

First faunistic investigation of semiterrestrial tardigrade fauna of North-West Russia using the method of DNA barcoding

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ABSTRACT: In this paper we present results of the first faunistic investigation of semiterrestrial tardigrade fauna of the North-West Russia using the methods of integrative taxonomy. For our analysis we collected moss samples in five different points of the region. For all the species found in addition to the morphological analysis using light and scanning electron microscopy we obtained data on mitochondrial COI gene sequences and in some cases additional data on 18S rRNA, 28rRNA and ITS-2 sequences. The number of tardigrade species known for the Russian fauna confirmed with molecular data is raised from 3 to 13. Among 11 species found during this investigation four are new for the fauna of Russia (*Milnesium dornensis*, *M. berladnicorum*, *Mesocrista revelata*, and *Paramacrobiotus fairbanksi*). For three species, previously noted for the Russian fauna their presence was for the first time supported with molecular data (*Adropion scoticum*, *Ramazzottius oberhaeuseri*, and *Macrobiotus hufelandi*). In the case of three other species found (*Diphascon* cf. *pingue*, *Pilatobius* cf. *bullatus*, and *Minibiotus* cf. *intermedius*) our analysis revealed significant genetic differences between the populations studied here and the data obtained from GenBank.

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KEY WORDS: Tardigrada, distribution, zoogeography, taxonomy, DNA-barcoding, fauna of Russia.

Первое исследование фауны наземных тихоходок Северо-Запада России с использованием метода ДНК-баркодинга

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РЕЗЮМЕ: В этой статье мы приводим результаты первого исследования фауны наземных тихоходок Северо-Запада России, выполненного с использованием методов интегративной таксономии. Для этой работы в пяти точках региона были собраны образцы мхов. Для всех обнаруженных видов, в дополнение к морфологическому анализу с использованием световой и сканирующей электронной микроскопии, были получены последовательности митохондриального гена COI и, в некоторых случаях, дополнительные последовательности генов 18S рРНК, 28S рРНК и ITS-2. В результате исследования число видов тихоходок, присутствие которых в фауне России подтверждено генетическими данными, увеличено с 3 до 13. Из 11 обнаруженных в ходе исследования видов четыре являются новыми для фауны России (*Milnesium dornensis*, *M. berladnicorum*, *Mesocrista revelata* и *Paramacrobiotus fairbanksi*). Для трех видов, ранее отмеченных для фауны России, впервые получено подтверждение с использованием молекулярных данных (*Adropion scoticum*, *Ramazzottius oberhaeuseri* и *Macrobiotus hufelandi*). В случае трех других видов, обнаруженных в ходе работы (*Diphyscon* cf. *pingue*, *Pilatobius* cf. *bullatus* и *Minibiotus* cf. *intermedius*) наш анализ выявил значительные различия между исследованными нами популяциями и данными, имеющимися для этих видов в GenBank.

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КЛЮЧЕВЫЕ СЛОВА: Tardigrada, тихоходки, распространение, зоогеография, таксономия, ДНК-баркодинг, фауна России.

Introduction

Tardigrades are a group of microscopical segmented animals widely distributed on the Earth (Nelson *et al.*, 2018). More than 1400 tardigrade species are known today (Degma, Guidetti, 2022). Most part of their species diversity is connected with semiterrestrial habitats containing drop-liquid water (moss, lichens, soil etc.) (Nelson *et al.*, 2018). Tardigrades can be abundant in such substrates reaching high

density values up to 537000 specimens/m² (Ito, 1999) similar to those of oribatid mites or collembolans (Nelson *et al.*, 2018), which suggests an important (and yet underestimated) role of these animals in the functioning of the occupied biotopes. Despite their wide distribution and abundance tardigrade fauna is still poorly investigated all over the world. During the pre-genomic era of the tardigrade studies only few European territories have been thoroughly studied from the faunistic point of view — Poland

Table 1. Sampling sites data.

Таблица 1. Информация о точках сбора материала.

Site number	Region	Coordinates, °N, °E	Material/ Substrate	Collection date	Collector
1	St.Peterburg, Kupchino	59.866043, 30.400251	moss/ brick wall	18.09.2020	Leontev A.A.
2	St.Peterburg, Pushkin	59.725531, 30.392167	moss/ stone	21.11.2019	Tumanov D.V.
3	Leningrad Obl., near Lembolovo station	60.4161193, 30.3426615	lichen and moss/ soil	20.09.2020	Androsova E.D.
4	Pskov Obl., Gdov distr.	58.784778, 28.150861	moss/ soil	15.07.2020	Mesentsev Y.S.
5	Leningrad Obl., Mikhailovskij	59.764114, 31.146594	moss/ soil	19.08.2020	Avdeeva G.S.

(Dastych, 1988), Italy (Maucci, 1986), Sicilia (Lisi, 2015). The study of the tardigrade fauna of the European part of Russia has been greatly advanced by the works of Biserov (1991; and references therein).

Since most of the above mentioned faunistic reviews were published, tardigrade taxonomy has undergone significant changes. Numerous forms, previously accepted as widely distributed and polymorphic species (e.g., *Macrobiotus hufelandi* C.A.S. Schultze, 1834, *M. pallarii* Maucci, 1954, *Paramacrobiotus richtersi* (Murray, 1911), *P. areolatus* (Murray, 1907), *Mesobiotus harmsworthi* (Murray, 1907), *Richtersius coronifer* (Richters, 1903), *Ramazzottius oberhaeuseri* (Doyère, 1840), *Hypsibius dujardini* (Doyère, 1840), *Milnesium tardigradum* Doyère, 1840, *Pseudechiniscus suillus* (Ehrenberg, 1853)), have now been proven to be complexes of similar species, poorly demarcated morphologically but often well-distinguishable with DNA barcoding (Bertolani *et al.*, 2011; Gąsiorek *et al.*, 2016, 2018a; Kaczmarek *et al.*, 2018b; Stec *et al.*, 2018, 2020b, c, 2021b; Guidetti *et al.*, 2019; Morek, Michalczyk, 2020; Cesari *et al.*, 2020; Grobys *et al.*, 2020; Roszkowska *et al.*, 2020). The presence of true cryptic species, completely undistinguishable in morphology, has been also revealed in tardigrades (Schill *et al.*, 2010; Bertolani *et al.*, 2011; Stec *et al.*, 2018; Guidetti *et al.*, 2019). As the result, most of the zoogeographical records of such species should be considered as doubtful, which means that the investigation of tardigrade fauna should be started *de novo*, using molecular methods.

Modern data on the tardigrade fauna of Russia are still extremely limited. Until now there are only three published works, for the entire territory of Russia, in which the zoogeographical records are supported with DNA barcoding using an integrative taxonomy approach, i.e., with the analysis of both morphology and gene sequences. Two of them confirm the presence of two species of the genus *Milnesium* — *M. tardigradum* and *M. inceptum* Morek, Suzuki, Schill, Georgiev, Yankova, Marley et Michalczyk, 2019 (Morek, Michalczyk, 2020; Maskin *et al.*, 2021) in Russian far East. The third publication confirms the presence of recently redescribed species *Notahypsibius pallidoides* (Pilato *et al.*, 2011) in the fauna of North-West Russia (Tumanov, 2021).

During the year 2020 moss samples were collected in five different points of North-West Russia. For each sample we collected all tardigrade specimens obtained and proceeded their identification using integrative approach – accepted here as a combination of light microscopy (LM), scanning electron microscopy (SEM), and DNA barcoding methods.

Material and methods

SAMPLING. The moss samples were collected in five different points of North-West Russia (Table 1). Material was stored within paper envelopes at room temperature until investigation. 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

Table 2. Primers and PCR programs used for amplification of the four DNA fragments sequenced in the study.
Таблица 2. Праймеры и программы ПЦР, использованные для амплификации фрагментов генов.

DNA fragment	Primer name	Primer direction	Primer sequence (5'–3')	Primer source	PCR programme
COI	COI_Mil.tar_Ff	forward	TATTTTATTTTGGTATTGATGTGC	Morek <i>et al.</i> , 2019	Michalczyk <i>et al.</i> , 2012
	COI_Mil.tar_Rr	reverse	CTCCCTCCGAGGATC	Morek <i>et al.</i> , 2019	
	COI_Mes.rev_Ff	forward	AATTGAGCTGCAACAGTAGG	Gasiorek <i>et al.</i> , 2016	
	COI_Mes.rev_Rr	reverse	GAATAAGTGTGGTATAAAATTGG	Gasiorek <i>et al.</i> , 2016	
	COI_Para_F	forward	TTTCAACAAACCCACAAGATATYGG	Gasiorek <i>et al.</i> , 2018a	
	COI_Eutar_R	reverse	TAAACTTCTGGGTGACCRARAAYCA	Gasiorek <i>et al.</i> , 2018a	
	LCO1490	forward	GGTCAACAATCATAAAGATATTGG	Folmer <i>et al.</i> , 1994	
	HCO2198	reverse	TAAACTTCAGGGTGACCAAAAATCA	Folmer <i>et al.</i> , 1994	
	18S_Tar_Ff1	forward	AGGCGAAACCGCGAATGGCTC	Stec <i>et al.</i> , 2017	
	18S_Tar_Rr1	reverse	GCCGCAGGCTCCACTCCTGG	Stec <i>et al.</i> , 2017	
18S rRNA	28S_Eutar_F	forward	ACCCGCTGAACTTAAAGCATAT	Gasiorek <i>et al.</i> , 2018b	Zeller (2010), in Stec <i>et al.</i> , 2015
28S rRNA	28S_R0990	reverse	CCTGGTGCCGTGTTTCAAGAC	Mironov <i>et al.</i> , 2012	Mironov <i>et al.</i> , 2012
	ITS2_Eutar_Ff	forward	CGTAACGTGAATTGCAGGAC	Stec <i>et al.</i> , 2018	Stec <i>et al.</i> , 2018
ITS-2	ITS2_Eutar_Rr	reverse	TGATATGCTTAAAGTTCAGCGG	Stec <i>et al.</i> , 2018	

29 µm mesh size; Tumanov, 2018). The contents of the finer sieve were examined under a Leica M205C stereomicroscope.

MICROSCOPY AND IMAGING. Tardigrades found were fixed with acetic acid or relaxed by incubating live individuals at 60 °C for 30 min (Morek *et al.*, 2016b) and mounted on slides in Hoyer's medium. 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 SEM specimens were thermally relaxed at 60 °C (Morek *et al.*, 2016b), dehydrated in an ascending ethyl alcohol series (10, 20, 30, 50, 70, 96%) and acetone, critical-point dried in CO₂, 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).

GENOTYPING. DNA was extracted from a single tardigrade specimen using QuickExtract™ DNA Extraction Solution (Lucigen Corporation, USA, see complete protocol description in Tumanov, 2020). Four markers were sequenced: fragment of the cytochrome oxidase subunit I (COI) gene, internal transcribed spacer (ITS-2), and fragments of a small ribosome subunit (18S rRNA) gene and a large ribosome subunit (28S rRNA) gene. PCR reactions included 5 µl template DNA, 1 µl of each primer, 1 µl DNTP, 5 µl Taq Buffer (10X) (–Mg), 4 µl 25 mM MgCl₂ and 0.2 µl Taq DNA Polymerase (Thermo Scientific™) in a final volume of 50 µl. The primers and PCR programs used are provided in Table 2. 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) with the help of 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). The COI sequences were translated to amino acids using the invertebrate mitochondrial code, implemented in MEGA11 (Tamura *et al.*, 2021), in order

Table 3. List of gene sequences obtained during the study.
Таблица 3. Список сиквенсов, полученных в ходе настоящего исследования.

Gene	Species	Accession number
COI	<i>Milnesium tardigradum</i>	OP009210, OP009210
	<i>Milnesium berladnicorum</i>	OP009212
	<i>Milnesium dornensis</i>	OP009213
	<i>Adropion scoticum</i>	OP013274
	<i>Mesocrista revelata</i>	OP013275
	<i>Pilatobius</i> cf. <i>bullatus</i>	OP013276, OP013277
	<i>Ramazzottius oberhaeuseri</i>	OP013278–OP013282
	<i>Macrobiotus hufelandi</i>	OP013285, OP013283, OP013284
	<i>Minibiotus</i> cf. <i>intermedius</i>	OP013286–OP013288
	<i>Paramacrobiotus fairbanksi</i>	OP013289–OP013291
18S rRNA	<i>Diphascon</i> cf. <i>pingue</i>	OP035716
	<i>Adropion scoticum</i>	OP035715
	<i>Mesocrista revelata</i>	OP035717
	<i>Pilatobius</i> cf. <i>bullatus</i>	OM304862, OP035720
	<i>Minibiotus</i> cf. <i>intermedius</i>	OP035718, OP035719
28S rRNA	<i>Diphascon</i> cf. <i>pingue</i>	OP035795, OP035796
	<i>Adropion scoticum</i>	OP035794
	<i>Mesocrista revelata</i>	OP035797
	<i>Pilatobius</i> cf. <i>bullatus</i>	OM304869, OP035799
	<i>Minibiotus</i> cf. <i>intermedius</i>	OP035798
ITS-2	<i>Diphascon</i> cf. <i>pingue</i>	OP035703, OP035704, OP035705
	<i>Adropion scoticum</i>	OP037896
	<i>Mesocrista revelata</i>	OP035706
	<i>Ramazzottius oberhaeuseri</i>	OP035714
	<i>Minibiotus</i> cf. <i>intermedius</i>	OP035707, OP035708
	<i>Paramacrobiotus fairbanksi</i>	OP035709–OP035713

to check for the presence of stop codons and therefore of pseudogenes. Complete list of the obtained gene sequences is presented in Table 3.

DATA PROCESSING. Homology comparison of the obtained sequence with GenBank records was performed using Blastn algorithm (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Sequences were aligned using the MAFFT algorithm (Katoh *et al.*, 2002) using AliView version 1.27 (Larsson, 2014). Uncorrected pairwise distances were calculated using MEGA11 with gaps/missing data treatment set to ‘pairwise deletion’.

Results and Discussion

Phylum Tardigrada Doyère, 1840
Class Eutardigrada Richters, 1926
Order Apochela Schuster, Nelson, Grigarick
et Christenberry, 1980

Family Milnesiidae Ramazzotti, 1962
Genus *Milnesium* Doyère, 1840

Milnesium tardigradum Doyère, 1840
Fig. 1.

Location 1, four adult specimens and seven newborns. Morphology of the adult specimens conforms the redescription of Michalczyk *et al.* (2012), newborn specimens morphology in accordance with the description of Morek *et al.* (2019). One specimen with rudimental basal spur on the posterior claw IV (Fig. 1J). Two COI sequences obtained in this study (GenBank Accessing numbers OP009210 and OP009210) were identical. Homology comparison of the obtained sequence with GenBank records (02 May 2022) indicated similarity to the genus *Milnesium*. The most closely related sequence, that of *M. tardigradum* from neotype population (JN664950, Michalczyk *et al.*, 2012), was identical by 99.37%

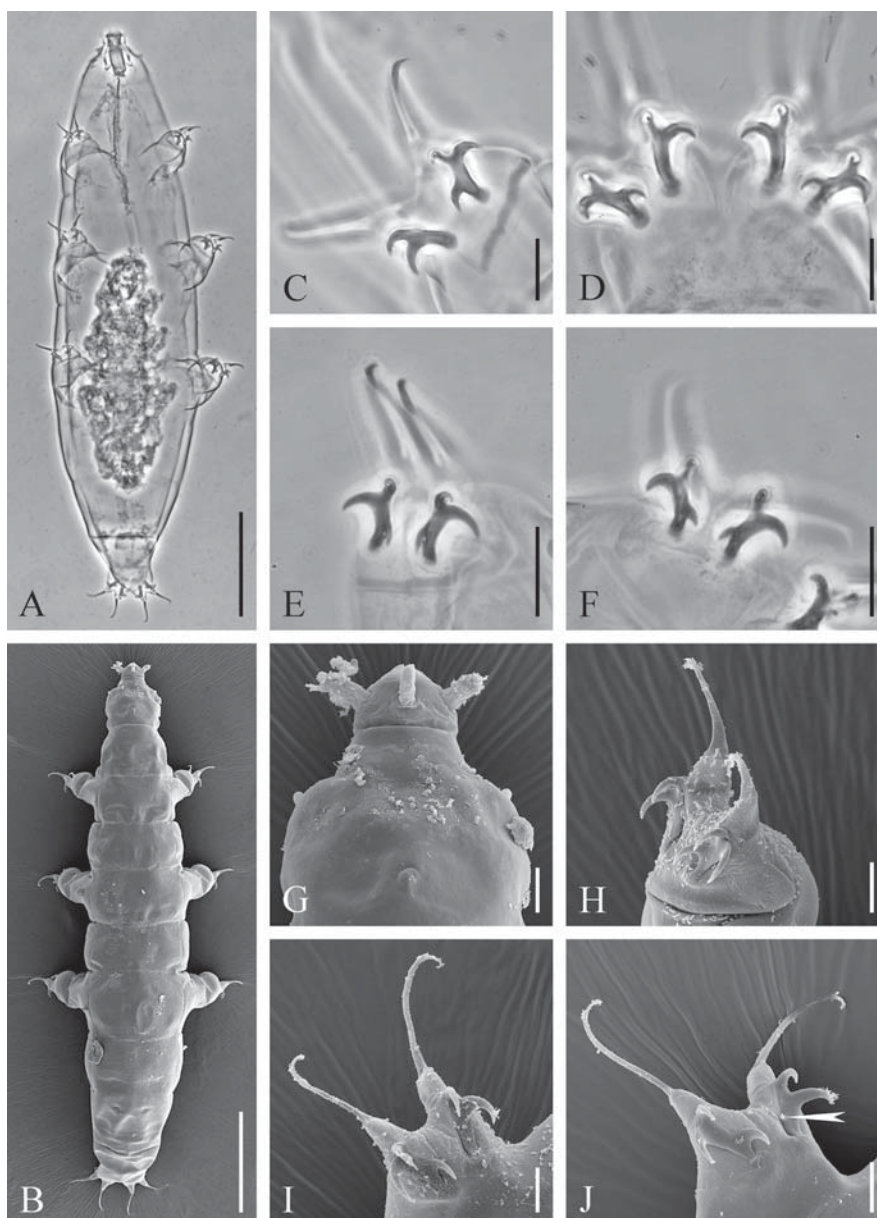


Fig. 1. *Milnesium tardigradum*. A, B — habitus; C — claws of the leg II, adult specimen; D — claws of the leg IV, adult specimen; E — claws of the leg III, hatchling; F — claws of the leg IV, hatchling; G — dorsal view of the head region; H — claws of the leg III, adult specimen; I — claws of the leg IV, adult specimen; J — abnormal claws of the leg IV, adult specimen, arrowhead points to the rudimentary basal spur. Scale bar: A, B — 100 µm; B-E, G-J — 10 µm. A, C-F — phase contrast; B, G-J — SEM.

Рис. 1. *Milnesium tardigradum*. A, B — общий вид; C — коготки ножки II пары, взрослый экземпляр; D — коготки ножки IV пары, взрослый экземпляр; E — коготки ножки III пары, новорожденный; F — коготки ножки IV пары, новорожденный; G — вид со спинной стороны на головной отдел; H — коготки ножки II пары, взрослый экземпляр; I — коготки ножки IV пары, взрослый экземпляр; J — аномальные коготки ножки IV пары, взрослый экземпляр, стрелка указывает на рудиментарную базальную ветвь коготка.

Масштаб: A, B — 100 µm; B-E, G-J — 10 µm. A, C-F — фазовый контраст; B, G-J — СЭМ.

and 99.52% (query coverage was 92% and 99% respectively and E-value was 0.0 for both sequences). The range of uncorrected genetic *p*-distances between the studied specimens of *M. tardigradum* and specimen from neotype population was 0.95%–0.81% (small difference in the *p*-distance value is caused due to the different length of the two obtained sequences).

Milnesium berladnicorum Ciobanu,
Zawierucha, Moglan et Kaczmarek, 2014
Fig. 2A–D.

Location 2, one adult specimen. Morphology of the adult specimens conforms the description of Ciobanu *et al.* (2014), with additions of Morek *et al.* (2016a). Thin reticulation of the dorsal pseudoplates (noted as trait detectible with SEM only by Morek *et al.* (2016a)) is visible in LM (Fig. 2C). The COI sequence was obtained from this single specimen (GenBank Accessing number OP009212). Homology comparison of the obtained sequence with GenBank records (13 May 2022) indicated similarity to the genus *Milnesium*. The most closely related sequence, that of *M. berladnicorum* from type population (KT951659, Morek *et al.*, 2016a), was identical by 100.00% (query coverage was 93% and E-value was 0.0). The range of uncorrected genetic *p*-distances between the studied specimen of *M. berladnicorum* and specimen from type population was 0.00%.

Species new for the fauna of Russia.

Milnesium dornensis Ciobanu, Roszkowska et
Kaczmarek, 2015
Fig. 2E–H.

Location 4, One juvenile specimen in simplex stage. The COI sequence was obtained from this single specimen (GenBank Accessing number OP009213). Homology comparison of the obtained sequence with GenBank records (13 May 2022) indicated similarity to the genus *Milnesium*. The most closely related sequence, that of *M. dornensis* from type population (MG923566, Morek *et al.*, 2019), was identical by 99.63% (query coverage was 90% and E-value was 0.0). The range of uncorrected genetic *p*-distances between the studied specimen of *M. dornensis* and specimen from type population was 0.37%. Thin reticulate pattern on the dorsal side of the body (Fig. 2G) was not mentioned in the original description of Ciobanu *et al.* (2015). That was likely because at the time of its publication the importance of tracking of morphological changes between the ontogenic stages was not revealed for the *Milnesium* taxonomy (Morek, Michalczyk, 2020), and as the result the description of the young stages

was not included in the species description. Presence of the thin reticulum on the dorsal side of the first postembryonic stage (hatchling) is confirmed to be present in *M. dornensis* type population (Łukasz Michalczyk, pers. comm.).

Species new for the fauna of Russia.

***Milnesium* species diversity in Russia**

The genus *Milnesium* is one of the most intensively studied tardigrade genera. Being for the long time accepted as monotypic now it includes 46 described species (Degma *et al.*, 2022) and more than 60 candidate species, which are not formally described but recognized with the genetic analysis. During the pre-genomic period of tardigrade taxonomy only the type species *M. tardigradum* was recognized on the territory of Russia (Biserov, 1991). Currently presence of two species of the genus *Milnesium* is confirmed for the territory of Russia using DNA barcoding. Population of *M. tardigradum* was recovered from the vicinity of Lake Baikal (Morek, Michalczyk, 2020) and the same species together with *M. inceptum* was recovered from Russkij Island (Vladivostok city) (Maskin *et al.*, 2021).

Our investigation for the first time revealed the presence of *M. tardigradum* in the European part of Russia. It should be noted that the haplotype found by us is most similar to the European cluster of populations (clades $\alpha+\beta$ in Morek *et al.*, 2019), while the Asian populations of *M. tardigradum* investigated by Sugiura *et al.* (2020), Morek and Michalczyk (2020), and Maskin *et al.* (2021) forms a clearly separate clade (clade γ in Morek *et al.*, 2019). The obtained phylogeographic pattern lead us to the conclusion that the hypothesis of unintentional introduction of *M. tardigradum* to the Far East from the European part of Russia should be rejected. On the contrary, presence of the haplotypes belonging to the γ -clade in Poland and Hungary (Morek *et al.*, 2019) can be an evidence of the backward introduction process.

Within the two other species found in our investigation, *M. berladnicorum* is known from several European localities (Romania, type locality, Ciobanu *et al.*, 2014; Ukraine, Morek *et al.*, 2021; Slovakia, Guil *et al.*, 2022) and from the Republic of South Africa, where it is probably a result of anthropogenic dispersal (Morek *et al.*, 2021). The second species – *M. dornensis* is known from Europe (Romania, type locality, Ciobanu *et al.*, 2015; Spain, Guil *et al.*, 2022) and possibly from Canary Islands (Morek, Michalczyk, 2020; Guil *et al.*, 2022).

New findings double the number of *Milnesium* species known for Russia, but it is for sure only the beginning. Taking into account records in Maskin *et al.* (2021) and Guil *et al.* (2022) at least three yet

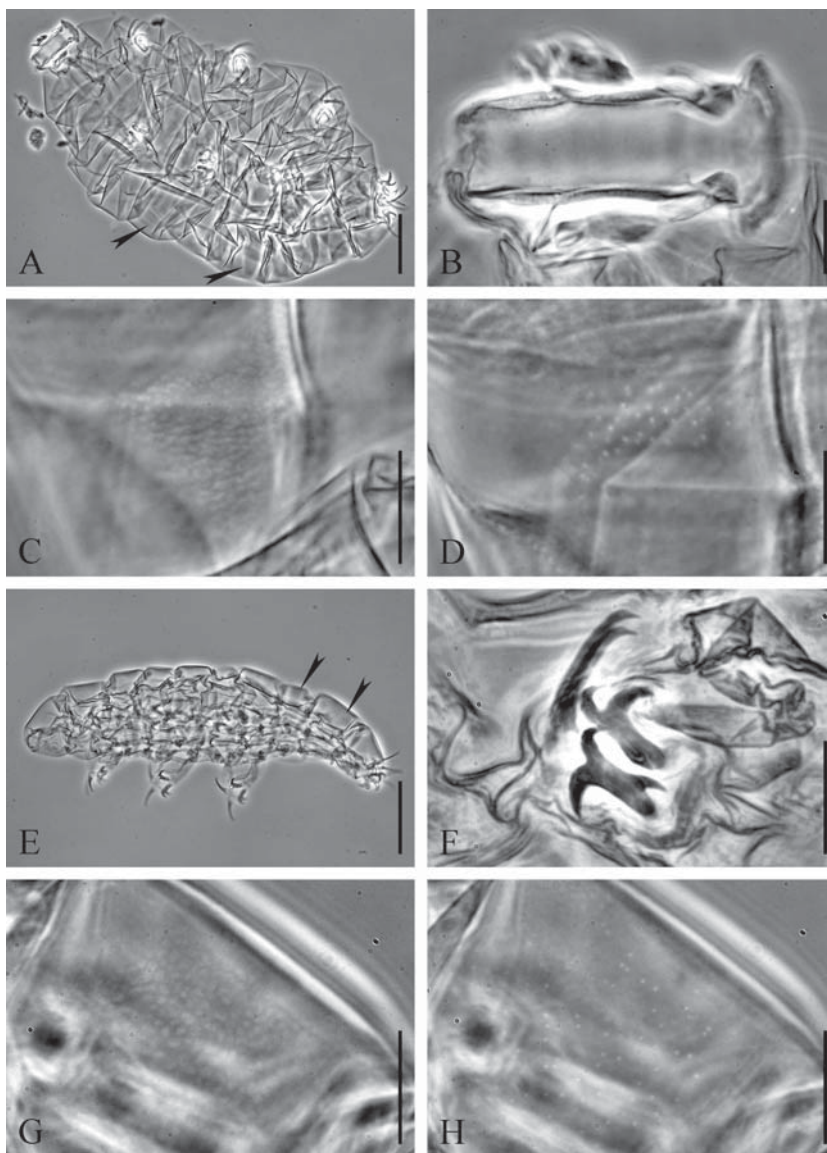


Fig. 2. *Milnesium berladnicorum* and *Milnesium dornensis*. A–D: *M. berladnicorum*. A — total view of the cuticular parts after DNA extraction, arrowheads point to the dorsal pseudoplates; B — buccal-pharyngeal apparatus; C — reticular sculpture on the pseudoplate surface; D — pseudopores in the dorsal pseudoplate. E–H: *M. dornensis*. E — total view of the cuticular parts after DNA extraction, arrowheads point to the dorsal pseudoplates; F — claws of the leg I; G — reticular sculpture on the pseudoplate surface; H — pseudopores in the dorsal pseudoplate. Arrowheads points to the dorsal pseudoplates. Scale bar: A, E — 50 μ m; B–D, F–H — 10 μ m. A–H — phase contrast.

Рис. 2. *Milnesium berladnicorum* и *Milnesium dornensis*. A–D: *M. berladnicorum*. A — общий вид кутикулярных структур после экстракции ДНК; B — рото-глоточный аппарат; C — сетчатая скульптура спинной псевдопластинки; D — псевдопоры спинной псевдопластинки. E–H: *M. dornensis*. E — общий вид кутикулярных структур после экстракции ДНК; F — коготки ножки I пары; G — сетчатая скульптура спинной псевдопластинки; H — псевдопоры спинной псевдопластинки. Стрелки указывают на спинные псевдопластинки.

Масштаб: A, E — 50 μ m; B–D, F–H — 10 μ m. A–H — фазовый контраст.

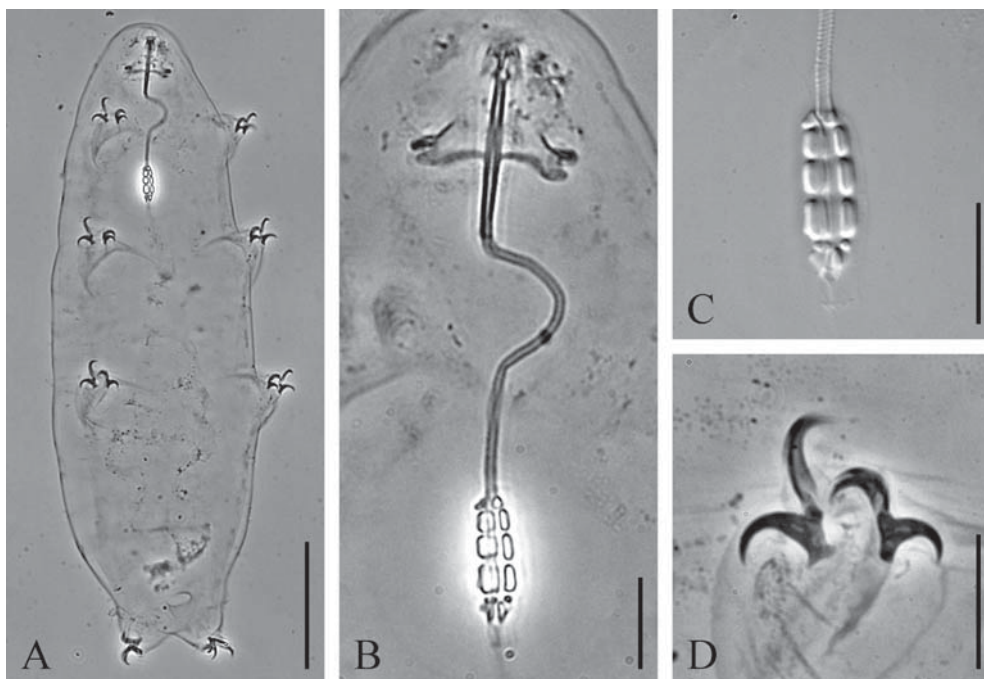


Fig. 3. *Diphascon* cf. *pingue*. A — habitus; B — buccal-pharyngeal apparatus; C — placoids; D — claws of the leg III.

Scale bar: A — 50 µm; B–D — 10 µm. A, B, D — phase contrast; C — differential interference contrast.

Рис. 3. *Diphascon* cf. *pingue*. A — общий вид; B — рото-глоточный аппарат; C — плакоиды; D — коготки ножки III пары.

Масштаб: A — 50 µm; B–D — 10 µm. A, B, D — фазовый контраст; C — дифференциальный интерференционный контраст.

undescribed species of this genus are present in the Russian Far East. Also our investigations revealed presence of two undescribed species in the European part of Russia (unpublished data).

Order Parachela Schuster, Nelson, Grigarick
et Christenberry, 1980

Superfamily Hypsibioidae Pilato, 1969

Family Hypsibiidae Pilato, 1969

Subfamily Diphasconinae Dastych, 1992

Genus *Diphascon* Plate, 1888

Diphascon cf. *pingue* (Marcus, 1936)

Fig. 3.

Location 3, 15 adult specimens and six exuvia containing eggs. Morphology of the adult specimen conforms the description of Pilato and Binda (1999). DNA sequences were obtained for 18S rRNA gene fragment (four specimens, GenBank OP035716), 28S rRNA gene fragment (three specimens, GenBank OP035795, OP035796), ITS-2 (three speci-

mens, GenBank OP035703, OP035704, OP035705), and COI gene fragment (four specimens, GenBank OP013273). All 18S and COI sequences belong to one haplotype, while the analysis of 28S and ITS-2 sequences revealed the presence of two haplotypes within the studied population. Homology comparison of the obtained sequences with GenBank records (15 May 2022) indicated similarity to the genus *Diphascon* (see Supplementary Table 1, except for the ITS-2 marker, because no *Diphascon* sequences were available). The range of uncorrected genetic *p*-distances between the studied specimens of *D. cf. pingue* and sequences of the genus *Diphascon* available in GenBank are presented in Supplementary Table 2.

***Diphascon pingue* taxonomic status in Europe**

Species with three elongated macroplacoids, microplacoids and septulum, smooth cuticle and legs without additional cuticular structures associated with claws (lunules or cuticular bars) constitute the so-called *pingue*-group within the genus *Diphascon* (Fontoura, Pilato, 2007). It is named by the first

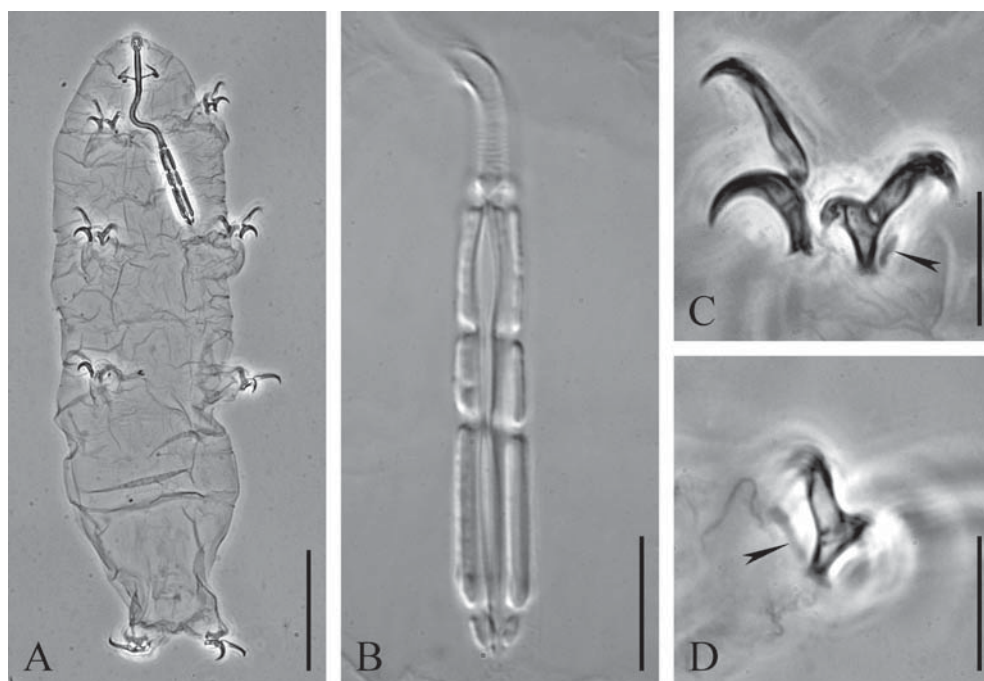


Fig. 4. *Adropion scoticum*. A — total view of the cuticular parts after DNA extraction; B — placoids; C — claws of the leg II; D — claws of the leg III. Arrowheads point to cuticular bar near internal claw base. Scale bar: A — 50 μ m; B–D — 10 μ m. A, C, D — phase contrast; B — differential interference contrast.

Рис. 4. *Adropion scoticum*. A — общий вид кутикулярных структур после экстракции ДНК; B — плакоиды; C — коготки ножки II пары; D — коготки ножки III пары. Стрелки указывают на кутикулярные полоски у основания коготков.

Масштаб: A — 50 μ m; B–D — 10 μ m. A, C, D — фазовый контраст; B — дифференциальный интерференционный контраст.

species of the group — *Diphascon pingue* (Marcus, 1936) which was described from Germany (Harz mountains). It is considered to be homogeneous group of species very difficult to separate (Pilato, Binda, 1997/98, 1999). Nowadays 12 species of this group are described, most of them known from the South Hemisphere (Antarctic region and South Africa). Only one recently described species (*Diphascon faialense* Fontoura et Pilato, 2007) is known from Azores. All other Palaearctic specimens conforming the *pingue*-group diagnosis until now were usually attributed to as *D. pingue*. No integrative investigations of this species including complete morphometric analyses and analyses of the genetic data were performed for this species. The only population of this species available for the genetic comparison derives from Spain (Guil, Giribet, 2012) and shows a significant difference from the studied population (see Supplementary Table 2). Uncorrected genetic *p*-distance for COI marker between the Spanish population and the population from Lembo-
lovo exceed 21%, which in our opinion is an evi-

dence for the presence of at least two different species of *Diphascon pingue*-group species in Europe. Unfortunately no morphological data were provided for the Spanish population. In the absence of the modern integrative redescription of *D. pingue* from the type locality correct attribution of the studied material is not possible.

Subfamily: Itaquasconinae Bartoš
in Rudescu, 1964
Genus *Adropion* Pilato, 1987

Adropion scoticum (Murray, 1905)
Fig. 4.

Location 3, one adult specimen. Morphology of the adult specimen conforms the description of Dastych (1988). DNA sequences were obtained for 18S rRNA (GenBank OP035715), 28S rRNA (GenBank OP035794), ITS-2 (GenBank OP037896), and COI (GenBank OP013274) gene fragments. Homol-

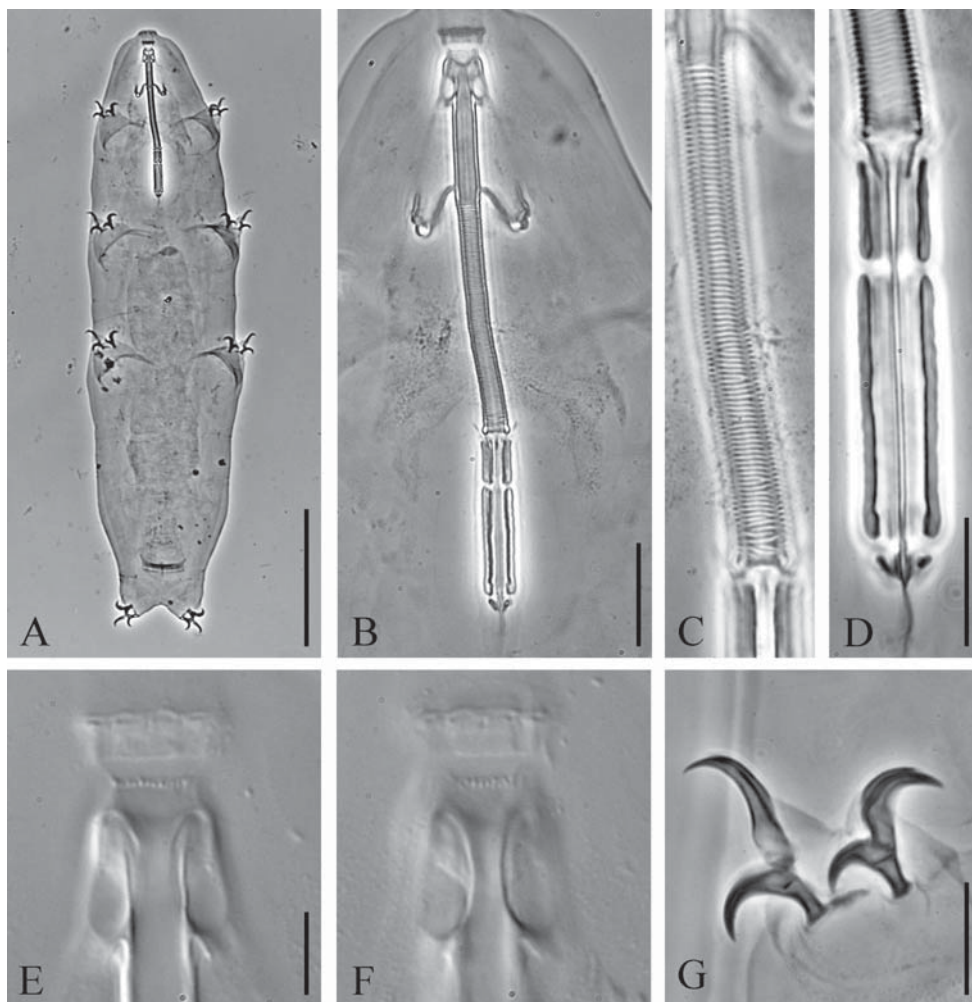


Fig. 5. *Mesocrista revelata*. A — habitus; B — buccal-pharyngeal apparatus; C — pharyngeal tube; D — placoids; E — oral cavity armature, dorsal; F — oral cavity armature, ventral; G — claws of the leg II. Scale bar: A — 100 µm; B — 20 µm; C, D, G — 10 µm; E, F — 5 µm. A–D, G — phase contrast; E, F — differential interference contrast.

Рис. 5. *Mesocrista revelata*. A — общий вид; B — рото-глоточный аппарат; C — глоточная трубка; D — плакоиды; E — ротовая арматура, дорсально; F — ротовая арматура, вентрально; G — коготки ножки II пары.

Масштаб: A — 100 µm; B — 20 µm; C, D, G — 10 µm; E, F — 5 µm. A–D, G — фазовый контраст; E, F — дифференциальный интерференционный контраст.

ogy comparison of the obtained sequences with GenBank records (13 June 2022) indicated similarity to the genus *Adropion* (see Supplementary Table 3, except for the ITS-2 marker, because no *Adropion* sequences were available). The range of uncorrected genetic *p*-distances between the studied specimens of *A. scoticum* and sequences of the genus *Adropion* available in GenBank are presented in Supplementary Table 4.

This species is considered as common and widely distributed in the European part of Russia, predominantly north to the 56° parallel (Biserov, 1991). Until now there is no integrative description of this old species, but small genetic *p*-distance (0.70–1.94% for COI marker) between the studied specimen and specimens from Scotland, the type locality of the species (Gąsiorek, Michalczyk, 2020) makes it possible to assume that our record is the first

genetically supported evidence of the presence of this species in Russia. On the other hand the presence of yet another undescribed *Adropion* species in Poland and Norway (Gąsiorek, Michalczyk, 2020) can be an evidence for the great hidden diversity within this morpho-species.

Genus *Mesocrista* Pilato, 1987

Mesocrista revelata Gąsiorek, Stec, Morek, Zawierucha, Kaczmarek, Lachowska-Cierlik et Michalczyk, 2016

Fig. 5.

Location 3, two adult specimens. Morphology of the adult specimen conforms the description of Gąsiorek *et al.* (2016). DNA sequences were obtained from one specimen for 18S rRNA (GenBank OP035717), 28S rRNA (GenBank OP035797), ITS-2 (GenBank OP035706), and COI (GenBank OP013275) gene fragments. Homology comparison of the obtained sequences with GenBank records (13 June 2022) indicated similarity to the genus *Mesocrista* (see Supplementary Table 5). The range of uncorrected genetic *p*-distances between the studied specimens of *M. revelata* and sequences of the genus *Mesocrista* available in GenBank are presented in Supplementary Table 6.

Species new for the fauna of Russia. For a long time after its separation from the genus *Diphascion* (Pilato, 1987) *Mesocrista spitzbergensis* (Richters, 1903) was considered as a single widely distributed species. In European Russia it was noted as present “Everywhere north of Moscow, south in Chuvashia only” (Biserov, 1991) and also it was noted for Taimyr peninsula (Biserov, 1996). Recently Gąsiorek *et al.* (2016) gave an integrative redescription of this species from its type locality (Spitsbergen) and revealed presence of a second species *M. revelata* in European fauna. These two species are poorly differentiated in their morphology, but distant in the genetic analysis. Till now *M. spitzbergensis* known to be present on Spitzbergen only, while *M. revelata* was found in Poland and continental Norway (Gąsiorek *et al.*, 2016; Kaczmarek *et al.*, 2018a). Our record is the third finding of *M. revelata* in Europe. In the light of these data all of the old records of *M. spitzbergensis* should be considered doubtful and need to be verified.

Subfamily Pilatobiinae Bertolani, Guidetti, Marchioro, Altiero, Rebecchi et Cesari, 2014

Genus *Pilatobius* Bertolani, Guidetti, Marchioro, Altiero, Rebecchi et Cesari, 2014

Pilatobius cf. bullatus (Murray, 1905)

Fig. 6.

Location 5, 32 adult specimens and two exuvia containing eggs on slides, 4 specimens on SEM stubs. Morphology of the adult specimen conforms the description of Murray (1905). Nine rows of poorly developed tubercles are present on the dorsal side of the animals (Fig. 6B, C, E), but the anterior most tubercles are usually poorly visible or indiscernible in light microscopy, being observed in SEM only. Another character which was not noted for this species previously is the presence of cuticular bars on legs IV (Fig. 6I). One short bar is present between the claws, the second bar is developed near the base of the anterior claw, and the third additional bar is located at right angles to the first bar. The last bar is clearly visible only in some specimens. It is also interesting to note that egg shell of this species possesses thin punctation, similar to the previously described for *P. recamieri* (Tumanov, 2020). This observation supports the hypothesis that this type of eggs chorion is typical for the genus *Pilatobius*, and thus is the only morphological character that unites *Pilatobius* and *Notahypsibius*, the only two genera of the Pilatobiinae subfamily (see discussion in Tumanov, 2020). DNA sequences for two specimens were obtained for 18S rRNA (GenBank OM304862, OP035720), 28S rRNA (GenBank OM304869, OP035799), and COI (GenBank OP013276, OP013277) gene fragments. Homology comparison of the obtained sequences with GenBank records (14 June 2022) indicated similarity to the genus *Pilatobius* (see Supplementary Table 7). The range of uncorrected genetic *p*-distances between the studied specimens of *Pilatobius cf. bullatus* and sequences of the genus *Pilatobius* available in GenBank are presented in Supplementary Table 8.

This species was previously noted (as *Diphascion*) for Karelia, the Ural, Leningrad and Yaroslavl district (Biserov, 1991) and Putoran Mts. region (Biserov, 1996), on the base of morphological identification. At the present state the taxonomic status of all of these records and of the material obtained during our investigation cannot be considered definitive. There is a confusion regarding the identity of the *Pilatobius* species with paired dorsal gibbosities (see Biserov, 1996) i.e. *P. bisbullatus* (Iharos, 1964), *P. bullatus* (Murray, 1905), *P. patanei* (Binda et Pilato, 1971), *P. elongatus* (Mihelčič, 1959), *P. nonbullatus* (Mihelčič, 1951), and *P. trachydorsatus* (Bartoš, 1937), latter three species are now considered as *nomina dubia* (Degma *et al.*, 2022). Descriptions of all of these species are outdated and do not

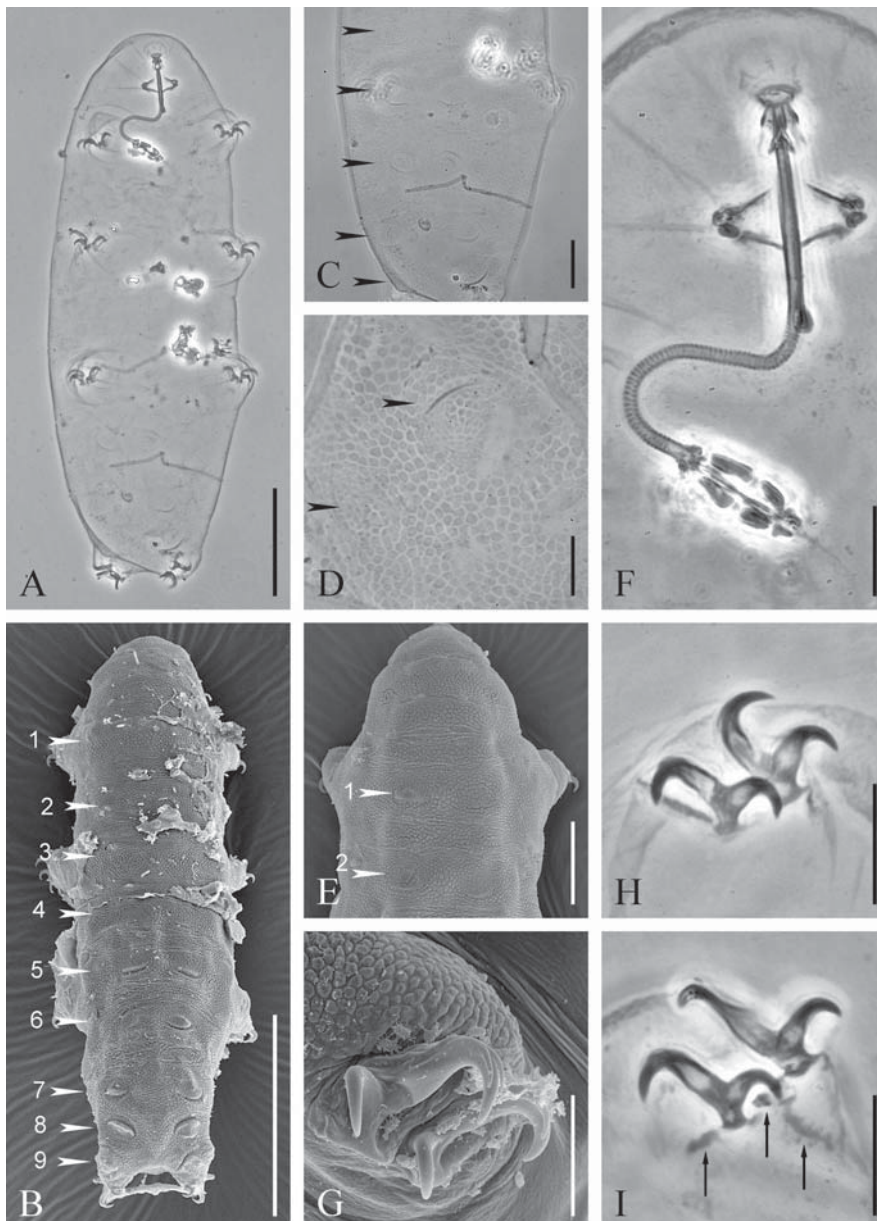


Fig. 6. *Pilatobius* cf. *bullatus*. A, B — habitus; C — caudal part of the dorsal surface; D — dorsal sculpture; E — anterior part of the dorsal surface; F — buccal-pharyngeal apparatus; G, H — claws of the leg II; I — claws of the leg IV. Arrowheads point to dorsal tubercles, numbers indicate the tubercles rows; arrows point to cuticular bars at the claw bases.

Scale bar: A, B — 50 μ m; C, E — 20 μ m; D, F, H — 10 μ m; G — 5 μ m. A, C, D, F, H, I — phase contrast; D, E, G — SEM.

Рис. 6. *Pilatobius* cf. *bullatus*. A, B — общий вид; C — каудальная часть дорсальной поверхности; D — дорсальная скульптура; E — передняя часть дорсальной поверхности; F — рото-глоточный аппарат; G, H — коготки ножек II пары; I — коготки ножки IV пары. Наконечники стрелок указывают на дорсальные бугорки, цифрами обозначены ряды бугорков; стрелки указывают на кутикулярные полоски у основания коготков.

Scale bar: A, B — 50 μ m; C, E — 20 μ m; D, F, H — 10 μ m; G — 5 μ m. A, C, D, F, H, I — фазовый контраст; D, E, G — СЭМ.

meet modern standards. Moreover the attempts to amend the descriptions and discriminative characters for those species were made on the base of material derived from localities distant from the type localities (Argue, 1974; Binda, Pilato, 1987) and cannot be accepted as reliable. The only DNA sequences available in GenBank belongs to *P. patanei* and represent two fragments of 18S rRNA (Bertolani *et al.*, 2014). Comparison of these sequences with the data obtained in our work revealed genetic distance (0.92–1.06%) which is comparable to the distance between some other *Pilatobius* species (see Supplementary Table 5), for example the distance for 18S rRNA sequences between *P. nodulosum* and *P. nuominensis* is 1.04% and the distance between *P. islandicus*, *P. glacialis*, and *P. recamieri* is 0.24%. In our opinion this is an evidence of the presence of at least two different species within *bullatus*-morphogroup, one of them is *P. patanei* and the second is tentatively recognised here as *Pilatobius* cf. *bullatus*. Without doubts only the integrative redescription of *P. bullatus* from the type locality will give a possibility to solve this problem.

Family Ramazzottiidae Sands, McInnes,
Marley, Goodall-Copestake, Convey
et Linse, 2008

Genus *Ramazzottius* Binda et Pilato, 1986

Ramazzottius oberhaeuseri (Doyère, 1840)
Fig. 7.

Location 1, 88 adult specimens and 16 eggs on slides, five specimens and two eggs on SEM stubs. Morphology of the adult specimen conforms the redescription of Stec *et al.* (2018), with only difference that the poorly developed reticular sculpture in our material is better visible in the cephalic region of the dorsal surface (Fig. 7B). DNA sequences were obtained for COI (five specimens GenBank OP013278–OP013282), and ITS-2 (one specimen GenBank OP035714) gene fragments. Homology comparison of the obtained sequences with GenBank records (17 June 2022) indicated similarity to the genus *Ramazzottius*. The most closely related COI sequence, that of *R. oberhaeuseri* from neotype population (MG573244, Stec *et al.*, 2018), was identical by 99.54% (query coverage was 94% respectively and E-value was 0.0). The most closely related ITS-2 sequence, that of *R. oberhaeuseri* from neotype population (MG573243, Stec *et al.*, 2018), was identical by 97.59% (query coverage was 92% respectively and E-value was 6e-154). Uncorrected genetic *p*-distance value for the studied specimens of *R. oberhaeuseri* and specimen from neotype population was 0.61% for COI and 3.47% for ITS-2.

Ramazzottius oberhaeuseri is one of the oldest described tardigrade species. For the long time it was considered as the single widely distributed species of the *oberhaeuseri*-group which is characterized by the presence of hemispherical egg processes, in contrast to the conical egg processes in other *Ramazzottius* species. The other two morphologically identified species of *oberhaeuseri*-group (*R. thulini* (Pilato, 1970) and *R. libycus* Pilato, D'Urso et Lisi, 2013) are known from type locations in Italy and Libya only. Presence of a hidden species diversity was supposed for this group of species (Pilato *et al.*, 2013), but has not been proven until the publication of Stec *et al.* (2018). In this work the presence of cryptic species within the morpho-species *R. oberhaeuseri* was revealed using the methods of genetic analysis, one of the genetically delimited species (derived from the type locality) was redescribed as a new type material, while seven other genetic entities (known from France, Germany, Italy, Poland, Portugal, Spain, Sweden, and Switzerland) until now are undescribed cryptic species. Recently, a new species of this group (*R. kretschmanni* Guidetti, Cesari, Giovannini, Ebel, Förschler et Schill, 2022) was described from Germany (Guidetti *et al.*, 2022).

Biserov (1991) noted *R. oberhaeuseri* as “a very common species”, known for the territory of Russia from Arkhangelsk region to Daghestan, but taking into account the cryptic speciation in this species complex it is not possible to be sure that all Biserov's records represent *R. oberhaeuseri* s.str. Our investigation for the first time revealed the presence of *R. oberhaeuseri* in the fauna of Russia using the method of DNA-barcoding.

Superfamily Macrobiotioidea Thulin, 1928

Family Macrobiotidae Thulin, 1928

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

Macrobiotus hufelandi C.A.S. Schultze, 1834
Fig. 8.

Locations 3 and 4, 195 adult specimens and 38 eggs on slides, 15 specimens and seven eggs on SEM stubs. Morphology of the adult specimen conforms the redescription of Bertolani & Rebecchi (1993). DNA sequences were obtained for COI (three specimens GenBank OP013285, OP013283, OP013284) gene fragment. Homology comparison of the obtained COI sequences with GenBank records (18 June 2022) indicated similarity to the genus *Macrobiotus*. The most closely related COI sequences, that of *M. hufelandi* from neotype population (HQ 876584–HQ876588, Bertolani *et al.*, 2011) were identical by 99.68–99.84% (query coverage was 83–87% and E-value was 0.0%). Uncorrected genetic *p*-distance value for COI marker of the studied speci-

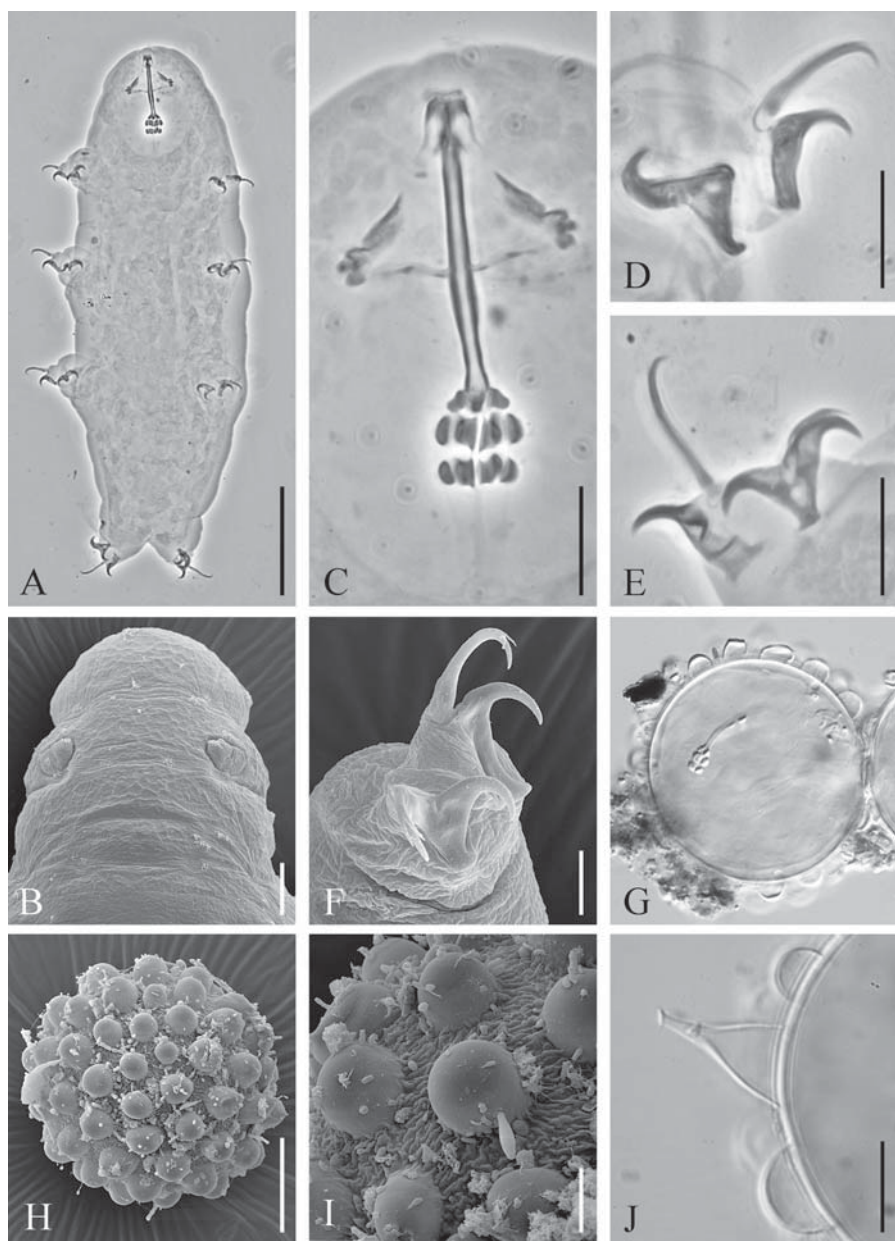


Fig. 7. *Ramazzottius oberhaeuseri*. A — habitus; B — anterior part of the dorsal surface with reticular sculpture; C — buccal-pharyngeal apparatus; D — claws of the leg II; E — claws of the leg IV; F — claws of the leg III; G — optical section through embrionated egg; H — egg; I — egg surface; J — egg shell processes. Scale bar: A — 50 μ m; G, H — 20 μ m; B–E, J — 10 μ m; F, I — 5 μ m. A, C, D, E — phase contrast; G, J — differential interference contrast; B, F, H, I — SEM.

Рис. 7. *Ramazzottius oberhaeuseri*. А — общий вид; В — передняя часть спинной поверхности с ретикулярной скульптурой; С — рото-глоточный аппарат; D — коготки ножки II пары; E — коготки ножки IV пары; F — коготки ножки III пары; G — оптический срез яйца с развитым эмбрионом; H — яйцо; I — поверхность яйца; J — выросты хориона яйца.

Масштаб: А — 50 μ m; G, H — 20 μ m; B–E, J — 10 μ m; F, I — 5 μ m. А, С, D, E — фазовый контраст; G, J — дифференциальный интерференционный контраст; B, F, H, I — СЭМ.

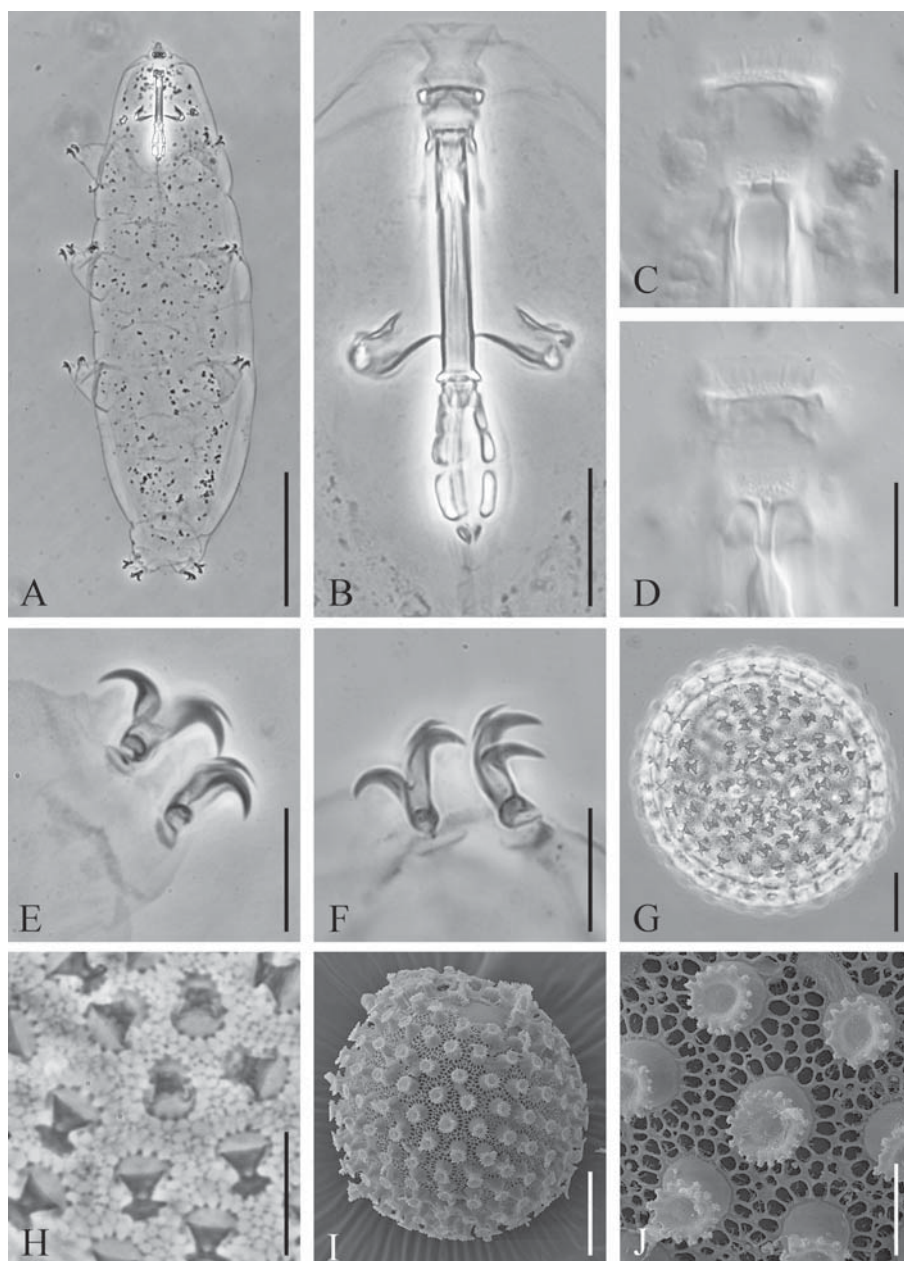


Fig. 8. *Macrobiotus hufelandi*. A — habitus; B — buccal-pharyngeal apparatus; C — oral cavity armature, dorsal; D — oral cavity armature, ventral; E — claws of the leg III; F — claws of the leg IV; G — egg; H — egg surface; I — egg; J — egg surface.

Scale bar: A — 100 μ m; G, I — 20 μ m; C–F, H — 10 μ m; J — 5 μ m. A, B, E–H — phase contrast; C, D — differential interference contrast; I, J — SEM.

Рис. 8. *Macrobiotus hufelandi*. A — общий вид; B — рото-глоточный аппарат; C — ротовая арматура, дорсально; D — ротовая арматура, вентрально; E — коготки ножки III пары; F — коготки ножки IV пары; G — яйцо; H — поверхность яйца; I — яйцо; J — поверхность яйца.

Масштаб: A — 100 μ m; G, I — 20 μ m; C–F, H — 10 μ m; J — 5 μ m. A, B, E–H — фазовый контраст; C, D — дифференциальный интерференционный контраст; I, J — СЭМ.

mens of *M. hufelandi* and specimens from neotype population was 0.16–0.34% for COI.

Being the first described tardigrade species *M. hufelandi* has a long and complicated history of findings. Morphological redescription of this species (Bertolani, Rebecchi, 1993) and receiving of genetic data for this species (Bertolani *et al.*, 2011) gave a start to the investigation of the real diversity of this group of species. Now *M. hufelandi* morpho-group is accepted as polyphyletic clade within monophyletic genus *Macrobiotus*, with variable morphology of the adult animals and egg chorion (Stec *et al.*, 2021a). It was shown that closely related and poorly differentiated species of this group can often be found in the same sample (Bertolani, Rebecchi, 1993; Bertolani *et al.*, 2011), and moreover there are cryptic species morphologically identical to *M. hufelandi*, but genetically different (Bertolani *et al.*, 2011).

Macrobiotus hufelandi was noted by Biserov (1991) a “the most common tardigrade” of the European Russia. But even in this work of pre-genomic period of tardigradology he supposed that many of older records of this species can be considered now as belonging to other species of *M. hufelandi* morpho-group. In the light of our current knowledge we must agree with this statement. Our finding is the first record of *M. hufelandi* s.str. for the territory of Russia supported with the DNA-barcode data.

Genus *Minibiotus* R.O. Schuster, 1980

Minibiotus cf. *intermedius* (Plate, 1888)

Fig. 9.

Location 3, eight adult specimens and one egg on slides. Morphology of the adult specimen conforms the redescription of Kaczmarek *et al.* (2022). DNA sequences were obtained for COI (three specimens GenBank OP013286–OP013288), 18S rRNA (two specimens GenBank OP035718, OP035719), 28S rRNA (one specimen GenBank OP035798), and ITS-2 (two specimens GenBank OP035707, OP035708) gene fragments. No genetic polymorphism within the population studied. Homology comparison of the obtained sequences with GenBank records (15 July 2022) indicated similarity to the genus *Minibiotus* (see Supplementary Table 9). The range of uncorrected genetic *p*-distances between the studied specimens of *Minibiotus* cf. *intermedius* and sequences of the genus *Minibiotus* available in GenBank are presented in Supplementary Table 10.

Minibiotus intermedius is an old species, described in XIX century as a species of the genus *Macrobiotus* (Plate, 1888). The description was short and poorly detailed, as it was usual for this period, but peculiar morphology of the buccal-pharyngeal apparatus and egg chorion make this species

easily recognizable and it was recorded from multiply locations all over the world (McInnes, 1994). Although the genus *Minibiotus* was separated from the genus *Macrobiotus* based on the morphological characteristics of *M. intermedius* (Schuster *et al.*, 1980) the diagnosis of this genus, its composition, and its possible polyphyletic nature are still the subject of a long-lasting discussion (Binda, Pilato, 1992; Claxton, 1998; Guidetti *et al.*, 2007; Stec *et al.*, 2015, 2020a, 2021a).

In Russia *M. intermedius* is considered to be widely distributed, with locality being noted as “everywhere” by Biserov (1991). Recently an integrative redescription of *M. intermedius* was published (Kaczmarek *et al.*, 2022), based on the material from the type locality (Germany). The comparison of gene sequences obtained in our investigation with sequences of neotype population revealed close similarity in conservative genes (*p*-distances: 0.0% for 18S rRNA and 0.13–0.81% for 28S rRNA). On the other hand the COI gene fragments demonstrate a larger *p*-distance value (14.20–14.54%) which should be considered as an evidence of the presence of yet undescribed cryptic or semicryptic *Minibiotus* species in the Russian fauna. Small number of specimens preclude the correct morphometric comparison of the populations. Additional material should be collected and analyzed to resolve this question. In our opinion all records of *M. intermedius* for the territory of Russia should be considered as doubtful, until the confirmation from genetic data is received.

Genus *Paramacrobiotus* Guidetti, Schill, Bertolani, Dandekar et Wolf, 2009

Paramacrobiotus fairbanksi Schill, Förster, Dandekar et Wolf, 2010

Fig. 10.

Location 5, 17 adult specimens and 10 eggs on slides, six specimens and three eggs on SEM stubs. Morphology of the adult specimen conforms the redescription of Guidetti *et al.* (2019). DNA sequences were obtained for COI (three specimens, GenBank OP013289–OP013291), and ITS-2 (five specimens, GenBank OP035709–OP035713) gene fragments. Homology comparison of the obtained sequences with GenBank records (15 July 2022) indicated similarity to the genus *Paramacrobiotus*. The most closely related COI sequence, that of *P. fairbanksi* from Poland (MH676011, Stec *et al.*, 2020c), was identical by 99.84% (query coverage was 98% respectively and E-value was 0.0). The most closely related ITS-2 sequence, that of *P. fairbanksi* from type population in Alaska (GQ 403678, Schill *et al.*, 2010), was identical by 100% (query coverage was 73% respectively and E-value was 7e-

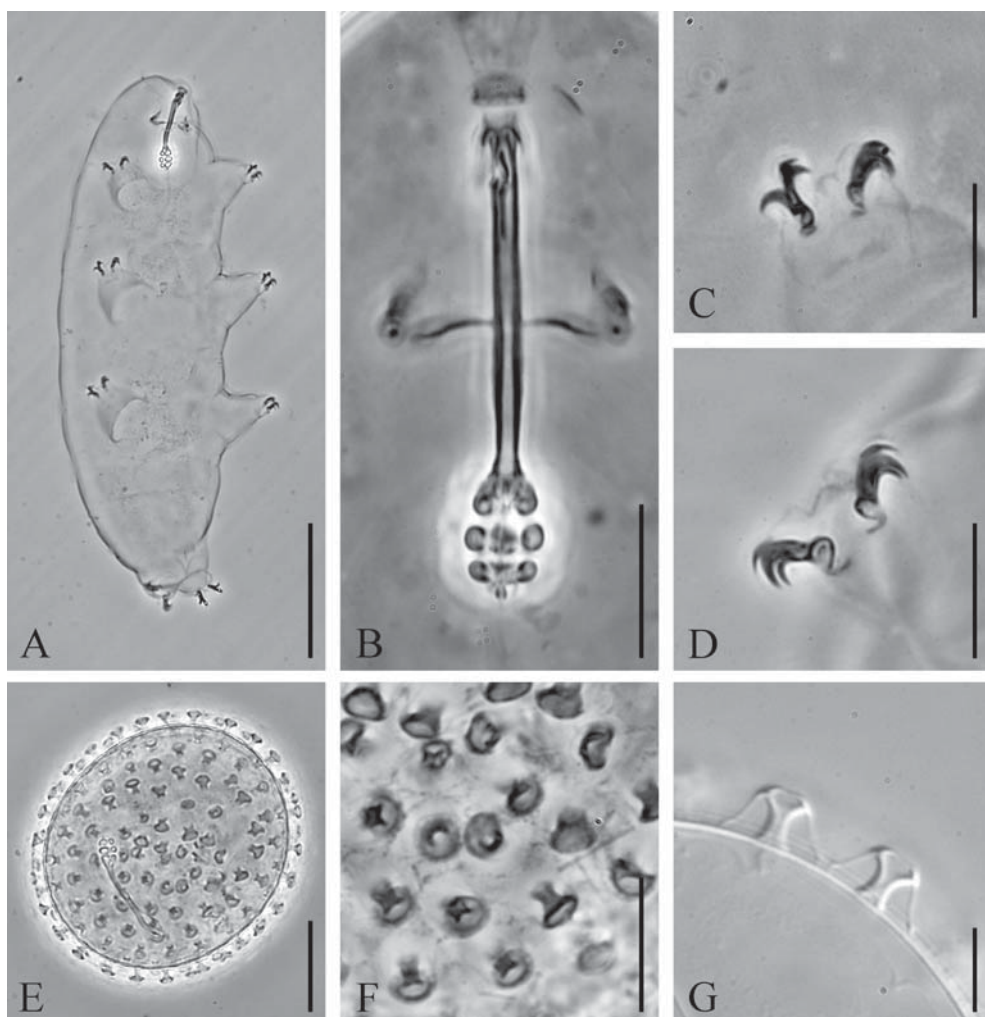


Fig. 9. *Minibiotus* cf. *intermedius*. A — habitus; B — buccal-pharyngeal apparatus; C — claws of the leg II; D — claws of the leg IV; E — egg; F — egg surface; G — egg processes.

Scale bar: A — 50 µm; E — 20 µm; B–D, F — 10 µm; G — 5 µm. A–F — phase contrast; G — differential interference contrast.

Рис. 9. *Minibiotus* cf. *intermedius*. A — общий вид; B — рото-глоточный аппарат; C — коготки ножки II пары; D — коготки ножки IV пары; E — яйцо; F — поверхность яйца; G — выросты хориона яйца. Масштаб: A — 50 µm; E — 20 µm; B–D, F — 10 µm; G — 5 µm. A–F — фазовый контраст; G — дифференциальный интерференционный контраст.

174). Uncorrected genetic *p*-distance values for the studied specimens of *P. fairbanksi* and specimens from populations from Europe, Alaska and Antarctica were 0.00–0.32% for COI and 0.00–0.29% for ITS-2 (see Supplementary Table 11).

Species new for the fauna of Russia. For a long time species *Paramacrobiotus richtersi* (Murray, 1911) described initially as a species of the genus

Macrobiotus and later transferred to the new genus *Paramacrobiotus* by Guidetti *et al.* (2009) was considered as widely distributed and extremely polymorphous species (Ramazzotti, Maucci, 1983; McInness, 1994). Recently Guidetti *et al.* (2019) re-described this species using material from the type locality and revealed the presence of a big complex of cryptic and semicryptic species within *P. richtersi* morpho-species. Our material represents a most wide-

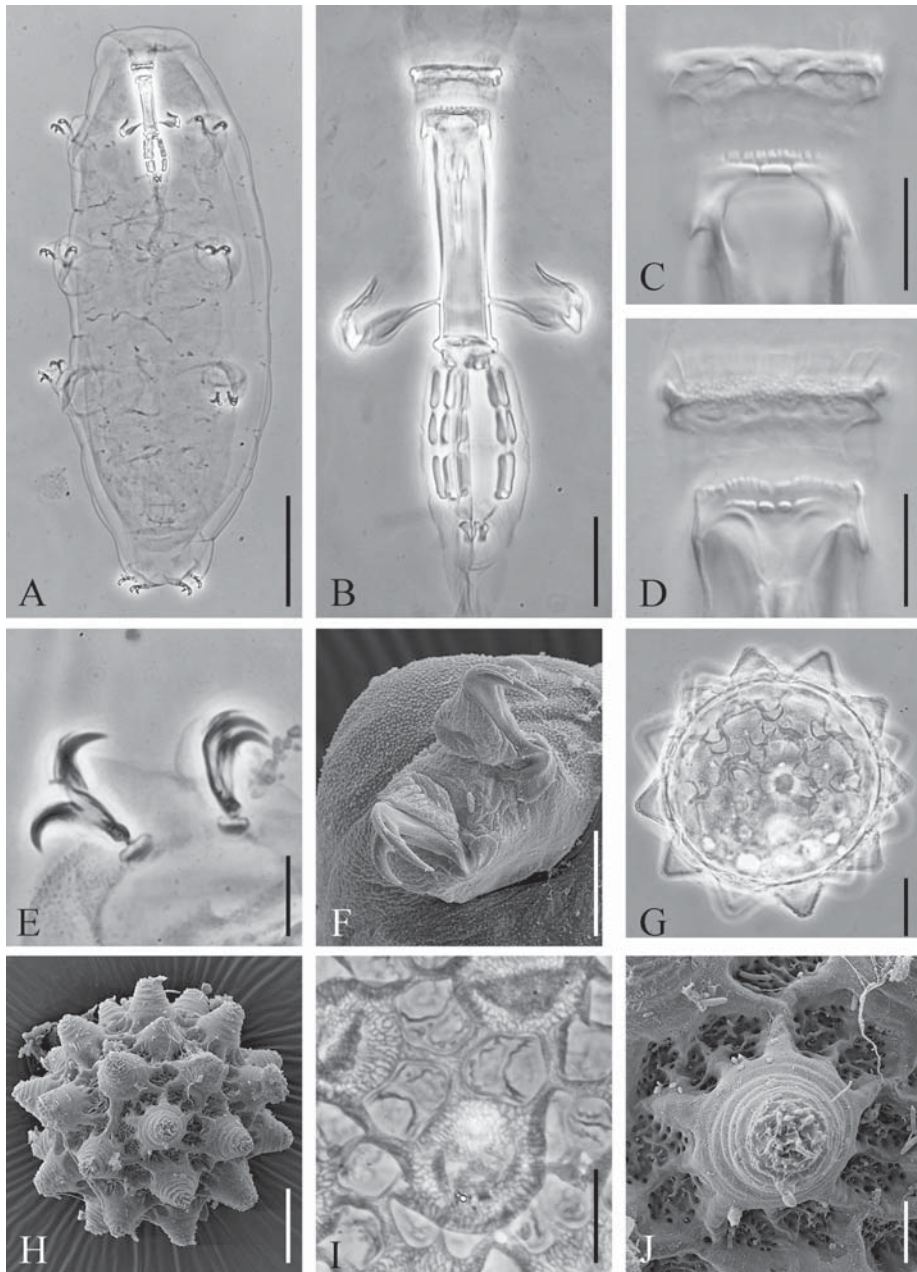


Fig. 10. *Paramacrobiotus fairbanksi*. A — habitus; B — buccal-pharyngeal apparatus; C — oral cavity armature, dorsal; D — oral cavity armature, ventral; E — claws of the leg II; F — claws of the leg IV; G, H — egg; I, J — egg surface.

Scale bar: A — 100 μ m; B, G, H — 20 μ m; C–F, I — 10 μ m; J — 5 μ m. A, B, E, G, I — phase contrast; C, D — differential interference contrast; F, H, J — SEM.

Рис. 10. *Paramacrobiotus fairbanksi*. A — общий вид; B — рото-глоточный аппарат; C — ротовая арматура, дорсально; D — ротовая арматура, вентрально; E — коготки ножки II пары; F — коготки ножки IV пары; G, H — яйцо; I, J — поверхность яйца.

Масштаб: A — 100 μ m; B, G, H — 20 μ m; C–F, I — 10 μ m; J — 5 μ m. A, B, E, G, I — фазовый контраст; C, D — дифференциальный интерференционный контраст; F, H, J — СЭМ.

ly distributed species of the *P. richtersi* complex — *P. fairbanksi*. This species was described from Alaska (Schill *et al.*, 2010) and currently is known also from Europe (Italy, Spain, Germany, Poland) and Antarctica (Kaczmarek *et al.*, 2020). Parthenogenetic reproduction of this species is the most probable explanation for such a big colonization success (Guidetti *et al.*, 2019; Stec *et al.*, 2020c).

Taking into account that this species cannot be morphologically differentiated from *P. richtersi* s.str. (Guidetti *et al.*, 2019), all previous numerous records of *P. richtersi* for the territory of Russia should be treated as doubtful, but presence of the *P. richtersi* s.str. in Russia is also possible, considering the recent finding of this species in Finland (Vecchi *et al.*, 2022).

Conclusion

Despite the small amount of studied samples from relatively small geographic region the results obtained during our study are of certain faunistic interest. The number of tardigrade species known for the Russian fauna confirmed with molecular data is raised from 3 to 13. Among 11 species found during this investigation four are new for the fauna of Russia (*Milnesium dornensis*, *M. berladnicorum*, *Mesocrista revelata*, and *Paramacrobiotus fairbanksi*). For three species, previously noted for the Russian fauna their presence was supported with molecular data (*Adropion scoticum*, *Ramazzottius oberhaeuseri*, and *Macrobiotus hufelandi*). In case of three other species found (*Diphascon* cf. *pingue*, *Pilatobius* cf. *bullatus*, and *Minibiotus* cf. *intermedius*) our analysis revealed significant genetic differences between the populations studied here and the data obtained from GenBank. It shows clearly, that the real species richness of tardigrades is deeply underestimated even in such relatively well-investigated region as Europe. It demonstrates that the modern integrative redescription of “old” tardigrade species is urgently needed for the future work in the field of tardigrade taxonomy and faunistics. And also our work demonstrates that the efforts made to redescribe some species in the recent past (e.g. Michalczyk *et al.*, 2012; Gąsiorek *et al.*, 2016; Morek *et al.*, 2016a; Stec *et al.*, 2018; Guidetti *et al.*, 2019; Kaczmarek *et al.*, 2022) already gave the possibility to obtain new well-supported faunistic data even if only a single specimen found. This is evidenced here by many exact hits with COI sequences of these taxa with populations found in this study.

Compliance with ethical standards

CONFLICTS OF INTEREST: The authors declare they have no conflict of interest.

Supplementary data. The following materials are available online.

Supplementary Table S1. Results of the Blastn search through the GenBank nucleotide database for the obtained DNA fragments of *Diphascon* cf. *pingue*.

Supplementary Table S2. The range of uncorrected genetic *p*-distances between the studied specimens of *D. cf. pingue* and sequences of the genus *Diphascon* available in GenBank.

Supplementary Table S3. Results of the Blastn search through the GenBank nucleotide database for the obtained DNA fragments of *Adropion scoticum*.

Supplementary Table S4. The range of uncorrected genetic *p*-distances between the studied specimen of *A. scoticum* and sequences of the genus *Adropion* available in GenBank.

Supplementary Table S5. Results of the Blastn search through the GenBank nucleotide database for the obtained DNA fragments of *Mesocrista revelata*.

Supplementary Table S6. The range of uncorrected genetic *p*-distances between the studied specimen of *M. revelata* and sequences of the genus *Mesocrista* available in GenBank.

Supplementary Table S7. Results of the Blastn search through the GenBank nucleotide database for the obtained DNA fragments of *Pilatobius* cf. *bullatus*.

Supplementary Table S8. The range of uncorrected genetic *p*-distances between the studied specimens of *Pilatobius* cf. *bullatus* and sequences of the genus *Pilatobius* available in GenBank.

Supplementary Table S9. Results of the Blastn search through the GenBank nucleotide database for the obtained DNA fragments of *Minibiotus* cf. *intermedius*.

Supplementary Table S10. The range of uncorrected genetic *p*-distances between the studied specimens of *Minibiotus* cf. *intermedius* and sequences of the genus *Minibiotus* available in GenBank.

Supplementary Table S11. The range of uncorrected genetic *p*-distances between the studied specimens of *Paramacrobiotus fairbanksi* and sequences of this species available in GenBank.

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References

- Argue C.W. 1974. Tardigrades from New Brunswick, Canada. 3 // *Can. J. Zool.* Vol.52. P.919–992.
- Bertolani R., Rebecchi L. 1993. A revision of the *Macrobiotus hufelandi* group (Tardigrada, Macrobiotidae), with some observations on the taxonomic characters of eutardigrades // *Zoologica Scripta*. Vol.22. P.127–152.
- Bertolani R., Rebecchi L., Giovannini I., Cesari M. 2011. DNA barcoding and integrative taxonomy of *Macrobiotus hufelandi* C. A. S. Schultze 1834, the first tardigrade species to be described, and some related species // *Zootaxa*. Vol.2997. P.19–36. <https://doi.org/10.11646/zootaxa.2997.1.2>
- 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.* Vol.76. P.110–126. <https://doi.org/10.1016/j.ympev.2014.03.006>
- Binda M.G., Pilato G. 1987. Tardigrada dell’Africa. V. Notizie sui Tardigradi del Nord-Africa e descrizione della nuove specie *Macrobiotus diffusus* // *Animalia*. Vol.14. P.177–191.
- Binda M.G., Pilato G. 1992. *Minibiotus furcatus*, nuova posizione sistematica per *Macrobiotus furcatus* Ehrenberg, 1859, e descrizione di due nuove specie // *Animalia*. Vol.19. P.111–120.
- Biserov V.I. 1991. An annotated list of Tardigrada from European Russia // *Zool. Jb. Syst.* Vol.118. P.193–216.
- Biserov V.I. 1996. Tardigrades of the Taimyr Peninsula with description of two new species // *Zool. J. Linn. Soc.* Vol.116. P.215–237. <https://doi.org/10.1111/j.1096-3642.1996.tb02345.x>
- Cesari M., Montanari M., Kristensen R.M., Bertolani R., Guidetti R., Rebecchi L. 2020. An integrated study of the biodiversity within the *Pseudechiniscus suillus-facettalis* group (Heterotardigrada: Echiniscidae) // *Zool. J. Linn. Soc.* Vol.188. P.717–732. <https://doi.org/10.1093/zoolinnean/zlzl045>
- Ciobanu D.A., Zawierucha K., Moglan I., Kaczmarek Ł. 2014. *Milnesium berladnicorum* sp. n. (Eutardigrada, Apochela, Milnesiidae), a new species of water bear from Romania // *ZooKeys*. Vol.429. P.1–11. <https://doi.org/10.3897/zookeys.429.7755>
- Ciobanu D.A., Roszkowska M., Kaczmarek Ł. 2015. Two new tardigrade species from Romania (Eutardigrada: Milnesiidae, Macrobiotidae), with some remarks on secondary sex characters in *Milnesium dornensis* sp. nov. // *Zootaxa*. Vol.3941. P.542–564. <https://doi.org/10.11646/zootaxa.3941.4.4>
- Claxton S.K. 1998. A revision of the genus *Minibiotus* (Tardigrada: Macrobiotidae) with descriptions of eleven new species from Australia // *Rec. Aust. Mus.* Vol.50. P.125–160.
- Dastych H. 1988. The Tardigrada of Poland // *Monografie Fauny Polski*, 16. Warszawa, Krakow: Państwowe Wydawnictwo Naukowe. 255 p.
- Degma P., Guidetti R. 2022. Actual checklist of Tardigrada species. https://doi.org/10.25431/11380_1178608. (Accessed 17.07.2022)
- Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates // *Mol. Marine Biol. Biotechnol.* Vol.3. P.294–299.
- Fontoura P., Pilato G. 2007. *Diphascon* (*Diphascon*) *faialense* sp. nov. a new species of tardigrada (Eutardigrada, Hypsibiidae) from Azores and a key to the species of the *D. pingue* group // *Zootaxa*. Vol.1589. P.47–55.
- Gąsiorek P., Michalczyk Ł. 2020. Phylogeny of Itaquasconinae in the light of the evolution of the flexible pharyngeal tube in Tardigrada // *Zool. Scr.* Vol.49. P.499–515. <https://doi.org/10.1111/zsc.12424>
- Gąsiorek P., Stec D., Morek W., Zawierucha K., Kaczmarek Ł., Lachowska-Cierlik D., Michalczyk Ł. 2016. An integrative revision of *Mesocrista* Pilato, 1987 (Tardigrada: Eutardigrada: Hypsibiidae) // *J. Nat. Hist.* Vol.50. P.2803–2828. <https://doi.org/10.1080/00222933.2016.1234654>
- Gąsiorek P., Stec D., Morek W., Michalczyk Ł. 2018a. An integrative redescription of *Hypsibius dujardini* (Doyère, 1840), the nominal taxon for Hypsibioidae (Tardigrada: Eutardigrada) // *Zootaxa*. Vol.4415. P.45–75. <https://doi.org/10.11646/zootaxa.4415.1.2>
- Gąsiorek P., Stec D., Zawierucha K., Kristensen R.M., Michalczyk Ł. 2018b. Revision of *Testechiniscus* Kristensen, 1987 (Heterotardigrada: Echiniscidae) refutes the polar-temperate distribution of the genus // *Zootaxa*. Vol.4472. P.261–297. <https://doi.org/10.11646/zootaxa.4472.2.3>
- Guidetti R., Bertolani R., Degma P. 2007. New taxonomic position of several *Macrobiotus* species (Eutardigrada: Macrobiotidae) // *Zootaxa*. Vol.1471. P.61–68.
- Guidetti R., Cesari M., Bertolani R., Altiero T., Rebecchi L. 2019. High diversity in species, reproductive modes and distribution within the *Paramacrobiotus richtersi* complex (Eutardigrada, Macrobiotidae) // *Zoological Letters*. Vol.5. Art.1. <https://doi.org/10.1186/s40851-018-0113-z>
- Guidetti R., Cesari M., Giovannini I., Ebel C., Förschler M.I., Rebecchi L., Schill R.O. 2022. Morphology and taxonomy of the genus *Ramazzottius* (Eutardigrada: Ramazzottiidae) with the integrative description of *Ramazzottius kretschmanni* sp. nov. // *Eur. Zool. J.* Vol.89. P.339–363. <https://doi.org/10.1080/24750263.2022.2043468>
- Guil N., Giribet G. 2012. A comprehensive molecular phylogeny of tardigrades – adding genes and taxa to a poorly resolved phylum-level phylogeny // *Cladistics*. Vol.28. P.21–49. <https://doi.org/10.1111/j.1096-0031.2011.00364.x>
- Guil N., Guidetti R., Cesari M., Marchioro T., Rebecchi L., Machordom A. 2022. Molecular phylogenetics, speciation, and long distance dispersal in tardigrade evolution: A case study of the genus *Milnesium* // *Mol. Phylogenet. Evol.* Vol.169. Art.107401. <https://doi.org/10.1016/j.ympev.2022.107401>
- Grobys D., Roszkowska M., Gawlak M., Kmita H., Kepel A., Kepel M., Parnikoza I., Bartylak T., Kaczmarek Ł. 2020. High diversity in the *Pseudechiniscus suillus-facettalis* complex (Heterotardigrada; Echiniscidae) with remarks on the morphology of the genus *Pseudechiniscus* // *Zool. J. Linn. Soc.* Vol.188. P.733–752. <https://doi.org/10.1093/zoolinnean/zlzl171>

- Ito M. 1999. Ecological distribution, abundance and habitat preference of terrestrial tardigrades in various forests on the Northern slope of Mt. Fuji, Central Japan // *Zool. Anz.* Vol.238. P.225–234.
- Kaczmarek Ł., Kosicki J., Roszkowska M. 2018a. Tardigrada of Bory Tucholskie National Park, Zaborski Landscape Park, and their surroundings (Pomerania Province, Poland) // *Turk. J. Zool.* Vol.42. Art.2. <https://doi.org/10.3906/zoo-1705-44>
- Kaczmarek Ł., Zawierucha K., Buda J., Stec D., Gawlak M., Michalczyk Ł., Roszkowska M. 2018b. An integrative redescription of the nominal taxon for the *Mesobiotus harmsworthi* group (Tardigrada: Macrobiotidae) leads to descriptions of two new *Mesobiotus* species from Arctic // *PLoS ONE*. Vol.13. P.e0204756. <https://doi.org/10.1371/journal.pone.0204756>
- Kaczmarek Ł., Mioduchowska M., Kačarević U., Kubska K., Parnikoza I., Góldyn B., Roszkowska M. 2020. New Records of Antarctic Tardigrada with Comments on Interpopulation Variability of the *Paramacrobiotus fairbanksi* Schill, Förster, Dandekar and Wolf, 2010 // *Diversity*. Vol.12. Art.108. <https://doi.org/10.3390/d12030108>
- Kaczmarek Ł., Kayastha P., Roszkowska M., Gawlak M., Mioduchowska M. 2022. Integrative Redescription of the *Minibiotus intermedius* (Plate, 1888) — The Type Species of the Genus *Minibiotus* R.O. Schuster, 1980 // *Diversity*. Vol.14. Art.356. <https://doi.org/10.3390/d14050356>
- Katoh K., Misawa K., Kuma K., Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform // *Nucleic Acids Res.* Vol.30. P.3059–3066. <https://doi.org/10.1093/nar/gkf43>
- Larsson A. 2014. AliView: a fast and lightweight alignment viewer and editor for large data sets // *Bioinformatics* Vol.30. P.3276–3278. <http://dx.doi.org/10.1093/bioinformatics/btu531>
- Lisi O. 2015. Current knowledge on the Sicilian tardigrade fauna // *Biodivers. J.* Vol.6. P.297–304.
- Marcus E. 1936. Tardigrada. Berlin/Leipzig: Walter de Gruyter & Co. 340 S.
- Maskin E.V., Grebenkin P.V., Zheleznova L.V., Tumanov D.V. 2021. [New data on tardigrades of the genus *Milnesium* (Tardigrada, Eutardigrada, Milnesiidae) from the Russian Far East, obtained using the method of molecular barkoding] // *Chteniya pamyati A.I. Kurentsova. Vladivostok*. Vol.32. P.114–122 [in Russian, with English summary].
- Maucci W. 1986. Tardigrada // *Fauna d'Italia*. Vol.24. Bologna: Calderini. 388 p.
- McInnes S.J. 1994. Zoogeographic distribution of terrestrial/freshwater tardigrades from current literature // *J. Nat. Hist.* Vol.28. P.257–352.
- Michalczyk Ł., Welnicz W., Frohme M., Kaczmarek Ł. 2012. Redescriptions of three *Milnesium* Doyere, 1840 taxa (Tardigrada: Eutardigrada: Milnesiidae), including the nominal species for the genus // *Zootaxa*. Vol.3154. P.1–20.
- Mironov S.V., Dabert J., Dabert, M. 2012. A new feather mite species of the genus *Proctophyllodes* Robin, 1877 (Astigmata: Proctophyllodidae) from the Long-tailed Tit *Aegithalos caudatus* (Passeriformes: Aegithalidae) – morphological description with DNA barcode data // *Zootaxa*. Vol.3253. P.54–61. <https://doi.org/10.11646/zootaxa.3253.1.2>
- Morek W., Michalczyk Ł. 2020. First extensive multilocus phylogeny of the genus *Milnesium* (Tardigrada) reveals no congruence between genetic markers and morphological traits // *Zool. J. Linn. Soc.* Vol.188. P.681–693. <https://doi.org/10.1093/zoolinnean/zlzo40>
- Morek W., Gąsiorek P., Stec D., Blagden B., Michalczyk Ł. 2016a. Experimental taxonomy exposes ontogenetic variability and elucidates the taxonomic value of claw configuration in *Milnesium* Doyère, 1840 (Tardigrada: Eutardigrada: Apochela) // *Contrib. Zool.* Vol.85. P.173–200.
- Morek W., Stec D., Gąsiorek P., Schill R.O., Kaczmarek Ł., Michalczyk Ł. 2016b. An experimental test of eutardigrade preparation methods for light microscopy // *Zool. J. Linn. Soc.* Vol.178. P.785–793. <https://doi.org/10.1111/zoj.12457>
- Morek W., Stec D., Gąsiorek P., Surmacz B., Michalczyk Ł. 2019. *Milnesium tardigradum* Doyère, 1840: The first integrative study of interpopulation variability in a tardigrade species // *J. Zoolog. Syst. Evol. Res.* Vol.57. P.1–23. <https://doi.org/10.1111/jzs.12233>
- Morek W., Surmacz B., López-López A., Michalczyk Ł. 2021. “Everything is not everywhere”: Time-calibrated phylogeography of the genus *Milnesium* (Tardigrada) // *Mol. Ecol.* Vol.30. P.3590–3609. <https://doi.org/10.1111/mec.15951>
- Murray J. 1905. The Tardigrada of the Forth Valley // *Ann. Scott. Nat. Hist.* Vol.55. P.160–164.
- Nelson D.R., Bartels P.J., Guil N. 2018. Tardigrade Ecology // Schill R. (ed.). *Water Bears: The Biology of Tardigrades*. Zoological Monographs, vol 2. Cham: Springer. P.163–210. https://doi.org/10.1007/978-3-319-95702-9_7
- Pilato G. 1987. Revision of the genus *Diphascon* Plate, 1889, with remarks on the subfamily Itaquasconinae (Eutardigra, Hypsibiidae) // Bertolani R. (ed.). *Biology of Tardigrades. Selected Symposia and Monographs U.Z.I., 1. Modena: Mucchi*. P.337–357.
- Pilato G., Binda M.G. 1997/1998. A comparison of *Diphascon* (D.) *alpinum* Murray, 1906, D. (D.) *chilenense* Plate, 1889 and D. (D.) *pingue* Marcus, 1936 (Tardigrada), and description of a new species // *Zool. Anz.* Vol.236. P.181–185.
- Pilato G., Binda M.G. 1999. Three new species of *Diphascon* of the *pingue* group (Eutardigrada, Hypsibiidae) from Antarctica // *Polar Biol.* Vol.21. P.335–342.
- Pilato G., D’Urso V., Lisi O. 2013. *Ramazzottius thulini* (Pilato, 1970) bona species and description of *Ramazzottius libycus* sp. nov. (Eutardigrada, Ramazzottiidae) // *Zootaxa*. Vol.3681. P.270–280. <https://doi.org/10.11646/zootaxa.3681.3.6>
- Plate L. 1888. Beiträge zur Naturgeschichte der Tardigraden // *Zool. Jahrb. (Anat.)*. Bd.3. S.487–550.
- Ramazzotti G., Maucci W. 1983. Il phylum Tardigrada. Verbania Pallanza: Istituto Italiano di Idrobiologia. 1012 p.
- Roszkowska M., Grobys D., Bartylak T., Gawlak M., Kmita H., Kepel A., Kepel M., Parnikoza I., Kaczmarek Ł. 2020. Integrative description of five *Pseudochiniscus* species (Heterotardigrada: Echiniscidae: the *suillus-facettalis* complex) // *Zootaxa*. Vol.4763. P.451–484. <https://doi.org/10.11646/zootaxa.4763.4.1>

- Schill R.O., Forster F., Dandekar T., Wolf N. 2010. Using compensatory base change analysis of internal transcribed spacer 2 secondary structures to identify three new species in *Paramacrobiotus* (Tardigrada) // *Org. Divers. Evol.* Vol.10. P.287–296.
- Schuster R.O., Nelson D.R., Grigarick A.A., Christenberry D. 1980. Systematic criteria of Eutardigrada // *Trans. Am. Microsc. Soc.* Vol.99. P.284–303.
- Stec D., Smolak R., Kaczmarek Ł., Michalczyk Ł. 2015. An integrative description of *Macrobiotus paulinae* sp. nov. (Tardigrada: Eutardigrada: Macrobiotidae: *hufelandi* group) from Kenya // *Zootaxa*. Vol.4052. P.501–526. <https://doi.org/10.11646/zootaxa.4052.5.1>
- Stec D., Zawierucha K., Michalczyk Ł. 2017. An integrative description of *Ramazzottius subanomalus* (Bisrov, 1985) (Tardigrada) from Poland // *Zootaxa*. Vol.4300. P.403–420. <https://doi.org/10.11646/zootaxa.4300.3.4>
- Stec D., Morek W., Gąsiorek P., Michalczyk Ł. 2018. Unmasking hidden species diversity within the *Ramazzottius oberhaeuseri* complex, with an integrative redescription of the nominal species for the family Ramazzottiidae (Tardigrada: Eutardigrada: Paracheila) // *Syst. Biodivers.* Vol.16. P.357–376. <https://doi.org/10.1080/14772000.2018.1424267>
- Stec D., Kristensen R.M., Michalczyk Ł. 2020a. An integrative description of *Minibiotus ioculator* sp. nov. from the Republic of South Africa with notes on *Minibiotus pentannulatus* Londoño *et al.*, 2017 (Tardigrada: Macrobiotidae) // *Zool. Anz.* Vol.286. P.117–134. <https://doi.org/10.1016/j.jcz.2020.03.007>
- Stec D., Krzywański Ł., Arakawa K., Michalczyk Ł. 2020b. A new redescription of *Richtersius coronifer*, supported by transcriptome, provides resources for describing concealed species diversity within the monotypic genus *Richtersius* (Eutardigrada) // *Zoological Lett.* Vol.6. Art.2. <https://doi.org/10.1186/s40851-020-0154-y>
- Stec D., Krzywański Ł., Zawierucha K., Michalczyk Ł. 2020c. Untangling systematics of the *Paramacrobiotus areolatus* species complex by an integrative redescription of the nominal species for the group, with multilocus phylogeny and species delineation in the genus *Paramacrobiotus* // *Zool. J. Linn. Soc.* Vol.188. P.694–716. <https://doi.org/10.1093/zoolinnean/zlzl163>
- Stec D., Vecchi M., Calhim S., Michalczyk Ł. 2021a. New multilocus phylogeny reorganises the family Macrobiotidae (Eutardigrada) and unveils complex morphological evolution of the *Macrobiotus hufelandi* group // *Mol. Phylogenet. Evol.* Vol.160. Art.106987. <https://doi.org/10.1016/j.ympev.2020.106987>
- Stec D., Vecchi M., Dudziak M., Bartels P.J., Calhim S., Michalczyk Ł. 2021b. Integrative taxonomy resolves species identities within the *Macrobiotus pallarii* complex (Eutardigrada: Macrobiotidae) // *Zoological Lett.* Vol.7. Art.9. <https://doi.org/10.1186/s40851-021-00176-w>
- Sugiura K., Minato H., Matsumoto M., Suzuki A.C. 2020. *Milnesium* (Tardigrada: Apochela) in Japan: the first confirmed record of *Milnesium tardigradum* s.s. and description of *Milnesium pacificum* sp. nov. // *Zool. Sci.* Vol.37. P.476–495. <https://doi.org/10.2108/zs190154>
- Tamura R., Stecher G., Kumar D. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11 // *Mol. Biol. Evol.* Vol.38. P.3022–3027. <https://doi.org/10.1093/molbev/msab120>
- Tumanov D.V. 2018. *Hypsibius vaskelae*, a new species of Tardigrada (Eutardigrada, Hypsibiidae) from Russia // *Zootaxa*. Vol.4399. P.434–442. <https://doi.org/10.11646/zootaxa.4399.3.12>
- Tumanov D.V. 2020. Integrative redescription of *Hypsibius pallidoides* Pilato *et al.*, 2011 (Eutardigrada: Hypsibioidea) with the erection of a new genus and discussion on the phylogeny of Hypsibiidae // *Eur. J. Taxon.* Vol.681. P.1–37. <https://doi.org/10.5852/ejt.2020.681>
- Tumanov D.V. 2021. Presence of *Notahypsibius pallidoides* (Tardigrada: Hypsibiidae) in the fauna of Russia confirmed with the methods of DNA barcoding // *Biological Communications*. Vol.66. P.274–280. <https://doi.org/10.21638/spbu03.2021.309>
- Vecchi M., Stec D., Vuori T., Ryndov S., Chartrain J., Calhim S. 2022. *Macrobiotus naginae* sp. nov., a new xerophilous tardigrade species from Rokua sand dunes (Finland) // *Zool. Stud.* Vol.61. Art.22. <https://doi.org/10.6620/ZS.2022.61-22>

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