal tube opening (Figure 2, empty arrowhead). The third band of teeth is discontinuous and divided into the dorsal and the ventral portion. It comprises three dorsal, distinctly separated, thin ridges (Figure 2, empty arrowhead) and three ventral teeth: two lateral ridges (Figure 2, lower insert) and one round or oval median tooth (Figure 2, lower insert, empty arrowhead). Pharyngeal bulb spherical, with triangular apophyses, two rod-shaped macroplacoids ($2 < 1$), and a triangular microplacoid (Figure 2). Both macroplacoids with slight central constrictions (in the second microplacoid being almost undetectable) (Figure 2, upper insert).

Claws small and slender, of the *hufelandi* type (Figures 3A and 3B). Primary branches with distinct accessory points. The common tract short and wide with an evident peduncle connecting the claw to the lunula (Figures 3A and 3B). Lunulae on legs I–III smooth (Figure 3A), but on legs IV slightly crenulated and occasionally with very faint indentations (Figure 3B). Bars under claws absent.

Table 1. Measurements (in μm) of selected morphological structures of individuals of *Macrobiotus vladimiri* Bertolani, Biserov, Rebecchi & Cesari, 2011 from the Polish population mounted in Hoyer's medium (N- number of specimens/structures measured; Range- the smallest and the largest structure among all measured specimens; SD- standard deviation).

Character	N	Range		Mean		SD	
		μm	<i>pt</i>	μm	<i>pt</i>	μm	<i>pt</i>
Body length	10	403–540	997–1254	457	1104	50	94
Buccopharyngeal tube							
Buccal tube length	10	39.6–43.9	–	41.3	–	1.4	–
Stylet support insertion point	10	30.6–34.7	77.0–81.9	32.4	78.4	1.3	1.6
Buccal tube external width	10	5.2–6.6	12.8–16.0	5.9	14.3	0.4	1.0
Buccal tube internal width	10	3.3–4.5	7.9–10.3	3.8	9.1	0.4	0.8
Ventral lamina length	8	26.1–29.8	63.2–69.1	27.4	65.6	1.2	2.1
Placoid lengths							
Macroplacoid 1	10	9.9–14.0	23.1–34.0	11.4	27.5	1.3	2.9
Macroplacoid 2	10	6.0–9.2	14.2–22.4	7.4	18.0	1.0	2.4
Microplacoid	10	2.3–3.9	5.7–9.9	3.0	7.3	0.5	1.2
Macroplacoid row	10	16.8–24.3	39.3–59.0	19.9	48.2	2.1	4.9
Placoid row	10	19.3–26.8	45.2–65.1	23.0	55.8	2.2	5.0
Claw 1 lengths							
External primary branch	8	10.8–12.7	26.8–29.4	11.6	28.2	0.6	0.9
External secondary branch	8	8.2–10.9	20.0–25.3	9.5	23.1	0.8	1.9
Internal primary branch	8	10.2–12.1	25.2–28.5	11.0	26.8	0.7	1.1
Internal secondary branch	8	7.0–11.2	17.6–25.9	8.6	20.9	1.2	2.4
Claw 2 lengths							
External primary branch	10	10.6–12.9	26.6–31.1	11.9	28.9	0.7	1.7
External secondary branch	10	9.3–10.8	22.1–26.5	10.0	24.3	0.5	1.5
Internal primary branch	10	10.6–12.3	26.2–29.8	11.5	27.8	0.5	1.2
Internal secondary branch	10	7.1–10.5	17.8–25.1	9.5	22.9	1.1	2.3
Claw 3 lengths							
External primary branch	10	11.1–13.3	28.0–32.3	12.4	29.9	0.7	1.4
External secondary branch	10	9.4–12.7	22.0–30.7	10.7	25.8	1.0	2.5
Internal primary branch	10	10.5–12.8	26.5–31.1	11.8	28.6	0.6	1.2
Internal secondary branch	10	6.9–10.9	17.4–26.5	9.6	23.3	1.2	2.8
Claw 4 lengths							
Anterior primary branch	10	13.1–15.1	32.5–36.7	14.1	34.1	0.7	1.1
Anterior secondary branch	10	10.5–11.8	25.4–27.5	10.9	26.4	0.4	0.7
Posterior primary branch	10	12.6–16.2	31.1–37.6	14.4	34.9	1.0	1.8
Posterior secondary branch	10	10.3–13.5	25.5–32.3	11.9	28.7	0.9	2.0

Eggs (measurements and statistics in Tables 3 and 4): Eggs laid freely, white, spherical (Figures 4A and 4B). The surface between processes of the *hufelandi* type, i.e. chorion covered with a reticulum with oval or round meshes, slightly larger and wider in the peribasal ring around the processes (Figures 4B and 4D). Processes

of inverted goblet shape (Figures 4A and 4C), and with concave distal disks that have jagged margins (Figure 4D).

To date, *M. vladimiri* has been recorded from three European countries: Italy (Andalo, the type locality), Germany (St. Ulrich), and Spain (Bertolani et al., 2011). The Spanish population was discovered only on the basis

Table 2. Measurements (in μm) of selected morphological structures of individuals of *Macrobiotus vladimiri* Bertolani, Biserov, Rebecchi & Cesari, 2011 from the type population mounted in polyvinyl-lactophenol medium (N- number of specimens/ structures measured; Range- the smallest and the largest structure among all measured specimens; SD- standard deviation).

Character	N	Range		Mean		SD	
		μm	<i>pt</i>	μm	<i>Pt</i>	μm	<i>pt</i>
Body length	3	322–419	805–1042	360	952	52	129
Buccopharyngeal tube							
Buccal tube length	3	33.5–40.2	–	37.9	–	3.8	–
Stylet support insertion point	3	26.5–31.1	76.6–79.1	29.5	77.8	2.6	1.2
Buccal tube external width	3	5.7–6.9	15.8–17.2	6.3	16.6	0.6	0.8
Buccal tube internal width	3	4.7–5.8	12.5–14.4	5.2	13.7	0.6	1.0
Ventral lamina length	3	22.9–25.8	61.4–68.4	24.5	64.8	1.5	3.5
Placoid lengths							
Macroplacoid 1	3	9.1–11.0	25.8–27.4	10.1	26.8	1.0	0.9
Macroplacoid 2	3	5.4–7.4	16.1–18.5	6.5	17.2	1.0	1.2
Microplacoid	3	1.9–2.6	5.5–6.5	2.2	5.9	0.4	0.5
Macroplacoid row	3	15.7–20.3	46.9–50.5	18.4	48.5	2.4	1.9
Placoid row	3	18.7–24.5	55.8–60.9	22.2	58.3	3.1	2.6
Claw 1 lengths							
External primary branch	2	8.9–9.6	23.9–26.6	9.3	25.2	0.5	1.9
External secondary branch	2	6.9–7.2	17.9–20.6	7.1	19.3	0.2	1.9
Internal primary branch	2	8.4–8.9	22.1–25.1	8.7	23.6	0.4	2.1
Internal secondary branch	2	6.7–7.0	17.4–20.0	6.9	18.7	0.2	1.8
Claw 2 lengths							
External primary branch	2	9.1–9.2	22.9–27.2	9.2	25.0	0.1	3.0
External secondary branch	2	7.1–7.9	17.7–23.6	7.5	20.6	0.6	4.2
Internal primary branch	2	8.5–9.1	21.1–27.2	8.8	24.2	0.4	4.3
Internal secondary branch	2	6.7–7.3	16.7–21.8	7.0	19.2	0.4	3.6
Claw 3 lengths							
External primary branch	3	9.2–10.5	22.9–28.4	9.7	25.8	0.7	2.8
External secondary branch	3	7.3–8.2	18.2–22.7	7.7	20.4	0.5	2.3
Internal primary branch	3	8.8–9.9	21.9–27.8	9.3	24.8	0.6	2.9
Internal secondary branch	3	7.1–8.1	17.7–22.7	7.6	20.2	0.5	2.5
Claw 4 lengths							
Anterior primary branch	3	10.7–11.4	26.6–34.0	11.2	29.7	0.4	3.9
Anterior secondary branch	1	10.4	26.0	10.4	26.0	?	?
Posterior primary branch	3	11.4–13.1	29.6–34.0	12.1	32.1	0.9	2.3
Posterior secondary branch	1	8.3	20.6	8.3	20.6	?	?

of DNA sequences (Bertolani et al., 2011; Guil and Giribet, 2012). Therefore, the Polish locality is the fourth record for this species and, at the same time, it is also a first record for the Polish tardigrade fauna. Thanks to the detailed morphological and morphometric examination of the discovered tardigrades, we were able to identify them as

M. vladimiri. Thus, now the number of known tardigrade species from Poland has risen to 103.

By comparing Polish individuals of *M. vladimiri* with paratypes from Italy, we have discovered several small morphometric differences in animals and eggs between the two populations. The type population is characterized

Table 3. Measurements (in μm) of selected morphological structures of eggs of *Macrobiotus vladimiri* Bertolani, Biserov, Rebecchi & Cesari, 2011 from the Polish population mounted in Hoyer's medium (N- number of eggs/structures measured; Range- the smallest and the largest structure among all measured eggs; SD- standard deviation).

Character	N	Range	Mean	SD
Egg bare diameter	2	76.6–81.2	78.9	3.2
Egg full diameter	2	90.0–96.9	93.4	4.9
Process height	6	7.1–8.2	7.5	0.5
Process base width	6	6.5–8.6	7.8	0.9
Process base/height ratio	6	92%–118%	104%	13%
Terminal disk width	6	5.4–6.4	6.0	0.5
Process base/terminal disk ratio	6	101%–148%	130%	20%
Distance between processes	6	3.6–5.0	4.3	0.5
Number of processes on the egg circumference	6	26–27	26.5	0.7

Table 4. Measurements (in μm) of selected morphological structures of eggs of *Macrobiotus vladimiri* Bertolani, Biserov, Rebecchi & Cesari, 2011 from the type population mounted in polyvinyl-lactophenol medium (N- number of eggs/structures measured; Range- the smallest and the largest structure among all measured eggs; SD- standard deviation).

Character	N	Range	Mean	SD
Egg bare diameter	3	96.8–99.3	98.3	1.3
Egg full diameter	3	104.5–109.2	106.7	2.4
Process height	9	4.6–6.9	5.7	0.8
Process base width	9	5.6–8.5	6.8	0.9
Process base/height ratio	9	99%–136%	119%	9%
Terminal disk width	9	4.8–6.3	5.6	0.5
Process base/terminal disk ratio	9	107%–149%	121%	13%
Distance between processes	9	3.5–6.5	4.9	1.1
Number of processes on the egg circumference	3	26–28	26.7	1.2

by shorter primary branches of external and internal claws on the first pair of legs (external primary branch length: 8.9–9.6 μm [$pt = 23.9\%–26.6\%$] in the type population vs. 10.8–12.7 μm [$pt = 26.8\%–29.4\%$] in the Polish population; internal primary branch length: 8.4–8.9 μm [$pt = 22.1\%–25.1\%$] in the type population vs. 10.2–12.1 μm [$pt = 25.2\%–28.5\%$] in the Polish population), a slightly wider buccal tube (buccal tube external width: 5.7–6.9 μm [$pt = 15.8\%–17.2\%$] in the type population vs. 5.2–6.6 μm [$pt = 12.8\%–16.0\%$] in the Polish population; buccal tube internal width: 4.7–5.8 μm [$pt = 12.5\%–14.4\%$] in the type population vs. 3.3–4.5 μm [$pt = 7.9\%–10.3\%$] in the Polish population), and larger eggs (egg bare diameter: 96.8–99.3 μm in the type population vs. 76.6–81.2 μm in the Polish population; egg full diameter: 104.5–109.2 μm in the type population vs. 90.0–96.9 μm in the Polish population) but with shorter processes (4.6–6.9 μm in the type population

vs. 7.1–8.2 μm in the Polish population) compared to the Polish population. Moreover, the processes of the eggs from the type population measured in our work are slightly shorter than the dimensions presented by Bertolani et al. (2011) (4.6–6.9 μm in the type population measured by us vs. 6.5–8.0 μm in the original description), which extends the range of the variability within this trait in *M. vladimiri*. The differences in the external and internal primary branch lengths of claws on the first pair of legs might be caused by low sample size, especially for the type population. Moreover, paratypes were also generally smaller than animals from the newly found population (mean body length: 360 \pm 52 μm in the type population vs. 457 \pm 50 μm in the Polish population). The recent study by Morek et al. (2016) showed that cover slip pressure may influence the buccal tube morphometrics, but the pressure has to be considerable to cause detectable deformation.

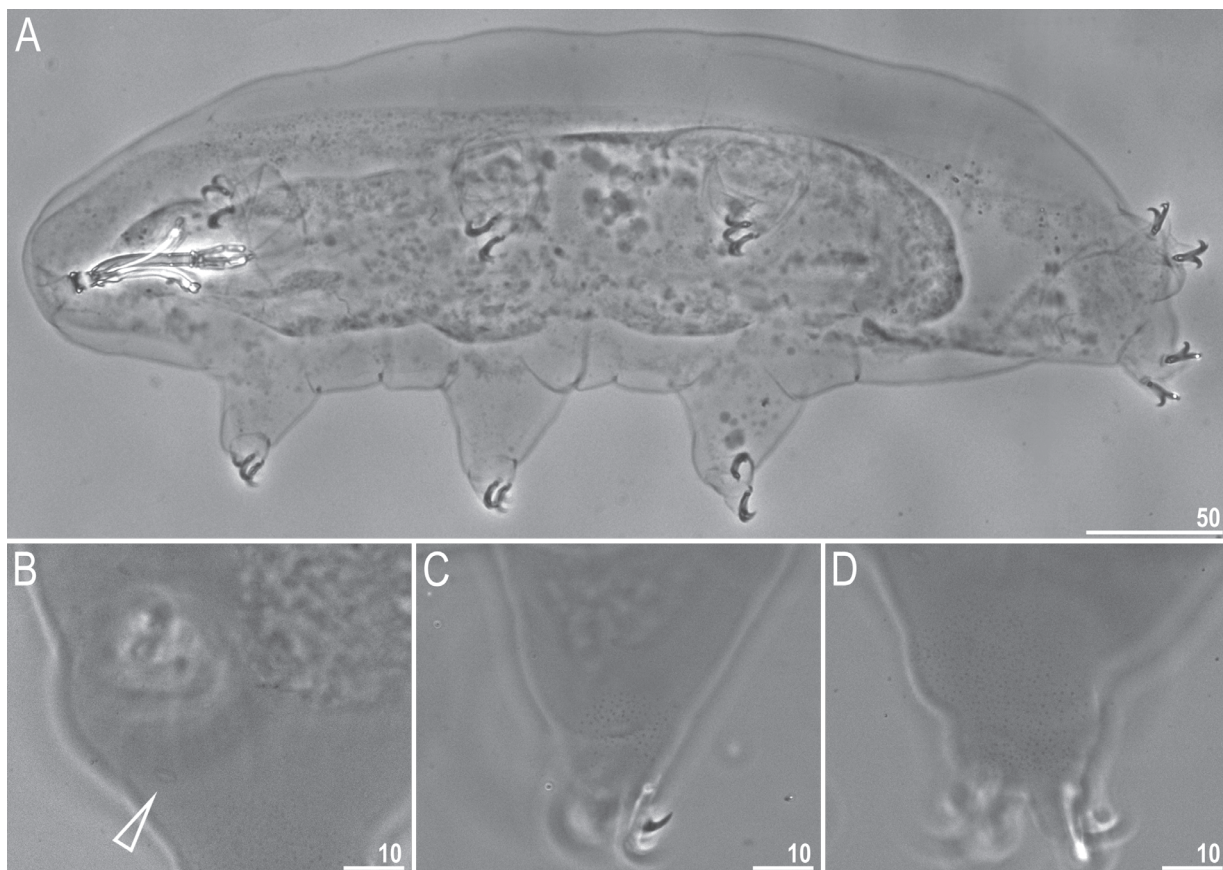


Figure 1. *Macrobiotus vladimiri* Bertolani, Biserov, Rebecchi & Cesari, 2011 from the Polish population A- habitus (PCM) dorsoventral projection; B- cuticular pores on the posterior part of the body indicated by empty arrowhead; C- granulation on leg II; D- granulation on leg IV. Scale bars in μm .

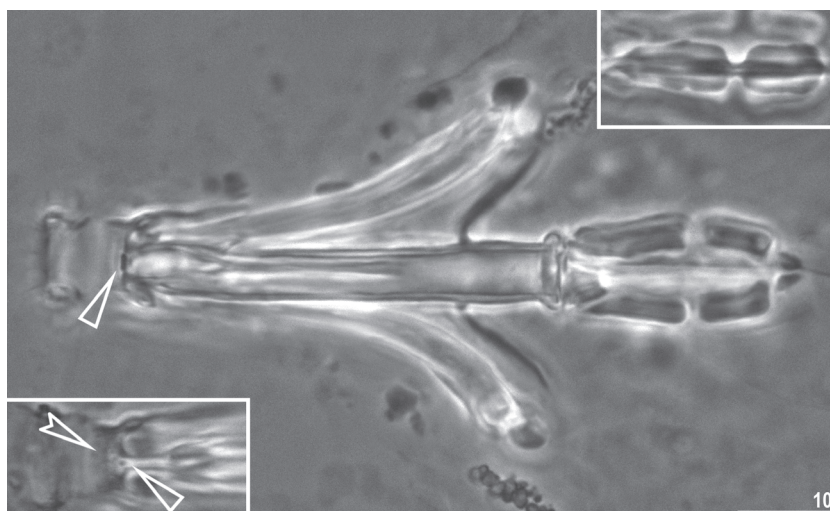


Figure 2. *Macrobiotus vladimiri* Bertolani, Biserov, Rebecchi & Cesari, 2011 from the Polish population – buccal apparatus (PCM), dorsoventral projection with dorsal teeth of the third band and dorsal placoids, the lower insert showing ventral teeth of the third band (of the same individual) whereas the upper insert shows ventral placoids (of the same individual), and empty indented arrowhead indicates second band of teeth whereas empty arrowheads indicate third band of teeth in the oral cavity. Figure 2 was assembled from several photos. Scale bars in μm .

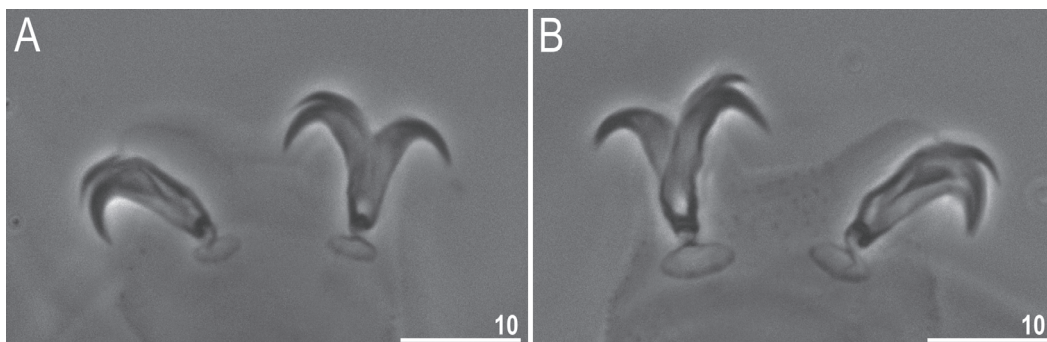


Figure 3. *Macrobiotus vladimiri* Bertolani, Biserov, Rebecchi & Cesari, 2011 from the Polish population – claws (PCM): A- claws II, with smooth lunula; B- claws IV, with barely visible weak and irregular indentation on lunula. Figures 3A and 3B were assembled from several photos. Scale bars in μm .

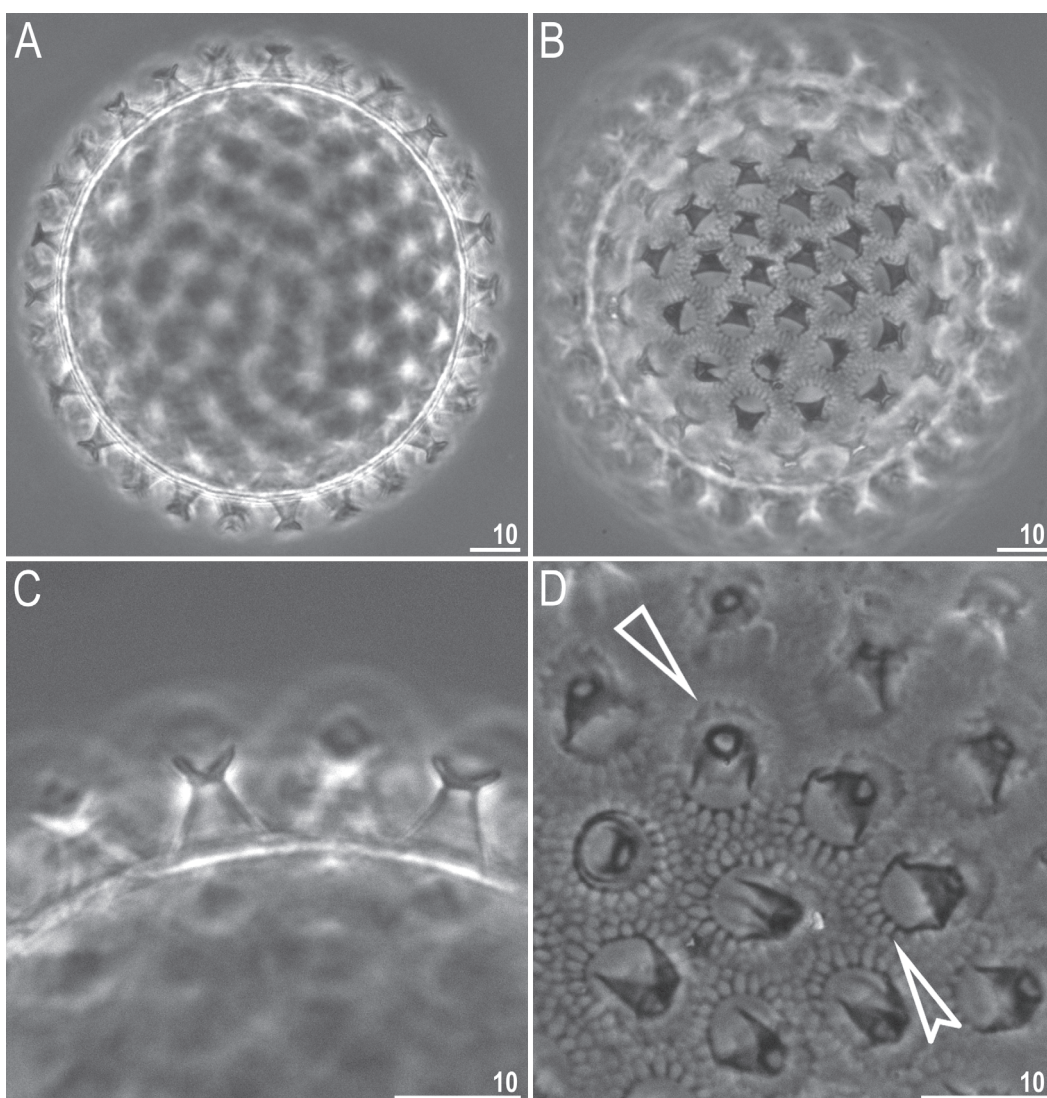


Figure 4. *Macrobiotus vladimiri* Bertolani, Biserov, Rebecchi & Cesari, 2011 from the Polish population – egg (PCM): A- midsection; B- surface; C- midsection under 1000 \times magnification; D- surface of egg hemisphere under 1000 \times magnification. Empty indented arrowheads indicate the ring of larger meshes around the process base, empty indented arrowhead indicates the jagged margins of distal disk. All photos show the details of the same egg. Scale bars in μm

It is, however, possible that some medium types could also affect cuticular structures by softening them and making them more prone to deformation (Morek et al., 2016). Given that the two populations were mounted in different media (polyvinyl-lactophenol and Hoyer's), it could be hypothesized that the observed difference in buccal tube width is a preparation methodology artifact (Nelson and Bartels, 2007). In fact, the buccal tube seems to have thicker walls with distinguishable external and internal walls when mounted in Hoyer's medium, whereas in polyvinyl-lactophenol, the walls seem thinner and with no clear external and internal boundaries. This might be the reason why *pt* values of internal widths of the buccal tube do not overlap in these two populations while *pt* values of external widths do. Specimens mounted in Hoyer's medium have shorter internal buccal tube width than specimens mounted in polyvinyl-lactophenol, which resulted in nonoverlapping *pt* ranges. Unfortunately, no studies investigating the effects of these media on tardigrade morphometric traits are available (Morek et al., 2016); thus, currently it cannot be stated whether the difference in buccal tube diameter is a preparation artifact or a true difference between the two populations.

The shell morphology of freely laid eggs is used widely for delimiting tardigrade species because it provides a number of morphological and morphometric traits that vary considerably between species, even closely related

ones (Bertolani and Rebecchi, 1993; Bertolani et al., 2010; Bertolani et al., 2011; Stec et al., 2015; Bąkowski et al., 2016; Roszkowska et al., in press). However, sometimes eggs may also exhibit significant intraspecific variability. For example, Stec et al. (2016) showed differences in chorion morphology between two haplotypes of a single parthenogenetic species, *Ramazzottius subanomalous* (Biserov, 1985), extracted from a single moss cushion. Moreover, the intraspecific variability in egg ornamentation between populations of *M. macrocalix* can also be seen in the work of Cesari et al. (2009). Thus, the minor differences between the Italian eggs (measured in this study as well as presented in the original description) and Polish eggs could be considered as intraspecific. To conclude, our specimens match the type specimens in all aspects except for some small inconsistencies in measurements, which we recognized as intraspecific morphological variability and/or a result of the preparation method employed.

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References

- Bąkowski M, Roszkowska M, Gawlak M, Kaczmarek Ł (2016). *Macrobiotus naskreckii* sp. nov., a new tardigrade (Eutardigrada: Macrobiotidae) of the *hufelandi* group from Mozambique. *Ann Zool* 66: 155-164.
- Bertolani R, Biserov V, Rebecchi L, Cesari M (2011). Taxonomy and biogeography of tardigrades using an integrated approach: new results on species of the *Macrobiotus hufelandi* group. *Invertebrate Zoology* 8: 23-36.
- Bertolani R, Guidetti R, Marchioro T, Altiero T, Rebecchi L, Cesari M (2014). Phylogeny of Eutardigrada: new molecular data and their morphological support lead to the identification of new evolutionary lineages. *Mol Phylogenet Evol* 76: 110-126.
- Bertolani R, Rebecchi L (1993). A revision of the *Macrobiotus hufelandi* group (Tardigrada, Macrobiotidae), with some observations on the taxonomic characters of eutardigrades. *Zool Scr* 22: 127-152.
- Bertolani R, Rebecchi L, Cesari M (2010). A model study for tardigrade identification. In: Nimis PL, Vignes LR, editors. *Tools for Identifying Biodiversity: Progress and Problems: Proceedings of the International Congress, Paris, 20-22 September 2010*. Trieste, Italy: EUT, Edizioni Università di Trieste, pp. 333-339.
- Biserov V (1985). *Hypsibius subanomalous* sp. n. (Eutardigrada, Hypsibiidae) from the Astrakhan District. *Zool Zh* 64: 131-135.
- Cesari M, Bertolani R, Rebecchi L, Guidetti R (2009). DNA barcoding in Tardigrada: the first case study on *Macrobiotus macrocalix* Bertolani & Rebecchi 1993 (Eutardigrada, Macrobiotidae). *Mol Ecol Resour* 9: 699-706.
- Dastych H (1980). Niesporczaki (Tardigrada) Tatrzańskiego Parku Narodowego. *Monografie Fauny Polski* 9. Warsaw, Poland: Państwowe Wydawnictwo Naukowe (in Polish).
- Dastych H (1988). The Tardigrada of Poland. *Monografie Fauny Polski* 16: 1-255.
- Dastych H (1997). Niesporczaki – Tardigrada. In: Razowski J, editor. *Checklist of Animals of Poland, Vol. 4*. Krakow, Poland: Institute of Systematics and Evolution of Animals, pp. 141-144.
- Degma P, Guidetti R (2007). Notes to the current checklist of Tardigrada. *Zootaxa* 1579: 41-53.
- Degma P, Bertolani R, Guidetti R (2016). Actual checklist of Tardigrada species (2009–2016, Version 30: 15-09-2016). Modena, Italy: University of Modena and Reggio Emilia.
- Doyère LMF (1840). Memoire sur les Tardigrades. I. *Ann Sci Nat Paris Series 2* 14: 269-362.

- Guidetti R, Bertolani R (2005). Tardigrade taxonomy: an updated check list of the taxa and a list of characters for their identification. *Zootaxa* 845: 1-46.
- Guidetti R, Peluffo JR, Rocha AM, Cesari M, Moly de Peluffo MC (2013). The morphological and molecular analyses of a new South American urban tardigrade offer new insights on the biological meaning of the *Macrobotus hufelandi* group of species (Tardigrada: Macrobiotidae). *J Nat Hist* 47: 2409-2426.
- Guil N, Giribet G (2012). A comprehensive molecular phylogeny of tardigrades: adding genes and taxa to a poorly resolved phylum-level phylogeny. *Cladistics* 28: 21-49.
- Hansen J, Katholm AK (2003). A study of the genus *Amphibolus* from Disko Island with special attention on the life cycle of *Amphibolus nebulosus* (Eutardigrada, Eohypsibiidae). Arctic Biology Field Course - Qeqertarsuaq, 2002. Copenhagen, Denmark: H.C.Ø.-Tryk, 2003.
- Hęciak S (1976). Niesporczaki (Tardigrada) Gór Świętokrzyskich. *Bad Fizjogr Pol Zach* 29: 111-128 (in Polish).
- Jakubski A (1915). Opis fauny wrotków (Rotatoria) powiatu sokalskiego z uwzględnieniem gromad brzuchorzęsków (Gartroprioga) i niesporczaków (Tardigrada). *Wiadom Mus Dzieduszyckich Lwów* 1: 1-166 (in Polish).
- Kaczmarek Ł (2008). Niesporczaki (Tardigrada). In: Fauna Polski, Charakterystyka i wykaz gatunków (Bogdanowicz W, Chudzicka E, Pilipiuk I, Skibińska E). Muzeum i Instytut Zoologii PAN Warszawa 3: 543-548 (in Polish).
- Kaczmarek Ł, Cytan J, Zawierucha K, Diduszko D, Michalczyk Ł (2014). Tardigrades from Peru (South America), with descriptions of three new species of Parachela. *Zootaxa* 3790: 357-379.
- Kaczmarek Ł, Gołdyn B, Czyż M, Michalczyk Ł (2010). The first records of *Isohypsibius pushkini* Tumanov, 2003 (Eutardigrada, Hyspibiidae) from Poland. *Biol Lett* 47: 81-85.
- Kaczmarek Ł, Michalczyk Ł (2004). *Macrobotus macrocalix* Bertolani & Rebecchi, 1993 – a species of water bear (Tardigrada) new to the fauna of Poland. *Badania Fizjograficzne nad Polską Zachodnią Seria C – Zoologia* 50: 39-43.
- Michalczyk Ł, Kaczmarek Ł (2003). A description of the new tardigrade *Macrobotus reinhardti* (Eutardigrada, Macrobiotidae, *harmsworthi* group) with some remarks on the oral cavity armature within the genus *Macrobotus* Schultze. *Zootaxa* 331: 1-24.
- Michalczyk Ł, Kaczmarek Ł (2013). The Tardigrada Register: a comprehensive online data repository for tardigrade taxonomy. *J Limnol* 72: 175-181.
- Minkiewicz S (1914). Przegląd fauny jezior tatrzańskich. *Sprawozdania Komisji fizyograficznej Akad. Umiej w Krakowie* 48: 114-137 (in Polish).
- Morek W, Stec D, Gąsiorek P, Schill RO, Kaczmarek Ł, Michalczyk Ł (2016). An experimental test of eutardigrade preparation methods for light microscopy. *Zool J Linn Soc-Lond* 178: 785-793.
- Nelson DR, Bartels PJ (2007). "Smoky Bears": tardigrades of Great Smoky Mountains National Park. *Southeast Nat* 6: 229-238.
- Nelson DR, Guidetti R, Rebecchi L (2015). Phylum Tardigrada. In: Thorp J, Rogers DC, editors. *Ecology and General Biology: Vol. 1: Thorp and Covich's Freshwater Invertebrates*. 4th Revised Edition. San Diego, CA, USA: Academic Press Inc., pp. 347-380.
- Pigoń A, Węglarska B (1953). The respiration of Tardigrada: a study in animal anabiosis. *Bull Acad Pol Sci* 1: 69-72.
- Pilato G (1981). Analisi di nuovi caratteri nello studio degli Eutardigrada. *Animalia* 8: 51-57 (in Italian).
- Pilato G, Dastych H (1974). *Diphascion montigenum* sp. nov., a new species of Tardigrada from Poland. *Bull Acad Pol Sci* 22: 325-327.
- Pilato G, Kaczmarek Ł, Michalczyk Ł, Lisi O (2003). *Macrobotus polonicus*, a new species of Tardigrada from Poland (Eutardigrada, Macrobiotidae, 'hufelandi group'). *Zootaxa* 258: 1-8.
- Richters F (1926). Tardigrada. In: Kükenthal W, Krumbach T, editors. *Handbuch der Zoologie*, Vol. 3. Berlin, Germany: Walter de Gruyter & Co., pp. 58-61 (in German).
- Roszkowska M, Ostrowska M, Stec D, Janko K, Kaczmarek Ł (in press). *Macrobotus polypoformis*, a new tardigrade (Macrobiotidae; *hufelandi* group) from the Ecuadorian Pacific coast, remarks on the claw abnormalities and the taxonomic status of *Mesobiotus armatus* (Pilato & Binda, 1996). *Eur J Taxon* (in press).
- Sands CJ, McInnes SJ, Marley NJ, Goodall-Copestake W, Convey P, Linse K (2008). Phylum Tardigrada: an "individual" approach. *Cladistics* 24: 1-18.
- Schultze CAS (1834). *Macrobotus Hufelandii* animal e crustaceorum classe novum, reviviscendi post diuturnam asphixiam et ardiatem potens, etc. 8, 1 tab. Berlin, Germany: C. Curths (in Latin).
- Schuster RO, Nelson DR, Grigarick AA, Christenberry D (1980). Systematic criteria of the Eutardigrada. *T Am Microsc Soc* 99: 284-303.
- Stec D, Gąsiorek P, Morek W, Koszyła P, Zawierucha K, Michno K, Kaczmarek Ł, Prokop ZM, Michalczyk Ł (2016). Estimating optimal sample size for tardigrade morphometry. *Zool J Linn Soc-Lond* 178: 776-784.
- Stec D, Morek W, Gąsiorek P, Kaczmarek Ł, Michalczyk Ł (2016). Determinants and taxonomic consequences of extreme egg shell variability in *Ramazzottius subanomalous* (Biserov, 1985) (Tardigrada). *Zootaxa* 4208: 176-188.
- Stec D, Smolak R, Kaczmarek Ł, Michalczyk Ł (2015). An integrative description of *Macrobotus paulinae* sp. nov. (Tardigrada: Eutardigrada: Macrobiotidae: *hufelandi* group) from Kenya. *Zootaxa* 4052: 501-526.
- Thulin G (1928). Über die Phylogenie und das System der Tardigraden. *Hereditas* 11: 207-266 (in German).

- Węglarska B (1959). Tardigraden Polens II. Vest Csl Spol Zool 23: 354-357 (in German).
- Węglarska B (1973). Tardigrada in High Tatra localities bare of snow with a description of *Itaquascon pawlowskii* sp. nov. Vest Cs Spol Zool 37: 150-154.
- Węglarska B, Korecka T (1983). Tardigrada from Dobczyce area (Poland). Zes Nauk Univ Jagiell 29: 83-92.
- Zawierucha K, Grzelak K, Kotwicki L, Kaczmarek Ł, Kristensen RM (2015). First observation of the marine tardigrades *Batillipes mirus* and *Batillipes noerrevangi* (Arthrotardigrada, Batillipedidae) from a strongly brackish part of Polish Baltic Sea coast. Mar Biol Res 11: 859-868.