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Minibiotus bernhardi sp. n. (Eutardigrada, Macrobiotidae) – A New Tardigrade Species from the Black Forest (Baden-Württemberg)

Minibiotus bernhardi sp. n. (Eutardigrada, Macrobiotidae) – eine neue Tardigradenart aus dem Schwarzwald (Baden-Württemberg)

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Summary: In many moss samples collected in the Black Forest region Breisgau-Hochschwarzwald in the communities Breitnau and Hinterzarten in Germany, over the last twenty years a new tardigrade species *Minibiotus bernhardi* sp. n. was found. The new species is most similar to *Minibiotus formosus*, but differs from it mainly by the egg processes, the presence of one to four (mostly two) large cuticular pores (in *Minibiotus formosus* there is only one) on legs I to III and a wider buccal tube. The new species is also most similar to *Minibiotus orthofasciatus*, but differs from it by the distribution and form of the pores in the cuticle (regular in form and distribution in *Minibiotus orthofasciatus,* irregular in form and distribution in *Minibiotus bernhardi* sp. n.).

Key words: *Minibiotus bernhardi* sp. n., taxonomy, Germany

Zusammenfassung: In vielen Moosproben, gesammelt in den letzten zwanzig Jahren in der Region Breisgau-Hochschwarzwald in den Gemeinden Breitnau und Hinterzarten (Deutschland), wurde eine neue Tardigradenart *Minibiotus bernhardi* sp. n. entdeckt und beschrieben. Die neue Art ist *Minibiotus formosus* sehr ähnlich, unterscheidet sich aber hauptsächlich durch die Eifortsätze, die Präsenz von 1 bis 4 (meistens zwei) großen Poren auf der Außenseite der Beine I bis III (bei *Minibiotus formosus* ist es nur eine Pore) und einen größeren Durchmesser der inneren Schlundröhre. Die neue Art ist ebenfalls *Minibiotus orthofasciatus* sehr ähnlich, unterscheidet sich aber durch die Verteilung und Form der Poren in der Kutikula (regelmäßige Form und Verteilung in *Minibiotus orthofasciatus,* unregelmäßige Form und Verteilung in *Minibiotus bernhardi* sp. n.).

Schlüsselwörter: *Minibiotus bernhardi* sp. n., Taxonomie, Deutschand

1. Introduction

Since the first description of a tardigrade in 1773 by Goeze 32 families, 153 genera in the phylum tardigrada have been described (Degma et al., 2021). The genus *Minibiotus* R.O. Schuster, 1980 was erected by Schuster et al. (1980) and re-defined by Claxton (1998). For one hundred years the genus *Minibiotus* contained only the species *Minibiotus intermedius* (Plate, 1888) described as *Macrobiotus intermedius*. In Germany *Min. intermedius* was found in the Black Forest (Heinis 1910) and near Marburg, in the Taunus-mountains, in the Eifel-region and on the island Rügen (Marcus 1936; Grabowski 1995). Actually, the genus *Minibiotus* R.O. Schuster, 1980 comprises 54 species (Degma et al. 2021), but from Germany only *Minibiotus intermedius* is known.

The Black Forest is a low mountainous area (up to 1500 m a.s.l.) in the south-west of Germany near the border to Switzerland and France. Within a very small region great differences in climate and vegetation, depending on the altitude, can be found, which is

also reflected by the richness of tardigrade species in this area (SCHUSTER 2002/2003). HEINIS (1910) reported 14 tardigrade species from the southern Black Forest. SCHUSTER (2002/2003) examined the more central part of the region (upper Black Forest, region Hochschwarzwald) and recorded 56 species. Four of the 14 species reported by HEINIS were not found by SCHUSTER (l.c.). These are *Cornechiniscus cornutus* (Richters, 1907), *Claxtonia wendti* (Richters, 1903), *Fractonotus verrucosus* (Richters, 1900) and *Murrayon hastatus* (Murray, 1907), but two new species were added. These are *Diphascon birklehofi* Schuster, 1999 (SCHUSTER 1999) from the central part and more recently *Crenubiotus ruhesteini* Guidetti, Schill, Giovannini, Massa, Goldoni, Ebel, Förschler, Rebecchi, Cesari, 2021 (GUIDETTI et al. 2021) from the northern part of the Black Forest.

In this article I describe a new species belonging to the genus *Minibiotus* – this species was still listed as *Minibiotus* cf. *poricinctus* in my earlier article (see SCHUSTER 2002/2003) – collected from various substrates from the Black Forest. This description increases the number of new species from this area to three.

2. Material and methods

Moss samples from rocks and other substrates (for details see also chapter 3.3. below) were collected in different localities in the region Breitnau/Hinterzarten between 1998 and 2020. Mosses collected were placed in a Petri dish in water and left to soak for ca. 10 hours. The samples were agitated and then squeezed out. Individual animals were mounted on microscope slides with Polyvinyl-Lactophenol.

The tardigrades were examined and photographed under the phase- (PHC) and differential interference contrast (DIC) microscopes (Zeiss Axiovert 200 M; Olympus Vanox TAH-2). Some DIC-micrographs (Figs 1 A, B, 2 C, D, E) were edited improved by stacking up to three micrographs with the program PICOLAY.

All measurements in the text are given in micrometers (µm), in the figures by scale bars. Structures were measured only if their orientation was appropriate. The body length was measured from the anterior tip to the end of the body, excluding the hind legs. The buccal tube length and the level of the stylet support insertion point were measured according to PILATO (1981). In addition, the *pt* ratio (i.e. the ratio of the length of a given structure to the length of the buccal tube expressed as a percentage) was determined (PILATO 1981). Morphometric data were handled as shown in KACZMAREK and MICHALCZYK (2017). The buccal tube width (external/internal diameter) was measured at the level of the stylet insertion point. The lengths of the claw branches were measured from the base of the claw to the top of the primary branch, including accessory points. The macroplacoid length sequence is stated according to KACZMAREK et al. (2014), i.e.macroplacoids are listed from the shortest to the longest one and their relative sizes are denoted with appropriate inequality, approximation and/or equality signs (<, ≤, ≈, =).

For comparison the type material of *Minibiotus xavieri* Fontoura, Pilato, Morais & Lisi, 2009 (FONTOURA et al. 2009a), *Minibiotus orthofasciatus* Fontoura, Pilato, Lisi & Morais, 2009 (FONTOURA et al. 2009b) (both deposited in the collection of PAULO FONTOURA (Department of Zoology and Anthropology, Faculty of Sciences, University of Port, Portugal)) and a paratype of *Minibiotus formosus* Zawierucha, Dziamięcki, Jakubowska, Michalczyk & Kaczmarek, 2014 (ZAWIERUCHA et al. 2014) (collection of ŁUKASZ KACZMAREK, Adam Mickiewicz University in Poznań, Poland) were examined. The comparisons with species not personally examined are based on original descriptions. For the identification and differentiation of the new species the keys of CLAXTON (1998), DASTYCH (1988) and RAMAZZOTTI

Tab. 1: Measurements (in µm) and *pt* values of selected morphological structures of ten specimens from the type population of *Minibiotus bernhardi* sp. n. mounted in Polyvinyl-Lactophenol (N – number of specimens/structures measured, Range refers to the smallest and the largest structure among all measured specimens, SD – standard deviation, ? – trait oriented unsuitably for measurements).
Tab. 1: Messwerte (in µm) und *pt*-Werte von ausgewählten morphologischen Strukturen von zehn Exemplaren der Typenpopulation von *Minibiotus bernhardi* sp. n., eingebettet in Polyvinyl-Lactophenol. (N – Zahl der gemessenen Individuen/Strukturen, Range bezieht sich auf den geringsten und den höchsten Wert bei allen vermessenen Individuen, SD – Standardabweichung, ? – Merkmal, welches für Messungen nicht benutzbar war).

Character	N	RANGE		MEAN		SD		Holo type
		µm	*pt*	µm	*pt*	µm	*pt*	µm
Body length	10	181.5 - 265.6	824 - 1140	229	1008.8	27	96.9	208
Buccal tube								
Length	10	20.4 - 24.2	-	22.7	-	1.8		22.2
Stylet support insertion point	10	11.7 - 13.4	54.4 - 57.4	12.7	55.9	0.9	1.0	12.2
External width	10	1.7 - 1.9	7.4 - 9.3	1.9	8.3	0.1	0.5	1.8
Internal width	10	0.9 - 1.2	3.8 - 5.2	1.0	4.4	0.1	0.5	0.9
Placoid lengths								
Macroplacoid 1	9	1.5 - 1.9	6.6 - 9.0	1.7	7.7	0.1	0.7	1.8
Macroplacoid 2	9	1.6 - 2.2	7.0 - 9.4	1.9	8.4	0.2	0.7	2.0
Macroplacoid 3	9	1.7 - 2.3	7.2 - 9.7	1.9	8.1	0.2	0.8	1.8
Microplacoid	9	0.8 - 1.1	3.2 - 4.6	0.9	3.9	0.1	0.4	0.9
Macroplacoid row	9	5.7 - 7.4	24.8 - 31.8	6.5	28.8	0.5	2.0	6.7
Placoid row	9	6.9 - 8.4	30.5 - 35.9	7.7	34.0	0.5	1.6	7.7
Claw 1 lengths								
External primary branch	6	5 - 5.5	21.3 - 23.9	5.2	22.7	0.2	0.8	?
External secondary branch	6	3.4 - 4.3	14.8 - 18.8	3.9	16.9	0.3	1.4	?
Internal primary branch	6	4.4 - 5.8	20.1 - 26	5.2	23.2	0.5	2.1	4.6
Internal secondary branch	6	3.3 - 4.4	15.2 - 18.2	3.7	16.6	0.4	0.9	3.4
Claw 2 lengths								
External primary branch	8	4.7 - 6.3	21.3 - 27.2	5.5	24.5	0.5	1.9	4.7
External secondary branch	7	3.3 - 4.7	15.4 - 19.3	3.8	16.9	0.4	1.4	4.1
Internal primary branch	9	4.9 - 6.1	21.8 - 25.2	5.4	23.9	0.4	1.2	5.0
Internal secondary branch	8	3.1 - 4.6	13.7 - 19.9	3.8	17.0	0.5	2.2	3.7
Claw 3 lengths								
External primary branch	10	5 - 6.5	23.2 - 26.7	5.6	24.7	0.4	1.0	5.3
External secondary branch	9	3.4 - 4.5	15.5 - 19.9	3.9	17.1	0.4	1.5	3.6
Internal primary branch	8	4.8 - 6.3	21.6 - 25.8	5.5	24.4	0.5	1.2	4.8
Internal secondary branch	7	3.2 - 4.9	14.4 - 20.1	3.9	17.2	0.5	1.7	3.2
Claw 4 lengths								
Anterior primary branch	6	5.7 - 7.6	25.8 - 32.5	6.6	28.9	0.6	2.7	5.7
Anterior secondary branch	6	3.73 - 5.2	17.3 - 22.4	4.2	18.6	0.5	1.7	3.9
Posterior primary branch	4	5.7 - 7.5	25.5 - 34.1	6.8	30.2	0.7	3.2	5.7
Posterior secondary branch	4	3.6 - 5.5	17.5 - 23.0	4.6	20.2	0.9	2.7	3.9

& MAUCCI (1983), and original descriptions (MICHALCZYK & KACZMAREK 2004; MICHALCZYK et al. 2005; GUIL & GUIDETTI 2005; MEYER & HINTON 2009; ZAWIERUCHA et al. 2014; CIOBANU et al. 2015; FONTOURA et al. 2009 a, b; LONDOÑO et al. 2017) were used.

3. Results

3.1. Taxonomic account

Order: *Parachela* Schuster, Nelson, Grigarick & Christensen, 1980

Superfamily: Macrobiotoidea Thulin, 1928 in Marley et al. 2011

Family: Macrobiotidae Thulin, 1928

Genus: *Minibiotus* R.O. Schuster, 1980

Species: *Minibiotus bernhardi* sp. n.

Type material: Holotype, 48 paratypes, 5 non-embryonated eggs

3.2. Description

3.2.1. Description of the adults

Measurements in µm, *pt* ratios and statistics (see Tab. 1): body white/transparent (Fig. 1 A). Posterior eyes present in live specimens and in ca. 60% of the specimens mounted in Polyvinyl-Lactophenol. Cuticle covered with pores of various sizes and shape, mostly round or oval, rarely multilobated. Dorsally pores arranged in eleven well defined transverse bands (Fig. 1 B), but ventrally only in eight. In the cephalic region the transverse bands are only dorsally visible, ventrally only some single pores present. Pores on the dorsal cuticle arranged more densely than on the ventral cuticle. In the anterior and the posterior sections of the body (Fig. 1 D) some of the pores distinctly larger (>2 µm). Additionally one to four – mostly two – larger pores (larger (1.8-2.6 µm) than the other pores in the same band) present on the external side of legs I-III (Fig. 1 C). A ring of pores around the mouth opening absent. Granulation on cuticle and on the legs absent.

Mouth opening located antero-ventrally. The bucco-pharyngeal apparatus of the *Minibiotus*-type (PILATO & BINDA 2010). An oral cavity armature is absent or not visible under PHC. Buccal tube with anterior and posterior bend (both visible only in lateral view, Fig. 2 B). Dorsal buccal tube walls are thickened just posterior the stylets insertion point of the stylet supports (Fig. 2 A, B). Pharyngeal apophyses triangular and very close to the first macroplacoids. Three granular macroplacoids and a minute microplacoid present. All macroplacoids of similar size (macroplacoid length sequence: 3≥2≥1). Septulum absent.

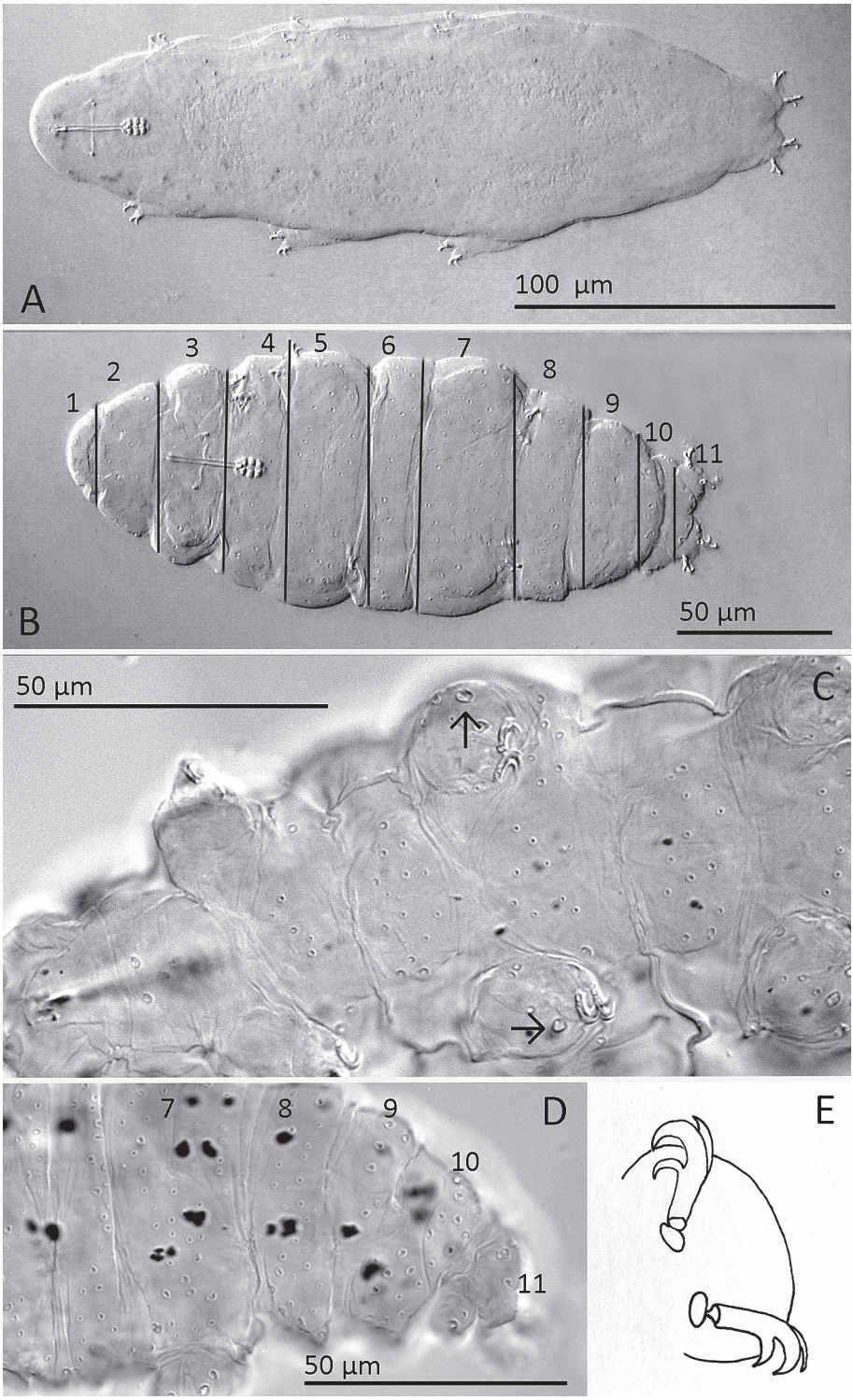
Claws slender, *Macrobiotus*-type (Fig. 1 E). Primary branches of the claws with thin, but obvious accessory points. A round refractive zone present at the base of claws. Smooth lunules present on all legs, distinctly larger under the external and posterior claws of leg IV (Fig. 1 E).

3.2.2. Eggs

Measurements (see Tab. 2): white/transparent, laid freely. The processes are screw-like,

Fig. 1 A-E: *Minibiotus bernhardi* sp. n.. **A** Habitus. **B** Habitus with eleven bands of pores. **C** Ventral cuticle with larger pores on the outside of the legs (arrows). **D** Posterior part of the body with bands 7 to 11. **E** Leg IV with claws. DIC-images.

Abb. 1 A-E: *Minibiotus bernhardi* sp. n.. **A** Habitus. **B** Habitus mit elf Porenbändern. **C** Ventrale Kutikula mit größeren Poren auf der Außenseite der Beine (Pfeile). **D** Posteriorer Abschnitt des Körpers mit den Porenbändern 7 bis 11. **E** Krallen von Bein IV. DIC-Bilder.


A
100 µm
B
1
2
3
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5
6
7
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9
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11
50 µm
C
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8
9
10
11
50 µm
E

each surrounded by a membrane (Fig. 2 C-E). No dots visible on the shellsurface between the processes. Five non-embryonated eggs have been found alongside the described specimens. *Min. bernhardi* sp. n. was the only *Minibiotus* species in the samples.

3.2.3. Locus typicus, additional material and etymology

47° 56' 23" N; 08° 01' 01" E, 550m a.s.l.: Germany, region Breisgau-Hochschwarzwald, community Breitnau, beside the main road B31, moss from stones.
Minibiotus bernhardi sp. n. was also found at 47°-53'-26"N; 08°-03'- 52"E, 1075m a.s.l. (Häuslebauer-Hof) (7 specimens, 6 non-embryonated eggs); 47° 53' 23" N, 08° 05' 45" E, 980m a.s.l. (Mathislehof) (13 specimens, 1 non-embryonated egg); 47° 54' 24" N, 08° 04' 35"E, 920m a.s.l. (Bisten) (13 specimens). These three locations belong to the community Hinterzarten, region Breisgau-Hochschwarzwald.
The new species *Min. bernhardi* sp. n. was named after BERNHARD HUBER, who introduced me to the tardigrades.

3.2.4. Type depositories

The holotype and nine paratypes (slide N. 699), one egg and four paratypes (slide N. 698) are deposited in the Bertolani Collection of the University of Modena and Reggio Emilia (Italy). One egg and four paratypes (NHMD-916469 for the slide with code 694), ten paratypes (NHMD-916470 for the slide with code 701) are deposited in the tardigrade collection of the Natural History Museum of Denmark, University of Copenhagen. Ten paratypes and one egg are deposited in the collection of ŁUKASZ KACZMAREK (Department of Animal Taxonomy and Ecology at the Adam Mickiewicz University, Poznań, Poland). The remaining paratypes, specimens and eggs are in my private collection.

3.2.5. Differential diagnosis

The new species *Min. bernhardi* sp. n. is very similar to *Min. formosus* Zawierucha, Dziamięcki, Jakubowska, Michalczyk & Kaczmarek, 2014 (ZAWIERUCHA et al. 2014), but it differs from it by the shape of egg processes (screw-like, each surrounded by a membrane in *Min. bernhardi* sp. n. vs short, smooth, slightly flexible cones on the eggs of *Min. formosus*). Larger internal width of the buccal tube (0.9-1.2 µm, *pt 3.8-5.2* in *Min. bernhardi* sp. n. vs 0.5-0.7 µm, *pt 2.4-3.4* in *Min. formosus*). In general higher number of pores on legs I-III (one to four (mostly two) in *Min. bernhardi* sp. n. vs only one in *Min.* formosus). Pores arranged in eleven well defined bands in *Min. bernhardi* sp. n. vs pores arranged in 9-10 poorly defined transverse bands in *Min. formosus*. The absence of granulation on all legs in *Min. bernhardi* sp. n. vs fine and regular granulation (better developed on legs IV), visible only in larger specimens, on all legs in *Min. formosus* (see also MICHALCZYK & KACZMAREK 2013).
Minibiotus bernhardi sp. n. is also very similar to *Min. orthofasciatus* Fontoura, Pilato, Lisi & Morais, 2009 (FONTOURA et al. 2009 b), but it differs by an irregular distribution and a randomly number of pores in the eleven dorsal bands in *Min. bernhardi* sp. n. vs a very regular straight disposition of the bands with pores arranged in dorsal and ventral bands constant in number and almost constant in pore distribution in *Min. orthofasciatus*, in having the stylet supports inserted on the buccal tube in a more anterior position (11.7-13.4 µm, *pt 54.4-57.4* in *Min. bernhardi* sp. n. vs 13.4-15.1 µm, *pt 66.5-67.8* in *Min. orthofasciatus*). The eggshell between the egg processes of *Min. bernhardi* sp. n. is even vs an areolated eggsshell between the eggprocesses in *Min.orthofasciatus*. *Minibiotus bernhardi* sp. n. has got no small cuticular bars on the legs, in *Min. orthofasciatus* small cuticular bars near the lunules on the first three pairs of the legs are present. The macroplacoid

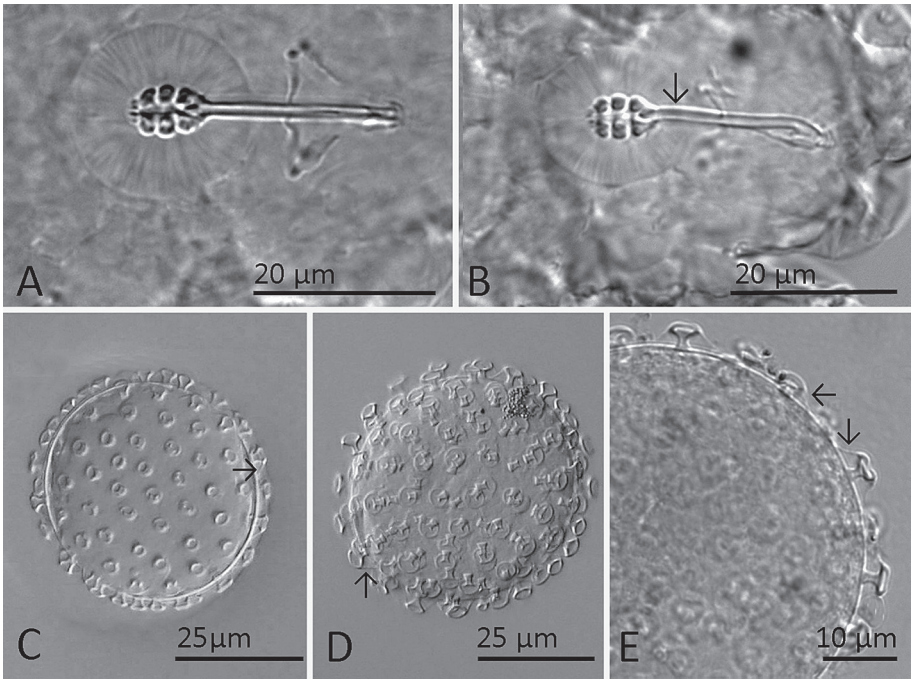

Fig. 2 A- E: *Minibiotus bernhardi* sp. n.. **A** Bucco-pharyngeal apparatus; dorso-ventral view. **B** Lateral view, the arrow points the thickened dorsal wall posterior the insertion point of the stylet supports. A, B: (PHC)-images. **C-E** Various aspects of eggs and their processes. Note the "membrane-like" structures covering the processes (arrows). DIC-images.
Abb. 2 A- E: *Minibiotus bernhardi* sp. n.. **A** Buccalapparat; dorsoventrale Ansicht. **B** laterale Ansicht, der Pfeil zeigt auf die verdickte dorsale Schlundröhre posterior des Ansatzpunktes der Stiletthalter. **C-E** Verschiedene Ansichten der Eier und ihrer Fortsätze. Man beachte die „membranähnlichen" Strukturen, welche die Fortsätze bedecken (Pfeile). DIC-Bilder.

Tab. 2: Measurements (in µm) of selected morphological structures of 5 *Minibiotus bernhardi* sp. n. eggs mounted in Polyvinyl-Lactophenol. The number of measured egg processes per egg in brackets.
Tab. 2: Messwerte (in µm) für ausgewählte Strukturen der 5 Eier von *Minibiotus bernhardi* sp. n., welche in Polyvinyl-Lactophenol eingebettet sind. In Klammern die Anzahl der gemessenen Eifortsätze pro Ei.

Character	**egg 1**	**egg 2**	**egg 3**	**egg 4**	**egg 5**
Diameter of egg without processes	50	64	58	48	45
Diameter of egg with processes	57	71	63	54	53
Process height (8)	3.7 - 4.5	3.7 - 4.9	3.4 - 4.4	3.8 - 4.6	3.9 - 4.6
Process base width (8)	1.7 - 2.3	1.5 - 2.6	1.6 - 2.2	1.9 - 2.5	1.6 - 2.4
Process base/height ratio (8)	41% - 56%	39% - 55%	37% - 52%	42% - 59%	37% - 53%
Diameter of the top of the processes (10)	3.2 – 4.0	3.1 – 4.0	3.5 – 4.0	3.1 – 4.2	3.4 – 4.1
Distance between processes (10)	4.8 - 7.4	3.8 - 7.4	4.5 - 6.8	4.7 - 7.7	3.9 - 5.9
Number of processes on the egg circumference	23	22	26	20	19

length sequence is 3≥2≥1 in *Min. bernhardi* sp. n. vs 1>2≈3 in *Min. orthofasciatus*. The first macroplacoid in *Min. bernhardi* sp. n. is shorter than in *Min. orthofasciatus* (1.5-1.9 µm, *pt 6.6-9.0* vs 1.9-3.1 µm, *pt 9.5-13.7*).

Other species to which *Min. bernhardi* sp. n. is similar in some characteristics of the adult or the egg morphology (e.g. pores in transverse bands, eggs with membrane coated screw-like processes) include species listed below. *Minibiotus bernhardi* sp. n., however, differs specifically from:

Minibiotus diversus Ciobanu, Roszkowska & Kaczmarek, 2015 (CIOBANU et al. 2015) by: the arrangement of pores on the dorsal side of the body (pores arranged in 11 transverse bands in *Min. bernhardi* sp. n. vs randomly arranged pores in *Min. diversus*), the absence of a triangular formation of pores on the ventral side of the head, smaller single pores on legs I–III (1.8-2.6 µm in *Min. bernhardi* sp. n. vs 3.1-4.8 µm in *Min. diversus*), the absence of a fine granulation on all legs, wider buccal tube (0.9-1.2 µm, *pt 3.8-5.2* in *Min. bernhardi* sp. n. vs 0.5-0.7 µm, *pt 2.1-3.0* in *Min. diversus*).

Minibiotus eichhorni Michalczyk & Kaczmarek, 2004 (MICHALCZYK & KACZMAREK 2004) by: the arrangement of pores on the dorsal side of the body (eleven dorsal transverse bands of pores in *Min. bernhardi* sp. n. vs pores arranged in six transverse bands in *Min. eichhorni*), the absence of star-shaped pores, the absence of four pores around the mouth opening, the absence of fine granulation on all legs, the absence of large elliptical lunules, a different macroplacoid length sequence (3≥2≥1 in *Min. bernhardi* sp. n. vs 1>3>2 in *Min. eichhorni*), stylet supports inserted in a more anterior position (11.7-13.4 µm, *pt 54.4-57.4* in *Min. bernhardi* sp. n. vs 16.2-23.8 µm, *pt 65.4-70.6* in *Min. eichhorni*), a shorter first macroplacoid (1.5-1.9 µm, *pt 6.6-9.0* in *Min. bernhardi* sp. n. vs 2.9-4.3 µm, *pt 10.3-12.5* in *Min. eichhorni*) and a shorter placoid row (6.9-8.4 µm, *pt 30.5-35.9* in *Min. bernhardi* sp. n. vs 9.0-14.3 µm, *pt 36.2-44.1* in *Min. eichhorni*).

Minibiotus floriparus Claxton, 1998 (CLAXTON 1998) by: the absence of red-purple pigment in cuticle, the presence of pores and presence of screw-shaped processes without flared distal ends and pores around central discs.

Minibiotus gumersindoi Guil & Guidetti, 2005 (GUIL & GUIDETTI 2005) by: pores of different sizes and forms in *Min. bernhardi* sp. n. vs the presence of large and round pores in the transversal bands in *Min. gumersindoi*, one to four larger pores (1.8-2.6 µm) in *Min. bernhardi* sp. n. vs a very large pore close to the end of each leg (2.7-3.7 µm) in *Min. gumersindoi*. In figure 2 of the original description the scale bars are marked =50 µm, which in my opinion should be changed to 10 µm.

Minibiotus harrylewisi Meyer & Hinton, 2009 (MEYER & HINTON 2009) by: the absence of tri- and quadrilobed cuticular pores, the presence of two types of pores (small and large) over the entire cuticle in *Min. bernhardi* sp. n. vs small pores present only in the anterior part of the body and large pores present only in the posterior part of the body in *Min. harrylewisi* and in a different shape of egg processes (screw-like, each surrounded by a membrane in *Min. bernhardi* sp. n. vs elongated tapering cones with bulbous bases in *Min. harrylewisi*).

Minibiotus pentannulatus Londoño, Daza, Lisi & Quiroga, 2017 (LONDOÑO et al. 2017) by: the absence of multilobated and star-shaped pores and the presence of eleven transverse rows of pores in *Min. bernhardi* sp. n.. In adult specimens of *Min. pentannulatus* there are no transverse rows visible. The macroplacoid length sequence is 3≥2≥1 in the new species vs 1>2=3 in *Min. pentannulatus*. Cuticular bars are absent in *Min. bernhardi* sp. n. but present in *Min. pentannulatus*. The eggs of *Min. bernhardi* sp. n. have screw-like processes vs eggs with numerous short conical processes in *Min. pentannulatus*.

Minibiotus poricinctus Claxton, 1998 (Claxton 1998) by: eleven transverse bands of pores in *Min. bernhardi* sp. n. vs eight bands of pores in *Min. poricinctus*. *Min. bernhardi* sp. n. has pores of different sizes and forms vs *Min. poricinctus* with just small round pores, the absence of fine granulation on top and both sides of legs I-III just above claws in *Min. bernhardi* sp. n., but the presence of one to four larger pores on the outside of legs I-III in the new species.
Minibiotus weglarskae Michalczyk, Kaczmarek & Claxton, 2005 (Michalczyk et al. 2005) by: eleven transverse bands of pores in *Min. bernhardi* sp. n. vs ten transverse bands of pores in *Min. wegklaskae*. The absence of cribiform areas and of larger triangular pores on the hind legs in *Min. bernhardi* sp. n. There is no fine granulation near the claws on all legs in *Min. bernhardi* sp. n. which is present in *Min. wegklaskae*. *Min. bernhardi* sp. n. has a larger second macroplacoid (1.6-2.2 µm, *pt 7.0-9.4* vs 1.0-1.4 µm, *pt 4.2-6.5* in *Min. wegklaskae*), a larger microplacoid (0.8-1.1 µm, *pt 3.2-4.6* vs 0.3-0.5 µm, *pt 2.0-2.3*) and a longer placoid row (6.9-8.4 µm, *pt 30.5-35.9* vs 5.2-6.8 µm, *pt 24.4-30.4*).
Minibiotus xavieri Fontoura, Pilato, Morais & Lisi, 2009 (Fontoura et al. 2009 a) by: eleven transverse bands of pores in *Min. bernhardi* sp. n. vs pores in the cuticle which are arranged in nine transverse bands in *Min. xavieri*. In *Min. bernhardi* sp. n. the pores are round to oval, *Min. xavieri* has trilobated pores on the external side of the legs. *Minibiotus bernhardi* sp. n. has a shorter second macroplacoid 1.6-2.2 µm, *pt 7.0-9.4* vs 2.9-3.6 µm, *pt 10.3-11.1* in *Min. xavieri*. The macroplacoid length sequence in *Min. bernhardi* sp. n. is 3≥2≥1 vs 1>2≈3 in *Min. xavieri*. Absolute measurements of the macroplacoids, the microplacoid and (macro)placoidrow in *Min. bernhardi* sp. n. are shorter than in *Min. xavieri*. The eggs of *Min. bernhardi* sp. n. with membrane coated screw-like processes vs *Min. xavieri* with long conical processes.

3.3. Notes on the habitat

Minibiotus bernhardi sp. n. was found in mosses growing on rocks or the bark of trees, which dry out easily and fast. However, the frequency of *Min. bernhardi* sp. n. does not depend on whether the site (e.g. the rock or tree bark) is exposed to the sun or is located in the shade. The investigated area comprises 120 km^2. 200 samples were collected in varying types of microhabitats such as: on tree bark (both in sunny as in shady places), on rocks (both sun-exposed and shady), on meadows, on tree stumps, on the ground in the forest, among foliage on the ground and in anthropogenic habitats such as roofs, walls, streets etc.. In all habitats *Min. bernhardi* sp. n. proved significantly to occur most frequently and dominantly (Schuster, 2002/2003).

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