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Tardigrades and oribatid mites in bryophytes from geothermally active lava fields (Krafla, Iceland) and the description of *Pilatobius islandicus* sp. nov. (Eutardigrada)

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Abstract: In polar regions, apart from tundra and glaciers, geothermally active areas with elevated temperatures are important elements of ecosystems. One such geothermally active region characterized by mosaic ecosystems and vast areas covered by recent lava fields is Iceland. The aim of our study was to explore the diversity of invertebrates inhabiting geothermally active lava fields in the Krafla area (Iceland). Eight bryophyte samples were collected from a warm surface, mainly from the steaming areas. We have found Nematoda, Rotifera, Tardigrada and Oribatida in the samples. Habitat analysis demonstrated there to be 12 bryophyte species (five liverworts and seven mosses). The diversity of bryophytes in a single sample ranged from one to six species. The most common bryophyte was *Racomitrium lanuginosum* (Hedw.) Brid. Four species of tardigrades were found, including one that was new. *Pilatobius islandicus* sp. nov. is described herein by morphological, morphometric and molecular approaches (COI, 28S rRNA, 18S rRNA). Oribatida mites were identified as two species (*Malaconothrus monodactylus* (Michael, 1888) and *Camisia foveolata* Hammer, 1955). The average density of invertebrates was 13.1 ind./g with a maximum of 40.8 ind./g calculated per dry material. The tardigrades found in our study belonged to herbivores, microbivores and omnivores, whereas the mites belonged to saprophages, which indicates complex trophic networks in geothermally active lava fields.

Key words: Subarctic, Oribatida, Tardigrada, Nematoda, Rotifera, thermophiles.

Introduction

Iceland is a geologically young, isolated island located near the border of the Atlantic and Arctic Oceans, which makes it a robust model for studies on animal dispersion, diversity and evolutionary history (*e.g.*, Shain *et al.* 2016; Potapov *et al.* 2018). The island is characterised by the presence of ice caps and tundra, but also active volcanoes of which eruptions are relatively frequent (Thordarson and Larsen 2007). In lava-covered areas, known as lava fields, vegetation and fauna have been studied sporadically (Bjarnason 1991; Svavarsson and Kristjansson 2006) apart from frequent geological surveys (*e.g.*, Thordarson and Larsen 2007; Pedersen *et al.* 2017). Even young and hot Icelandic lava fields are often covered by moss patches (Bjarnason 1991). Mosses worldwide are inhabited by various invertebrates, like mites, nematodes, rotifers, springtails or tardigrades (*e.g.*, Norton and Behan-Pelletier 2009; Nelson *et al.* 2015; Glime 2017). In spite of the fact that moss-fauna is small in size, owing to high density, its total biomass may be relatively large (*e.g.*, Sohlenius 1977). Thus, these organisms play an important role as decomposers, influencing the circulation of nutrients (*i.e.*, carbon, nitrogen, phosphorus) in ecosystems, and transferring energy to higher trophic levels (Jennings 1976; Sohlenius 1977).

The current knowledge of invertebrates associated with geothermally active lava fields worldwide is limited to a few studies, and this research topic is vastly underinvestigated. Only comprehensive research on invertebrate abundance and diversity was conducted in the Surtsey island (Iceland), which was formed after the eruption of a volcano in 1963 (Ólafsson 1982; Gjelstrup 2000; Svavarsson and Kristjansson 2006). One of the most active volcanic regions in Iceland is Krafla, where the last eruption of a volcano occurred between 1975 and 1984 (Árnadóttir *et al.* 1998). To fill the knowledge gap concerning invertebrates inhabiting these extreme and arid systems, we collected bryophytes from modern lava fields and focused on invertebrates, with a particular emphasis placed on two common groups of moss-associated fauna: Tardigrada and Oribatida.

Tardigrada, also referred to as water bears, are a phylum that belongs to Panarthropoda (Nelson *et al.* 2015). At present, more than 1270 species from around the world are known (Degma *et al.* 2018). Most species are herbivorous, detritivorous or omnivorous, although carnivores exist as well (Guidetti *et al.* 2012). Tardigrades are known for their capability to live and reproduce in extreme conditions, such as the High Arctic and Antarctic ecosystems or high mountains (Marcus 1936; Nelson *et al.* 2015; Gąsiorek *et al.* 2017b). They are one of the few animals that can survive unfavourable conditions (*e.g.*, high temperatures, freezing, desiccation) in the state of cryptobiosis (Ramazzotti and Maucci 1983; Nelson *et al.* 2015). Sometimes, tardigrades, along with rotifers, may exceptionally dominate ecosystems (Convey and McInnes 2005). Despite their ubiquity worldwide (*e.g.*, Kaczmarek and Michalczyk 2017; McInnes *et al.* 2017), tardigrade fauna from geothermal places are still poorly known (*e.g.*, Kristensen 1982).

Oribatid mites (Oribatida) are one of the most numerous and systematically diverse groups of mites (Acari). Their widespread occurrence and high abundance is associated with their significant role in the transformation of organic matter and participation in soil-forming processes. They make up the largest group of soil mites and in certain environments constitute 60–90% of total mites. They exhibit great ecological diversity and in many habitats are found in high abundances and in a large number of taxa (Subías *et al*. 2017).

Our research presents novel data on the diversity and density of invertebrates on the Krafla lava fields along with the description of a new species of water bear, namely *Pilatobius islandicus* sp. nov.

Study area

Iceland. — Iceland is geologically young island, situated on the top of a mantle plume at the boundary of North American and Eurasian plates (Fig. 1A). Even though Iceland is located in the northern latitudes, it is characterised by mild climate due to the conjunction of warm and cold waters of the North Atlantic Current. The island was formed during volcanic eruptions lasting from mid Miocene to the Holocene (Saemundsson 1979).

Vegetation on geothermal active lava fields. — Geothermal areas constitute harsh habitats for plant persistence. An extremely high temperature of soil usually combined with a permanent saturation of air with hot steam (especially close to a geothermal vent), and a significant concentration of heavy metals and sulphur compounds in soil are the main factors affecting vegetation covering such places (Wilberscheid 2008). Vegetation close to geothermal areas is characterized by the occurrence of cyanobacteria with a significant contribution of liverworts and the frequent presence of various pioneer moss species (Bjarnason 1991; Wilberscheid 2008). These places are rather poor in terms of vascular plant (mainly small shrub species) abundance and dominated by bryophyte and lichen associations (Bjarnason 1991; Ottósson *et al*. 2016). The most common moss in such habitats is *Racomitrium lanuginosum* (Hedw.) Brid., often achieving significant coverage. Geothermal plant communities in Iceland occur mainly in the south (the Hekla and Eldhraun areas), the south-west (the Reykjanes Peninsula) and the north (the area near Mývatn Lake) of the island, and they are regionally variable (Ottósson *et al*. 2016).

Krafla. — Krafla has a 10-km wide caldera that was formed about 100 000 years ago. It was mostly filled with eruptive material. Up to 1984, total lava coverage amounted to 36 km^2. Krafla is still a geologically active site (Hjartardóttir *et al*. 2012). Vegetation on Krafla lava field are extremely poor as a consequence of the thin and hot layer of soil. Vegetation cover developed mainly close to steam vents (Wilberscheid 2008; Fig. 1B,C).

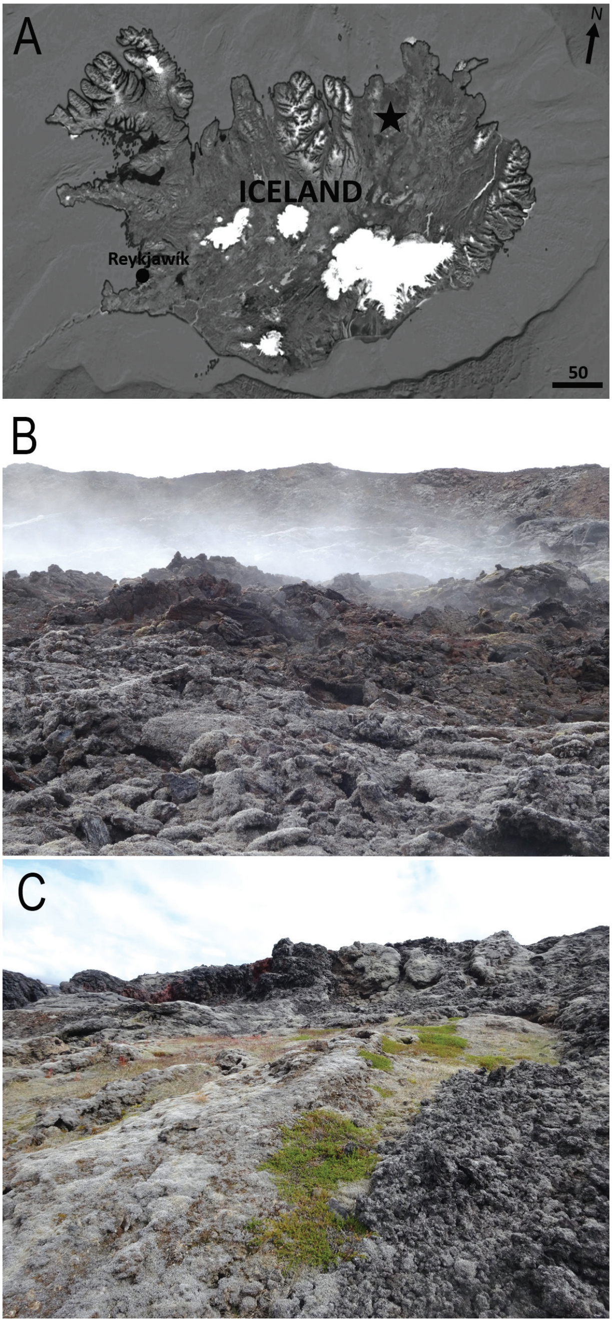

Fig. 1. A, Map of Iceland (Google Earth: Data SIO, NOAA, U.S. Navy, NGA, GEBCO). The Krafla volcano is marked with a star. Scale given in km. B, Lava field within the Krafla region. C, Bryophyte patches on the Krafla lava fields.

Material and Methods

Sampling and extraction of invertebrates. — Eight bryophytes samples were collected on 10th July 2016 from warm, steaming lava fields in the Krafla area, *i.e*. lava surface and cracks (Fig. 1B,C, Table 1). We did not measure temperatures directly in the field but plant communities were mostly typical for warm areas of lava fields excess 30°C (Wilberscheid 2008). Samples were collected from eruption sites from the active period between 1975 and 1984 in the Krafla region (Hjartardóttir *et al*. 2012). After collection, material was stored in envelopes, which allowed them to dry up slowly. Before analysis, the samples were weighed dry with a precision of 0.01 g. Mosses were examined for microinvertebrates using standard methods (Dastych 1980): animals were isolated by placing the material in a beaker filled with 200 ml of H_2O. After *ca*. six hours, mosses were vigorously shaken and comminuted within the beaker. Then, the material was separated optically in the beaker and the supernatant containing invertebrates inhabiting bryophytes was stirred and poured into a 250 ml cylinder. After sedimentation *ca*. 50 ml of precipitate was stirred, poured onto a glass Petri dishes and examined and counted under a stereomicroscope. In total, we analysed 28.4 grams of dry bryophytes.

Table 1

List of bryophyte species in samples collected on a lava fields (Krafla, Iceland).

sample	mosses	liverworts
1	*Pohlia nutans* (Hedw.) Lindb. *Racomitrium lanuginosum* (Hedw.) Brid.*	*Barbilophozia sudetica* (Nees ex Huebener) L. Söderstr., De Roo et Hedd. *Cephaloziella rubella* (Nees) Warnst.
2	*Ditrichum lineare* (Sw.) Lindb. *Pleurozium schreberi* (Willd. ex Brid.) Mitt. *Racomitrium lanuginosum* (Hedw.) Brid.	*Barbilophozia sudetica* (Nees ex Huebener) L. Söderstr., De Roo et Hedd. *Cephaloziella rubella* (Nees) Warnst.* *Nardia scalaris* Gray
3	*Racomitrium lanuginosum* (Hedw.) Brid.	*Gymnocolea inflata* (Huds.) Dumort.* *Cephaloziella rubella* (Nees) Warnst.
4		*Gymnocolea inflata* (Huds.) Dumort.
5	*Archidium alternifolium* (Hedw.) Mitt.	
6	*Bartramia ithyphylla* Brid. *Pohlia drummondii* (Müll.Hal.) A.L. Andrews	*Barbilophozia hatcheri* (A. Evans) Loeske
7	*Racomitrium lanuginosum* (Hedw.) Brid.*	*Nardia scalaris* Gray
8	*Racomitrium lanuginosum* (Hedw.) Brid.	

* – dominant species in the sample.

Tardigrada identification, morphometry, imaging and nomenclature. — Specimens of tardigrades for light microscopy and morphometry were mounted on microscope slides in a small drop of Hoyer's medium and examined under a Olympus BX53 phase contrast microscope (PCM) and differential interference contrast microscope (DIC). For species identification and differentiation, the keys in Ramazzotti and Maucci (1983) and original descriptions (Murray 1906; Marcus 1936; Bertolani and Rebecchi 1996; Biserov 1996; Gąsiorek *et al*. 2017b) were used. Morphometry of *Pilatobius secchi* (Bertolani et Rebecchi 1996) are given after Gąsiorek *et al*. (2017b). All figures were assembled in Corel Draw x7. For deep structures that could not be fully focused in a single photograph, a series of 2–8 images were taken every *ca*. 0.2 µm and then assembled into a single deep-focus image (using GIMP 2.8.18). Sample size for morphometrics was chosen as possible following recommendations by Stec *et al*. (2016). All measurements are given in micrometres (µm) and were performed under PCM with the Quick PHOTO CAMERA 3.0 software. Structures were measured only when their orientations were suitable. Body length was measured from the anterior to the posterior end of the body, excluding the hind legs. Macroplacoid length sequence is given according to Kaczmarek *et al*. (2014). Placoid row length was measured from the anterior portion of the first macroplacoid to the posterior end of the septulum. Claws were measured following Beasley *et al*. (2008). Claw morphology nomenclature follows Gąsiorek *et al*. (2017b). Bucco-pharyngeal tubes were measured following Pilato and Binda (1999). The *pt* ratio is the ratio of the length of a given structure to the length of the buccal tube (Pilato 1981). Tardigrade taxonomy is presented according to Bertolani *et al*. (2014). Morphometric data were handled using the "Parachela" ver. 1.2 template available from the *Tardigrada Register* (Michalczyk and Kaczmarek 2013). Trophic groups were established based on the scheme presented in Guidetti *et al*. (2012) and Guil and Sanchez-Moreno (2013). All samples and microscope slides are deposited at the Department of Animal Taxonomy and Ecology at Adam Mickiewicz University in Poznań, Poland.

Oribatid mites preparation, identification and nomenclature. — Oribatid mites were extracted under an Olympus SZ51 stereo microscope, cleaned for 24 hours in lactic acid, mounted on temporary cavity slides for the duration of the study and thereafter stored in 70% ethanol. Specimens were viewed and determined with an Olympus BX51 microscope using a keys and descriptions of Colloff (1993) and Weigmann (2006). Species nomenclature follows Subías (2017) and Weigmann (2006). The ratio of juveniles to adults is given following Niedbała and Rohloff (1972).

Bryophytes nomenclature. — Nomenclature for liverworts is given according to Söderström *et al*. (2016) and for mosses according to Hill *et al*. (2006).

Molecular analysis. — Two specimens of the new tardigrade species used for DNA analysis were collected from a bryophyte sample no. 6 (Krafla area, Iceland, 10 Jul. 2016). Total genomic DNA was extracted individually from two adult specimens using the method described in Dabert *et al*. (2008) and Mironov *et al*. (2012). In order to get exoskeletons, after three days of digestion, mix *i.e*. ATL buffer with proteinase K and tardigrade in the Eppendorf tube, was centrifuged at 7000 rpm. From each tube 90 μl for further analysis was carefully removed using pipette, remaining 10 μm on the bottom contained tardigrade specimen. The remaining 10 μl of mix was preserved with alcohol and after a few days exoskeleton was mounted in Hoyer medium for morphological analysis. DNA was isolated using the DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) as following the protocol in Dabert *et al*. (2008). A fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified with a bcdF01 forward primer (CATTTTCHACTAAYCATAARGATATTGG) and bcdR04 reverse primer (TATAAACYTCDGGATGNCCAAAAAA) (Dabert *et al*. 2008). Sequence of 18S rRNA was amplified in two overlapping fragments using 18Sf (CTTGTCTCAAAGATTAAGCCATGCA) with rev930 (GACGGTCCAAGAATTTCAC) (Dabert *et al*. 2016). D1–D3 region of the nuclear 28S rRNA was amplified with primers 28SF01 (ACCCVCYNAATTTAAGCATAT) and 28SR990 (CCTTGGTCCGTGTTTCAAGAC) (Mironov *et al*. 2012). Amplification of DNA fragment (PCR) for 18S rRNA and 28S rRNA was conducted in a total volume of 5.5 μl including: 3 μl Type-it Microsatellite PCR Kit (Qiagen), 0.5 μl of each primer, 0.5 μl Q-Solution (Qiagen) and 1 μl of the DNA template. For COI in a total volume of 5 μl were prepared, including: 3 μl Type-it Microsatellite PCR Kit (Qiagen), 0.5 μl of each primer and 1 μl of the DNA template. For PCR a thermocycling profile with one cycle of 5 min at 95°C followed by 40 steps of 30 s each at 95°C, 90 s at 50°C, 1 min at 72°C, and with a final step of 5 min at 72°C, for all amplicons was used. After amplification, the PCR products were diluted with 5 μl of MQ water; 2 μl of the diluted PCR product was analysed by electrophoresis on 1% agarose gel. Samples containing visible bands were purified with exonuclease I and Fast alkaline phosphatase (Fermentas). The PCR product was prepared for sequencing in a total volume of 6.0 μl containing: 1.5 μl primer, 1.5 μl purified PCRs product and 3 μl MQ water. Sequenced using the BigDye Terminator v3.1 kit and the ABI Prism 3130xl Genetic Analyzer (Applied Biosystems), following the manufacturer's instructions.

Molecular distances. — To calculate molecular distances for the COI fragments, three sequences were obtained from GenBank for *Pilatobius recamieri* (Richters, 1911) (GenBank accession numbers: KX347529, KX347531 and KX347530 deposited by Gąsiorek *et al*. (2017b)). To calculate molecular distances for the 28S rRNA fragments, one sequence was obtained from GenBank for *P. recamieri* (GenBank accession number: KX347528 deposited by Gąsiorek *et al*. (2017b)). Finally, to calculate molecular distances for the 18S rRNA fragments, four sequences were obtained from GenBank: one for *P. recamieri* (GenBank accession number: KX347526 deposited by Gąsiorek *et al*. (2017b)), one for *Pilatobius ramazzottii* (Robotti, 1970) (GenBank accession number: HQ604939 deposited by Bertolani *et al*. (2014)), one for *Pilatobius patanei* (Binda *et* Pilato, 1971) (GenBank accession number: HQ604936 deposited by Bertolani *et al*. (2014)) and one for *Pilatobius nodulosus* (Ramazzotti, 1957) (GenBank accession number: HQ604934 deposited by Bertolani *et al*. (2014)). Sequences were processed in BioEdit ver.7.2.5 (Hall 1997). Pairwise distances between nucleotide sequences were calculated using a distance model for all codon positions as implemented in MEGA 7 (Kumar *et al*. 2016). In COI, 18S and 28S p-distance calculations for all positions containing gaps and missing data were eliminated.

Results

Densities of invertebrates and species richness. — The analysis of eight bryophytes from lava fields around the Krafla volcano revealed a total of 47 tardigrades, four rotifers, 256 mites and 48 nematodes. No other microinvertebrates were found. The animals were recorded in six out of eight samples. The highest density of invertebrates per 1 g of dry material was found for mites – 29.2 individuals. The average density of animals in the lava fields was 13.1 ind./g. The maximum number of detected invertebrates was 40.8 ind./g. In 50% of the samples, all four taxa co-occurred. In just one sample, nematodes and tardigrades were present, and in another sample, only mites were recorded.

Table 2

Density of bryophyte associated invertebrates on a lava fields (Krafla, Iceland) calculated per 1 gram of dry sample mass (mass of sample – mass of analysed material after drying).

sample	mass of sample	Tardigrada	Rotifera	Nematoda	Acari	total
1	6.3	0	0	0	0	0
2	2.4	3.3	0.4	0.4	12.5	16.7
3	3.6	0.6	0.3	0.6	6.9	8.3
4	5.0	2.4	0.2	0.4	24.8	27.8
5	1.2	0	0	0	0.8	0.8
6	3.7	5.1	0	5.4	0	10.5
7	2.6	2.3	0.4	8.8	29.2	40.8
8	3.6	0	0	0	0	0

Detailed data for densities of each group are provided in Tables 2 and 3. Habitat analysis demonstrated there to be 12 bryophyte species (five liverworts and seven mosses). The diversity of bryophytes in a single sample ranged from one to six species. The most common bryophyte was *Racomitrium lanuginosum* (Table 1). The highest density of invertebrates was found in sample no. 7 consisting of the moss, *Racomitrium lanuginosum* and hepatic *Nardia scalaris*. In the same sample the highest diversity of invertebrates was found – two species of tardigrades, two species of oribatid mites, unidentified nematodes and rotifers (Table 2, 4 and 5).

Table 3

Invertebrate densities and frequency of occurrence in the bryophytes from lava fields (density was calculated per 1 gram of dry sample mass). Data are presented for all analysed samples. Min – minimum, Max – maximum, SE – standard error.

basic statistics	Tardigrada	Rotifera	Nematoda	Acari	total
Densities	13.7	1.3	15.6	74.2	104.9
Min	0	0	0	0	0
Max	5.1	0.4	8.8	29.2	40.8
Mean	1.7	0.2	2	9.3	13.1
SE	1.9	0.2	3.3	11.9	14.7
Frequency [%]	62.5	50	62.5	62.5	75

Table 4

Tardigrade species found in bryophyte samples collected from lava fields (Krafla, Iceland). An absolute number of isolated animals.

sample	*P. islandicus* sp. nov.	*D.* cf. *pingue*	*M.* cf. *crenulatus*	*E.* cf. m. *merokensis*	unidentified simplex
1	0	0	0	0	0
2	0	0	8	0	0
3	0	0	2	0	0
4	0	1	11	0	1
5	0	0	0	0	0
6	8	0	0	11	0
7	0	1	5	0	0
8	0	0	0	0	0
total	8	2	26	11	1

Table 5

Oribatid-mites species found in bryophyte samples collected from lava fields (Krafla, Iceland). An absolute number of isolated animals.

sample	*M. monodactylus* adults	*M. monodactylus* juv.	*C. foveolata* juv.
1	0	0	0
2	29	1	0
3	15	10	0
4	55	69	0
5	1	0	0
6	0	0	0
7	42	31	3 (1 deutonymph. 2 protonymphs)
8	0	0	0
total	142	111	3

The tardigrades in our study belong to two classes, four genera and four species, one of which is described here as *Pilatobius islandicus* sp. nov. (Figs 2–4). The others are: *Diphascon* cf. *pingue* (Marcus, 1936), *Macrobiotus* cf. *crenulatus* Richters, 1904 belonging to class Eutardigrada, and *Echiniscus* cf. *merokensis merokensis* Richters, 1904 which belongs to class Heterotardigrada. Additionally, one unidentified eutardigrade simplex stage was found. Species richness in the analysed material ranged from one to two tardigrade species per sample (Table 4). The new species was recorded in one sample only, no. 6 (Figs 2–4).

In five out of eight samples, 256 representatives of mites (Acari) were found, all belonging to the oribatid mites (Oribatida) of the Desmonomata group, and they represented two species: *Malaconothrus monodactylus* (Michael, 1888) (141 adults and 112 juveniles) and *Camisia foveolata* Hammer, 1955 (three juveniles: two protonymphs and one deutonymph). The ratio of juveniles to adults was 1:1.2. (Table 5).

Taxonomic

Phylum Tardigrada, Doyère, 1840
Class Eutardigrada Richters, 1926
Order: Parachela Schuster, Nelson, Grigarick et Christenberry, 1980
Superfamily Hypsibioidea Pilato, 1969 (in Marley *et al*. 2011)
Family Hypsibiidae Pilato, 1969

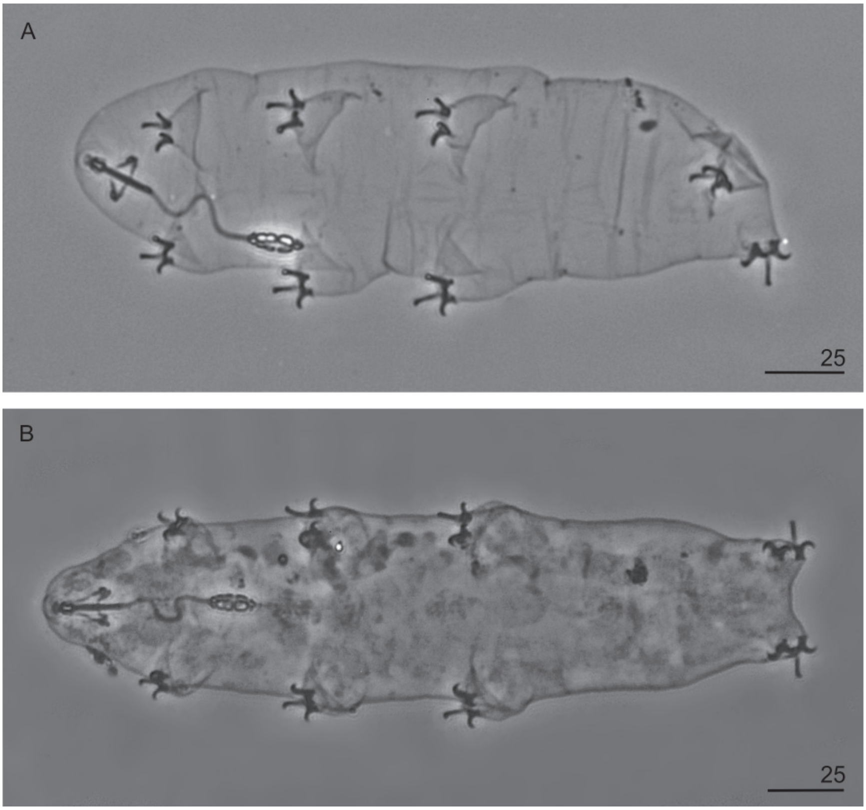

Fig. 2. *Pilatobius islandicus* sp. nov. (PCM). A, holotype, exoskeleton after DNA extraction, ventral view; B, paratype ventral view. Scales given in µm.

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

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

Pilatobius islandicus sp. nov. Buda *et* Zawierucha
(Figs 2–4, Table 6 and 7)

Material. — Holotype (slide no. 6/4, exoskeleton after digestion) and seven paratypes (slides: no. 6/1 (four specimens); 6/2 (one specimen); 6/3 (two specimens)), in total eight individuals, are deposited in the Department of Animal Taxonomy and Ecology at Adam Mickiewicz University, Poznań, Poland.

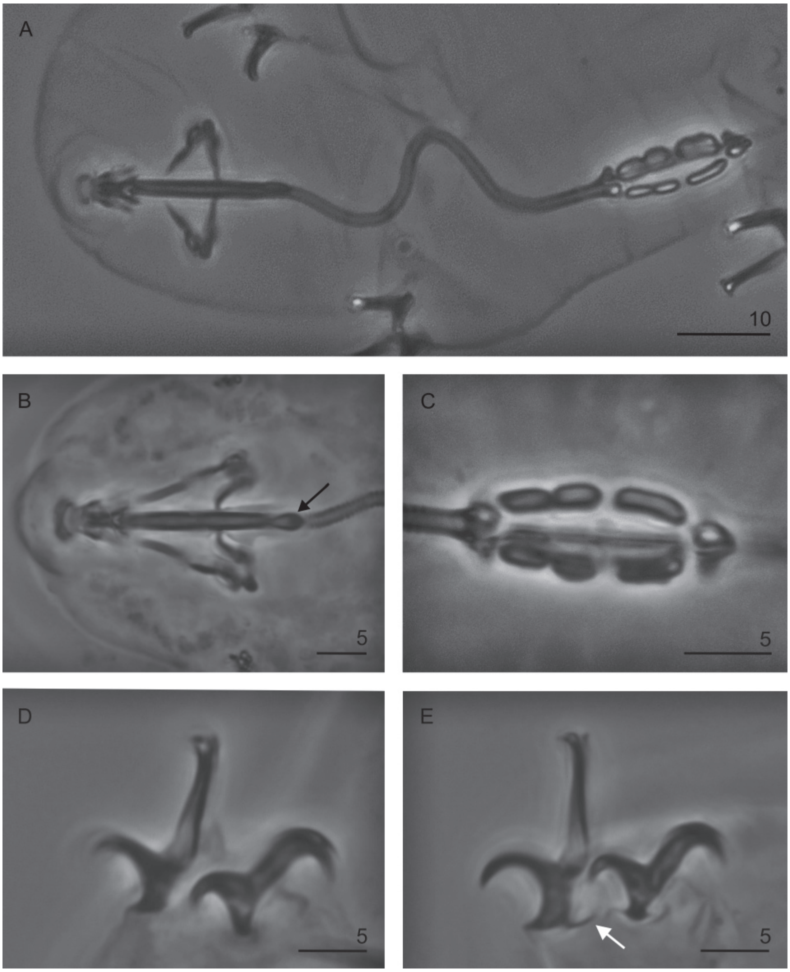

Fig. 3. *Pilatobius islandicus* sp. nov. (PCM). A, bucco-pharyngeal apparatus, holotype; B, buccal apparatus, black arrow indicates drop-like thickening, paratype; C, pharyngeal structures, paratype; D, claws III, paratype; E, claws IV, white arrow indicates cuticular bar, paratype. Scales given in µm.

Type locality. — Krafla volcano area, (*ca*. 65°46'N,16°47'W), bryophytes collected from solid lava type AA, Northeast Iceland.

Etymology. — Name *islandicus* refers to *Terra typica* for species – Iceland.

Description. — Body elongated, whitish, covered with smooth cuticle (Fig. 2). Eyes present in six specimens (Fig. 2B). Buccopharyngeal apparatus strongly elongated (Fig. 3A). The oral cavity armature absent or not visible under PCM. Stylet furcae of the *Hypsibius* type (see Pilato and Binda (2010)

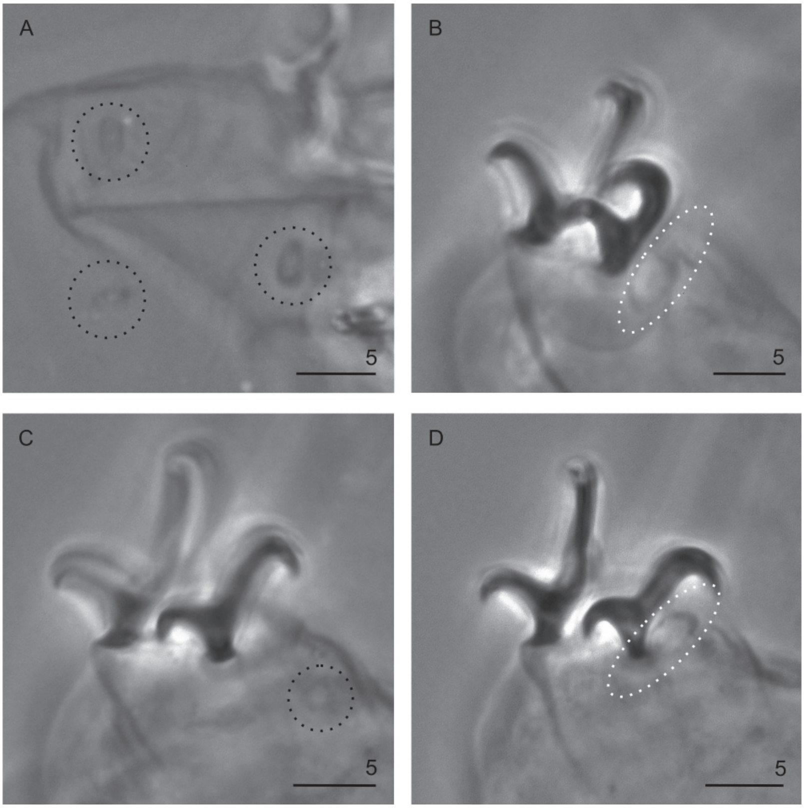

Fig. 4. *Pilatobius islandicus* sp. nov. (PCM). A, "pores" in cuticle, digested muscle attachment points (holotype); B,D bended cuticle which is reminiscent of oval structure (paratypes). C, "pore" in the leg (paratype); see remarks for details. Scales given in µm.

for definitions of furca types). Oval drop-like thickening on the border between the buccal and the pharyngeal tube present (Fig. 3B). Visible annulation dorsally and ventrally on pharyngeal tube (Fig. 3A). A short, very posterior part of the pharyngeal tube without annulation (Fig. 3A). Pharyngeal bulb with triangular apophyses and with two macroplacoids and a septulum (Fig. 3A,C). Macroplacoid length sequence 2<1; macroplacoids bar-shaped, arranged diagonally. The first macroplacoid with a strong mid-constriction (Fig. 3A,C), in some specimens the constriction being so strong that macroplacoid may seem to be divided into two parts (Fig. 3C). The second macroplacoid without constriction under PCM (Fig. 6A,C). Drop-shaped septulum present (Fig. 3A,C). Claws of the *Hypsibius* type, with widened bases and with apparent accessory points on the primary branches (Figs 3D, 4D). Internal and anterior claws with septum like structure dividing the claw into the basal portion, the secondary branch, and the primary branch (Fig. 3D,E, 4D). The base of the posterior claw extends

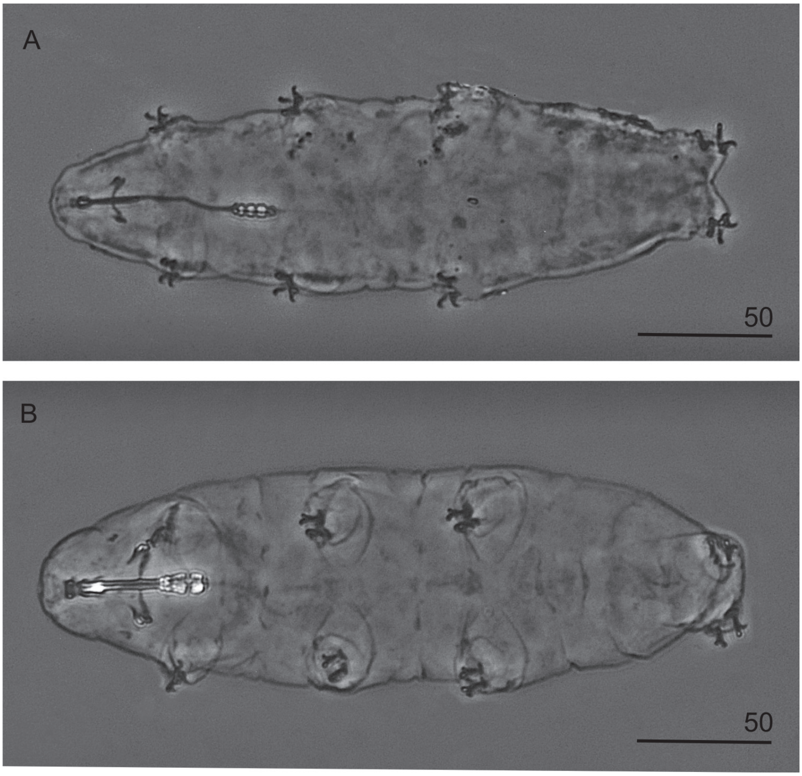

Fig. 5. Ventral view of: A, *Diphascon* cf. *pingue*; B, *Macrobiotus* cf. *crenulatus*. All in PCM. Scales given in µm.

towards the base of the anterior claw, forming a small cuticular bar (Fig. 3E). Anterior claws with pseudolunulae at their bases (Fig. 3E); pseudolunulae also sometimes weakly visible at the bases of internal claws I–III. External and posterior claws without pseudolunulae. No cuticular bars on legs I–III present but specific bended cuticle which reminiscent oval structure under internal claws (Figs 3D, 4A–D), see also Remarks. Eggs were not found.

Differential diagnosis

Morphology and morphometry. — By having smooth cuticle, two macroplacoids and septulum in the pharynx, the new species is most similar to the following *Pilatobius* species: *P. recamieri*, *P. brevipes* and *P. borealis*, but differs from:

– *P. recamieri*, known from many localities: Arctic, Europe, Asia, and North and South America (Ramazzotti and Maucci 1983; Gąsiorek *et al*. 2017b),

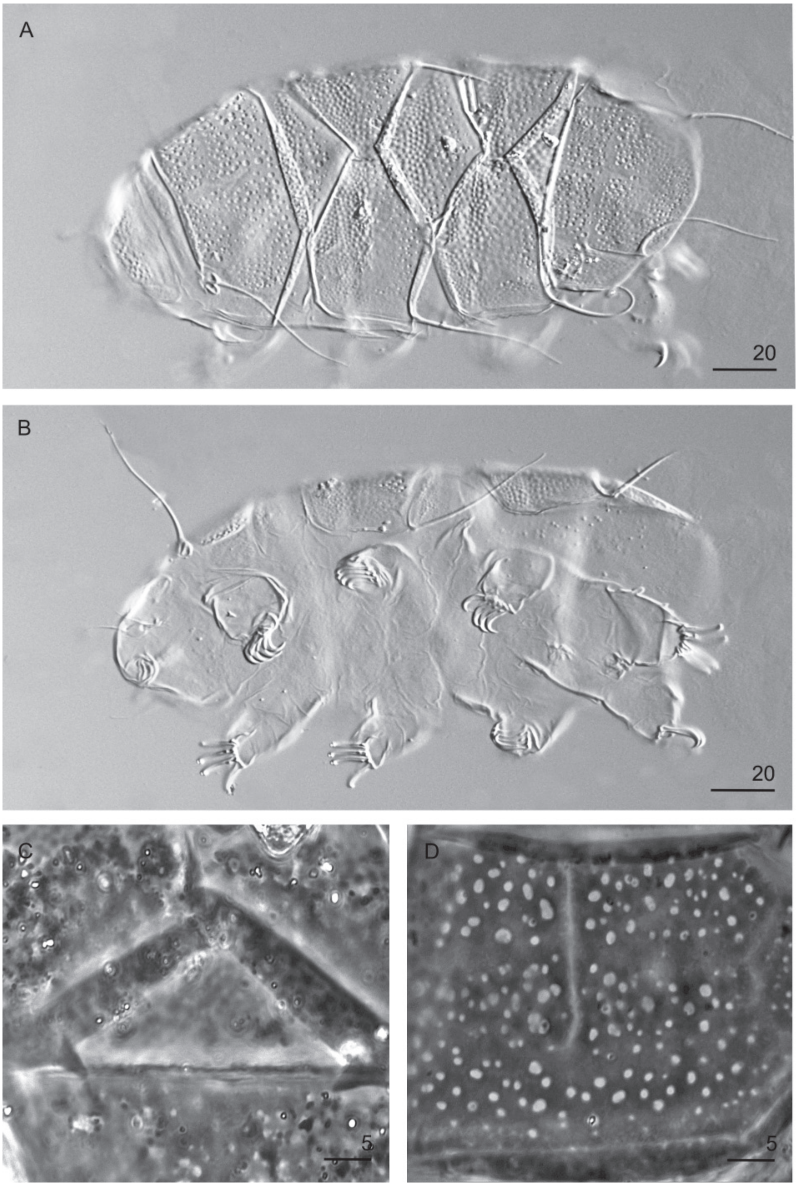

Fig. 6. *Echiniscus* cf. *merokensis merokensis*. A, dorsal view (DIC); B, ventral view (DIC) with cuticule reminicent plates (see Discussion); C, third median plate with dorsal appendices (PCM); D, sculpture on a terminal plate (PCM). Scales given in μm.

by a slightly longer first macroplacoid length (5.9 μm for 269 μm body length *P. islandicus* sp. nov. *vs* 5.3 μm for 272 μm body length *P. recamieri* (for details see tables 6 and 7), and lack of oval structures under internal claws (but see also Remarks).

Table 6

Measurements (in µm) of selected morphological structures of *Pilatobius islandicus* sp. nov. individuals mounted in Hoyer's medium (N – Number of the measured structures, range – the smallest and the largest structure among the measures, SD – standard deviation)

character	N	range		mean		SD		holotype	
		µm	*pt*	µm	*pt*	µm	*pt*	µm	*pt*
Body length	6	242–288	*1072–1280*	266	*1165*	18	*83*	–	–
Buccopharyngeal tube									
Buccal tube length	7	20.7–25.1	–	22.5	–	1.6	–	20.7	–
Pharyngeal tube length	6	40.9–49.3	*188.5–217.6*	45.0	*206.2*	3.2	*11.3*	43.2	*208.7*
Buccal/ pharyngeal tube length ratio	5	46%–53%	–	49%	–	3%	–	48%	–
Stylet support insertion point	7	12.8–17.7	*58.8–76.7*	15.1	*67.1*	1.8	*6.8*	13.2	*63.8*
Buccal tube external width	8	2.0–2.4	*8.6–11.4*	2.2	*9.8*	0.1	*1.0*	2.2	*10.6*
Buccal tube internal width	8	1.1–1.2	*4.4–5.7*	1.2	*5.2*	0.1	*0.4*	1.1	*5.3*
Placoid lengths									
Macroplacoid 1	8	5.0–6.4	*22.7–28.5*	5.7	*25.2*	0.4	*2.0*	5.9	*28.5*
Macroplacoid 2	8	3.9–5.0	*16.7–23.2*	4.4	*19.8*	0.4	*2.1*	4.8	*23.2*
Microplacoid	8	2.0–2.8	*10.1–13.5*	2.4	*10.9*	0.3	*1.2*	2.8	*13.5*
Macroplacoid row	8	9.8–12.2	*44.2–56.0*	10.9	*48.8*	0.9	*4.3*	11.6	*56.0*
Placoid row	8	12.6–14.9	*56.2–72.0*	13.8	*62.1*	0.9	*5.2*	14.9	*72.0*
Claw 1 lengths									
External base	2	3.2–4.5	*13.2–20.0*	3.9	*16.6*	0.9	*4.8*	–	–
External primary branch	2	6.6–7.8	*29.3–32.1*	7.2	*30.7*	0.8	*2.0*	–	–
External secondary branch	2	4.2–5.6	*17.3–24.9*	4.9	*21.1*	1.0	*5.4*	–	–
Internal base	6	2.6–3.5	*11.6–14.4*	2.9	*12.9*	0.3	*1.2*	2.8	*13.5*
Internal primary branch	6	3.9–5.0	*19.5–23.2*	4.6	*21.1*	0.5	*1.9*	4.8	*23.2*
Internal secondary branch	5	2.6–3.7	*11.6–17.9*	3.1	*13.7*	0.5	*2.9*	3.7	*17.9*
Claw 2 lengths									
External base	5	2.8–4.7	*13.5–20.9*	4.0	*18.5*	0.9	*3.4*	2.8	*13.5*
External primary branch	6	7.4–9.9	*35.2–44.0*	8.6	*39.8*	1.1	*3.1*	8.2	*39.6*
External secondary branch	6	4.4–6.5	*21.3–28.0*	5.5	*25.5*	0.9	*2.5*	4.4	*21.3*
Internal base	4	2.9–3.8	*13.4–15.0*	3.2	*14.1*	0.4	*0.8*	3.1	*15.0*
Internal primary branch	5	5.7–6.4	*26.7–29.5*	6.0	*27.9*	0.3	*1.3*	6.1	*29.5*
Internal secondary branch	4	2.6–5.3	*12.6–23.6*	3.8	*17.9*	1.1	*5.5*	2.6	*12.6*

Table 6 – continued

character	N	range		mean		SD		holotype	
		µm	*pt*	µm	*pt*	µm	*pt*	µm	*pt*
Claw 3 lengths									
External base	3	3.5–4.0	*16.1–17.8*	3.7	*17.2*	0.3	*0.9*	–	–
External primary branch	3	8.1–9.2	*37.3–40.9*	8.6	*39.6*	0.6	*1.9*	–	–
External secondary branch	3	4.6–6.2	*21.2–27.6*	5.2	*23.9*	0.9	*3.3*	–	–
Internal base	7	2.2–4.1	*8.8–18.2*	3.3	*14.8*	0.6	*3.5*	3.7	*17.9*
Internal primary branch	7	5.2–6.3	*24.0–28.1*	5.8	*26.2*	0.3	*1.5*	5.7	*27.5*
Internal secondary branch	6	3.5–4.7	*15.9–22.2*	4.1	*19.3*	0.5	*2.5*	4.6	*22.2*
Claw 4 lengths									
Anterior base	7	2.8–4.5	*13.3–19.1*	3.8	*16.9*	0.6	*2.3*	3.7	*17.9*
Anterior primary branch	7	4.2–6.6	*18.8–29.0*	5.7	*25.2*	0.7	*3.6*	5.5	*26.6*
Anterior secondary branch	7	3.2–5.0	*13.2–21.9*	3.9	*17.5*	0.7	*2.9*	3.2	*15.5*
Posterior base	6	3.9–5.0	*18.8–23.8*	4.6	*20.9*	0.4	*1.7*	3.9	*18.8*
Posterior primary branch	6	8.8–10.1	*37.9–44.9*	9.3	*41.9*	0.5	*2.5*	8.8	*42.5*
Posterior secondary branch	6	4.5–6.3	*18.5–28.5*	5.6	*25.4*	0.6	*3.6*	5.9	*28.5*

- *P. brevipes*, known from various localities in the Palearctic and from some Nearctic habitats (Ramazzotti and Maucci 1983), by the lack of bars under claws I–III, a longer pharyngeal tube (40.9–49.3 µm in 242–288 µm long specimens of *P. islandicus* sp. nov. *vs ca*. 30 µm in a 350 µm long specimen of *P. brevipes*), and lack of oval structures under internal claws (see Remarks). We must underline that morphometric difference in pharyngeal tube length may result from the use of a different measurement technique in terms of *P. brevipes*: Pilato and Binda (1999) proposed that, in taxa with a drop-like thickening, the buccal tube length should be measured down to the posterior end of the drop. Previously, some authors measured the buccal tube length to the anterior end of the drop, which translates to a shorter measurement.
- *P. borealis*, recorded only from the type locality in the sub-Arctic Taimyr Peninsula (Biserov 1996), by a significantly longer buccal and pharyngeal tube (respectively 20.7–25.1 µm and 40.9–49.3 µm in *P. islandicus* sp. nov. *vs* 14–16 µm and 22–26 µm in *P. borealis*), and lack of oval structures under internal claws (see Remarks).

Pilatobius islandicus is also similar to *P. secchi*. Gąsiorek *et al*. (2017b) found no morphological or morphometric differences between *P. recamieri* and *P. secchii*, a species described from Italy 85 years after *P. recamieri* (Bertolani

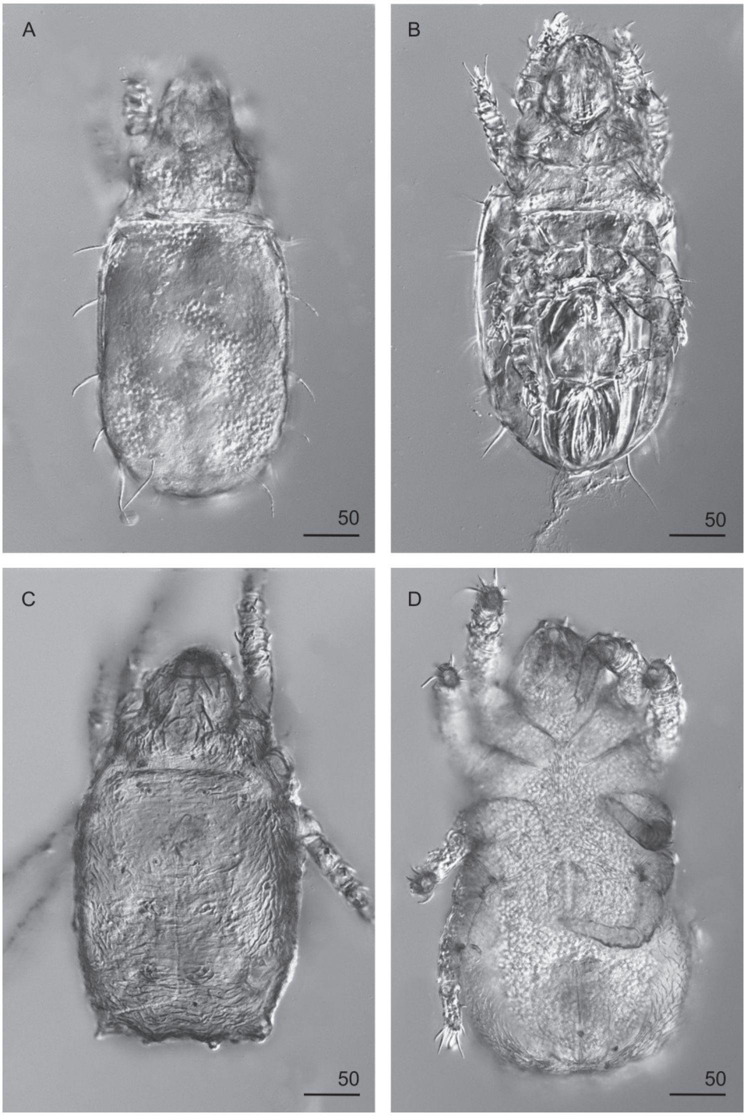

Fig. 7. Acari from lava fields (DIC). A,B, *Malaconothrus monodactylus*, adult: A, dorsal view; B, ventral view; C,D, *Camisia foveolata*, nymphal stages: C, protonymph, dorsal view; D, deutonymph, ventral view. Scales given in µm.

and Rebecchi 1996). Gąsiorek *et al*. (2017b) claimed the taxonomic status of *P. secchii* must be considered *nomen inquirendum* until DNA sequences become available for this taxon. The main difference between *P. islandicus* and *P. secchi* still is morphometry of buccal tube, a slightly longer in *P. islandicus* (24.3 µm for 283 µm body length *P. islandicus* sp. nov. *vs* 20.3 µm for 277 µm body length

P. secchi). However, more measurements of both, *P. islandicus* and *P. secchi* along with DNA barcodes are needed to establish status of *P. secchi* and present proper differential diagnosis. See also remarks and discussion.

Table 7

Comparison of first macroplacoid length of *Pilatobius islandicus* sp. nov. and *Pilatobius recamieri*, including the body length and buccal tube length.

Pilatobius islandicus sp. nov.			*Pilatobius recamieri*		
Body length	Buccal tube	First macroplacoid	Body length	Buccal tube	First macroplacoid
269 μm	25.1 μm	5.9 μm	272 μm	23.1 μm	5.3 μm
283 μm	24.3 μm	6.4 μm	306 μm	23.7 μm	6.2 μm
288 μm	22.5 μm	5.9 μm	312 μm	22.9 μm	5.7 μm

Remarks. — *Pilatobius islandicus* sp. nov. was found in the mixed samples containing one hepatic species (*Barbilophozia hatcheri*) and two mosses (*Bartramia ithyphylla*, *Pohlia drummondii*). *Pohlia drummondii* (a dominant moss identified in sample no. 6) occurs in various habitats but grows mainly in initial places. This species was recorded in a previous study conducted in similar microhabitats from other geothermal lava fields in the Hekla area (Bjarnason 1991). Other species in sample no. 6 (*Bartramia ithyphylla* and *Barbilophozia hatcheri*) have a wide habitat range and are common in Iceland, but not characteristic of geothermal areas. On a digested exoskeleton of a holotype and one paratype, we observed structures similar in shape to pores in a cuticle (Fig. 4A–D). However, in the holotype, they are visible under one leg only, and we suspect that these hollows are digested muscle attachment points (Fig. 4A). In the paratype, this "pore" probably constitutes an artefact (Fig. 4C).

By having a smooth cuticle, two macroplacoids and septulum in the pharynx, as well as lacking of the cuticular bars under internal claws, I–III *P. islandicus*, *P. recamieri* and *P. secchi* are the most similar. Despite the fact that *P. secchi* is considered to be *nomen inquirendum*, all three species differ in the type of habitats they were found in. These habitats comprise different temperature regimes, which may influence speciation. *P. recamieri* was originally described and re-described from the Arctic tundra, *P. secchi* was described from mountain grasslands in Italy and, finally, *P. islandicus* from lava fields in Iceland where temperatures may reach up to 50–80°C measured at a depth of 3 cm under the surface of soil covered by bryophytes (Wilberscheid 2008) (see the Discussion section for details). Taking into account that tardigrade morphometric traits may be influenced by the environment (Zawierucha *et al*. 2018), genetics (Stec *et al*. 2017), individual development/ontogeny (Morek *et al*. 2016a) and also by preparation techniques (Morek *et al*. 2016b), such minor morphometric differences in macroplacoid length should be treated with caution. The most important differences between *P. islandicus* and other similar species is genetic distance, which indicates that certain individuals of *P. recamieri* identified beyond type locality may be similar species belonging to one species complex.

Molecular markers and diagnosis. — COI and 28S rRNA sequences for *Pilatobius islandicus* sp. nov. obtained in this study were unique and distinct from all other sequences deposited in GenBank. The mitochondrial and nuclear markers were represented by single haplotypes. The analysis of COI involved five nucleotide sequences. There was a total of 589-bp positions in the final dataset. The p-distances for COI between *P. islandicus* (GenBank accession number: MH734939) and *P. recamieri* haplotypes (KX347529; KX347530; KX347531) varied between 17.0–17.2%.

The analysis of 28S rRNA involved three nucleotide sequences. There was a total of 448-bp positions in the final dataset. The p-distances for 28S rRNA between *P. islandicus* sp. nov. (GenBank accession number: MH682257) and *P. recamieri* (KX347527) was 0.7%.

The analysis of 18S rRNA involved five nucleotide sequences. There was a total of 768-bp positions in the final dataset. There were no differences in p-distances for 18S rRNA between *P. islandicus* sp. nov. (GenBank accession number: MH682258) and *P. recamieri* (KX347527). The p-distances for 18S rRNA between *P. islandicus* sp. nov., *P. ramazzotti* (HQ604939), *P. patanei* (HQ604936) and *P. nodulosum* (HQ604934) varied between 1.2 and 2.8%.

Discussion

Lava fields – unique ecosystems for studies on invertebrate and bryophyte diversity. — Geothermal active lava fields are a characteristic component of volcanically active regions, such as those observed in the Canary islands (Valet and Soler 1999) or Iceland (Thordarson and Larsen 2007). Despite the number of geological studies devoted to lava fields worldwide, biological characteristics of these places are poorly understood (*e.g*., Bjarnason 1991; Svavarsson and Kristjansson 2006).

Iceland began to emerge relatively recently on a geological scale: ~20 Myr (Allen *et al*. 2002). Meanwhile, there were glaciation periods with volcanic activity and eruptions (Mortensen *et al*. 2015). The fauna of Iceland is probably represented by recent colonizers inhabiting the area since the Last Glacial Maximum, as suggested for the Arctic by Pugh and McInnes (1998). It is also assumed that the survivors could found the shelters during the glaciation on nunataks or other refugia as vicinity of volcanoes. Thus, species recorded from Iceland until now may be both recent colonizers or endemic, long-persistent fauna. Such evidence was demonstrated by Shain *et al*. (2016) for Icelandic rotifers. According to the authors, the species inhabiting currently separated ice fields of Iceland are polyphyletic and their ancestors survived during the Pleistocene in separation. *Pilatobius islandius* sp. nov. could have evolved under specific conditions of lava fields and persistent volcanic eruptions in colder refugia closer to the glaciers. Further, *Echiniscus* cf. *merokensis merokensis* found in this study is characterised by the presence of unusual ventral structures. If they do not constitute an artefact, these specimens probably also belong to a new species.

One of the main factors differentiating bryophyte communities in geothermal active lava fields is the temperature of the soil upon which they grow. A poor bryophyte coverage consisting of species such as *Racomitrium elongatum*, *Calliergonella lindbergii*, *Dicranum bonjeanii* and *Archidium alternifolium* can still be observed on the soil surface with a temperature ranging between 50°C–80°C (Wilberscheid 2008). Ecological studies of Icelandic geothermal vegetation showed *Archidium alternifolium* (found in sample no. 5) growing in the soil at a mean temperature of *ca*. 46°C. The most frequent moss species in this study – *Racomitrium lanuginosum* (Hedw.) Brid. – was previously recorded from soil on a lava field with a mean temperature *ca*. 41°C. However, this species can grow beyond geothermal areas, even in cold places. The maximum temperature at which a number of the identified liverworts can occur amounts to: 51°C for *Gymnocolea inflata*, 39°C for *Barbilophozia sudetica* and 34°C for *Nardia scalaris* (Wilberscheid 2008).

Invertebrate densities and diversity. — In sample no. 2, the highest diversity of bryophytes was recorded with three mosses and three hepatic species. However, bryophyte species diversity did not influence invertebrate abundance as the greatest densities of tardigrades were found in sample no. 6 with just one moss and one hepatic species. Based on different methods of density estimations for various invertebrate groups, the comparison of data between different studies is unreliable. Comparative methods used to calculate the density of tardigrades in mosses of polar areas were used by Zawierucha *et al*. (2016). The density of tardigrades per 1 g of dry moss material in Svalbard reached the maximum of 955 ind./g (with an average density of water bears in the areas fertilised by seabirds 9.31 ind./g, and in areas beyond seabird influence 5.83 ind./g) as opposed to maximum *ca*. 5 ind./g (average 1.7) on the Krafla lava fields. We observed lower densities of tardigrades on lava fields than the other northern areas, but more studies are needed to understand and compare factors influencing tardigrade densities in various regions, especially poorly studied extreme ecosystems. Only eight samples from the lava fields were analysed, which does not constitute a comparable sample size.

Coulson and Midgley (2012) studied completely different bryophyte habitats in terms of thermal regime – moss balls on Icelandic glaciers. Contrary to the results of Coulson and Midgley (2012), we observed lower numbers of tardigrades and nematodes and we did not find any springtails. However, we did note mites. The lack of ubiquitous oribatid mites in glacier mice and their high abundance in mosses from a lava field indicate that oribatid mites may be selected by thermal regime. Nevertheless, these results require more attention as the oribatid mites inhabit even high Arctic tundra (Coulson *et al.* 2014) – a cold environment comparable to glacier mice.

Tardigrade species richness per positive sample recorded in the present study ranged from one to two species. Guil *et al.* (2009) recorded a maximum 14 tardigrade species per one analysed sample from Spain. Dastych (1985) reported up to 10 species and Zawierucha *et al.* (2015) up to eight species per moss sample in Svalbard. However, the observations of tardigrade species richness on the Krafla lava fields are rather in accordance with the previous studies carried out in various regions where tardigrade communities usually have low diversity and the number of tardigrade species per sample ranges from two to six (*e.g.*, Ramazzotti and Maucci 1983; Meyer 2006).

Succession processes were studied on Surtsey, a new volcanic island located in the vicinity of Iceland. Seven years after the island had appeared, only one oribatid mite species *Oribotritia faroensis* (Sellnick 1923) was found (Gjelstrup 2000). Twenty-five years later, the number of species increased significantly to 22 taxa. The total number of mites per square metre extracted from soil samples of different successional plant communities varied from 416 to 243 443 individuals. The oribatid mite species recorded in our study were not found on Surtsey, but are quite common on Iceland (Gjelstrup and Solhøy 1994), with *Malaconothrus monodactylus* usually seen in publications under the synonymized names, *M. gracilis* or *M. mollisetosus*.

The two identified oribatid mites in our studies are widely distributed parthenogenetic species. *Malaconothrus monodactylus* is quite common across the entire Holarctic, while the range of *Camisia foveolata* can be described as boreal (Alaska, Greenland, Norway, Svalbard) (Hågvar *et al.* 2009; Bayartogtokh *et al.* 2011; Subías *et al.* 2017). Data on their occurrence in the Neotropical region require confirmation. These species can be described as moisture-demanding but preferring various types of plant communities and microhabitats – mainly mosses and lichens in boreal regions (Solhøy 1976; Bayartogtokh *et al.* 2011).

The tardigrades found in our study belonged to herbivores, microbivores and omnivores, whereas mites belonged to saprophages. Even though lava fields are occasionally hostile (high temperatures) and relatively young ecosystems, complex trophic networks at an apex level in bryophytes on Icelandic lava fields are easily detectable.

Tardigrades taxonomy. — We did not delimit all tardigrades to the species level owing to the lack of sufficient amounts of material (*i.e.*, number of specimens and their eggs) and lack of DNA barcodes. According to the recently suggested optimal sample size for morphometric ranges in taxonomic traits for tardigrades (Stec *et al.* 2016), we decided not to formally delimit *D.* cf. *pingue* based on only two individuals. *Macrobiotus*. cf. *crenulatus* was found in this study without eggs, which inhibits a proper diagnosis based on the fact that eggs have crucial value in macrobiotid species delimitation (*e.g*., Kaczmarek and Michalczyk 2017). *Echiniscus* cf. *m. merokensis* possesses all features of typical *E. m. merokensis*. Yet, it differs by a slightly visible ventral ornamentation, which is characteristic for genus *Testechiniscus*. Up to now, tardigrades recorded from the Arctic and Subarctic were morphologically different from *E.* cf. *merokensis merokensis* found in this study. However, proper identification of these specimens without re-description of the other members of *Echiniscus* (Gąsiorek *et al*. 2017a; McInnes *et al*. 2017), especially members of the same group (*merokensis*), made an exact differential diagnosis difficult and unreasonable.

Correct species identification is of great value to environmental and experimental research as well as environmental management and protection. In the light of modern taxonomic techniques, including morphometric and molecular approaches, the status of many tardigrade species, especially those described in the past, seems to be unclear. The lack of morphometric and molecular data for type specimens is particularly problematic. However, morphometry was not commonly used in the past, and a molecular approach did not exist at the beginning of the 20th century. Thus, many species were considered to be cosmopolitan for decades and some of them still are (McInnes *et al*. 2017; Kaczmarek and Michalczyk 2017). The most recent examples are species from genus *Milnesium*, *Ramazzottius* or *hufelandi* group (genus *Macrobiotus*) or ‘harmsworthi’ group (genus *Mesobiotus*), which were regarded as cosmopolitan two decades ago and now constitute complexes of similar species differing in details of claws, cuticle and eggs (*e.g*., Vecchi *et al*. 2016; Stec *et al*. 2017). In this study, we showed that species recorded as *P. recamieri*, apart from type locality, may be in fact complexes of cryptic species identified only by molecular markers, proper morphometric design and careful morphological observation. According to our knowledge, we observed bended cuticles which is reminiscent of oval structure in Hypsibiidae for the first time (Fig. 7B, D).

Pilatobius islandicus is the most similar to *P. recamieri* and *P. secchi* that is considered to be *nomen inquirendum* until DNA sequences became available for this taxon (Gąsiorek *et al*. 2017b). Nevertheless, apart from its morphometry and molecular distances, *P. islandicus* still differs from its most similar congeners by the ecosystem it inhabits: (a) *P. recamieri* was originally described from mosses in Arctic tundra in the Svalbard archipelago (Richters 1911) and re-described from type locality by Gąsiorek *et al*. (2017b); (b) *P. secchi* was described from grass turf samples in the northern Apennines (Bertolani and Rebecchi 1996); and (c) *P. islandicus* was described from lava fields on Iceland. To date, cryptic species complexes among tardigrades have been revealed in various genera (*e.g*., Guidetti *et al*. 2009), and thus not only the *Pilatobius* genus, but also other taxa found in this study, are candidates of cryptic species complexes.

Conclusions

Despite the fact that geothermally active ecosystems constitute vast areas of the land, there are just a few studies devoted to invertebrate diversity in such habitats (Ólafsson 1982; Gjelstrup 2000; Svavarsson and Kristjansson 2006). Examination of the bryophyte samples collected on warm, steaming lava fields in the Krafla area uncovered the most common moss invertebrates, with one new tardigrade among them. Bryophyte species discovered in this study grow close to steam vents and are typical for geothermal warm areas of Iceland (Bjarnason 1991; Wilberscheid 2008). Our findings, along with those of previous studies (Gjelstrup 2000; Shain *et al*. 2016), highlight that biodiversity in areas characterised by strong selective pressures associated with extreme environmental conditions (*e.g*., high temperature) and rapid geological events (*e.g*., volcanic eruption) are still underestimated. Apart from bryophytes, there are other potential habitats for invertebrates on lava fields, *i.e*., small water reservoirs in solid lava or cracks with soil on the bottom. All of them remain unstudied. Morphologically similar tardigrades inhabiting various environments (High Arctic tundra, warm lava fields) may be in fact different species that have varying thermal niches, and the tardigrade genus, *Pilatobius*, might comprise an excellent example. Owing to the progress of earth and space research, many analogies have been noted between the Earth and other celestial bodies in our solar system (*e.g*., Gilichinsky *et al*. 2007; Cottin *et al*. 2017; Northup et al. 2011). Based on their uniqueness (*e.g*., geothermal activity and topography), lava fields are of great interest to astrobiologists (*e.g*., EAC-COST Summer School "Biosignatures and the Search for Life on Mars" 2016). Animals inhabiting these ecosystems may serve as a model for future surveys on animal survival (especially those able to enter cryptobiosis) on other planets and moons as was suggested for tardigrades (Bertolani *et al*. 2001). Thus, the description of their diversity in extreme ecosystems is highly relevant.

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