Tardigrada from Arctic tundra (Spitsbergen) with description of *Isohypsibius karenae* sp. n. (Isohypsibiidae)

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Abstract: Five species of Tardigrada were found in two moss samples collected from the Hornsund area (Ariekammen, Spitsbergen) including one new to science. The new species, *Isohypsibius karenae* sp. n., differs from the other similar congeners mainly by having a different type of cuticular sculpture, a different macroploid length sequence, by the presence of lunules and cuticular bars under claws as well as by some morphometric characters. The current study increases the number of *Isohypsibius* species known from Svalbard to thirteen.

Key words: Arctic, Hornsund, Ariekammen, Eutardigrada, moss fauna, taxonomy.

Introduction

Tardigrada, also known as water bears, is a phylum with *ca.* 1100 known species of micrometazoans (50–2112 μm in size) that inhabit a great variety of ecosystems throughout the globe (Ramazzotti and Maucci 1983; Nelson 2002; Guil 2008; Degma et al. 2012). On the Arctic archipelago of Svalbard, tardigrades are found in marine environments (e.g. Smykla et al. 2011), areas of terrestrial tundra (e.g. Węglarska 1965; Dastych 1985; Maucci 1996; Kaczmarek et al. 2012), freshwater bodies (e.g. Janiec 1996), as well as in extreme glacier microecosystems such as cryoconite holes (e.g. Dastych 1985; De Smet and Van Rompu 1994). Despite earlier, more than a century ago, polar exploration and associated reports of water bears (e.g. Richters 1911), new tardigrades are still being discovered in both the Arctic and the Antarctic (e.g. Miller et al. 2005; Tumanov 2007; McInnes 2010; Dastych 2011; Kaczmarek et al. 2012; Kaczmarek et al. 2013).

The genus *Isohypsibius* Thulin, 1928 has a worldwide distribution and can be found both in freshwater and terrestrial habitats, in all climatic zones, including polar regions (McInnes 1994).

In this study, two moss samples collected from the Ariekammen area near a little auk (*Alle alle* (Linnaeus, 1758)) colony were examined. This resulted in five species, including one new for science, which are reported and described below.

**Material and methods**

Two moss samples were collected from the Ariekammen area, near a large colony of little auks (*Alle alle*) located on the northern coast of Hornsund (Spitsbergen, Svalbard archipelago), 77°00’ N, 15°32’ E, ca. 7 m asl. The samples were collected by K. Wojczulanis-Jakubas and D. Jakubas, and later examined for tardigrades according to Ramazzotti and Maucci (1983) and Dastych (1985).

All specimens and eggs were mounted in microscopic slides in Hoyer’s medium and then examined and photographed with a Phase Contrast Microscope (PCM) (Olympus BX 40). Species were identified using the key to the world Tardigrada (Ramazzotti and Maucci 1983) and later original descriptions (Binda 1988; Bertolani and Balsamo 1989). All measurements (performed with QuickPhoto Camera 2.3 software) are given in micrometers [μm]. Structures were measured only if 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 the Tardigrada Register (Michalczyk and Kaczmarek 2013). Claws were measured according to Beasley *et al.* (2008). In eutardigrades, the *pt* ratio is the ratio of the length of a given structure to the length of the buccal tube, expressed as a percentage (Pilato 1981). Morphometric data were handled using the “Hypsibioidea and Isohypsibioidea” ver. 1.1 template available from the Tardigrada Register (Michalczyk and Kaczmarek 2013).

The new species has also been compared with two paratypes of *I. laevis* and measurements and microphotographs of *I. marii*. The investigated material is held in the Department of Animal Taxonomy and Ecology, Adam Mickiewicz University, Poznań, Poland. Raw data underlying the description of *Isohypsibius karenae* sp. n. are deposited in the Tardigrada Register (Michalczyk and Kaczmarek 2013, under http://www.tardigrada.net/register/0008.htm).
Taxonomy

Phylum Tardigrada (Spallanzani, 1777)
Class Eutardigrada Richters, 1926
Order Parachela Schuster, Nelson, Grigarick et Christenberry, 1980
Superfamily Hypsibioidae Pilato, 1969 in Marley et al. 2011
Family Hypsibiidae Pilato, 1969
Subfamily Diphasconinae Dastych, 1992
Genus Diphascon Plate, 1888
Diphascon (Diphascon) recamieri Richters, 1911

Material examined. — 39 specimens + 13 exuvia.

Remarks. — Holarctic, recorded from sparsely distributed localities in Europe, Asia and North America (McInnes 1994). Previously reported from Svalbard archipelago for Spitsbergen (Richters 1911; Marcus 1936; Węglarska 1965; Dastych 1985; De Smet and Van Rompu 1994; Janiec 1996; Maucci 1996; Kaczmarek et al. 2012) and for Hopen (Richters 1911; Van Rompu and De Smet 1996).

Family Hypsibiidae Pilato, 1969
Subfamily Hypsibiinae Pilato, 1969
Genus Hypsibius Ehrenberg, 1848
Hypsibius dujardini (Doyère, 1840)

Material examined. — 90 specimens + 19 exuvia.

Remarks. — Species belongs to the cosmopolitan convergens–dujardini complex of species (McInnes 1994; Miller et al. 2005; Kaczmarek and Michalczyk 2009). The original description is inadequate and unsatisfactory therefore the examined specimens were compared with the later descriptions (e.g. Ramazzotti and Maucci 1983; Dastych 1988). Previously reported from Svalbard archipelago for Spitsbergen (Richters 1903, 1904, 1911; Marcus 1936; Węglarska 1965; Dastych 1985; Janiec 1996; Maucci 1996; Kaczmarek et al. 2012), for Prins Karls Forland (Murray 1907), for Bjørnøya (Van Rompu and De Smet 1988), for Barentsøya (Van Rompu and De Smet 1991), for Edgeøya (De Smet et al. 1988), Hopen (Van Rompu and De Smet 1996), and for Amsterdamøya (Richters 1911).

Subfamily Itaquasconinae Rudescu, 1964
Genus Mesocrista Pilato, 1987
Mesocrista spitzbergensis (Richters, 1903)

Material examined. — 1 specimen.

Remarks. — Holarctic, recorded from localities in Europe, Asia and North America (McInnes 1994). Previously reported from Svalbard archipelago for Spitsbergen (Marcus 1936, recounting Richters 1903 and 1904; Węglarska 1965; Dastych 1985; Klekowski and Opaliński 1989), for Prins Karls Forland (Murray 1907), for Edgeøya (De Smet et al. 1988), for Bjørnøya (Van Rompu and De Smet 1988), and for Amsterdamøya (Richters 1903, 1904).
Superfamily Isohypsibioidea Marley, McInnes et Sands, 2011
Family Isohypsibiidae Marley, McInnes et Sands, 2011
Genus *Isohypsibius* Thulin, 1928

*Isohypsibius karenæ* sp. n.
(Figs 1–3, Table 1)

**Material.** — Holotype (slide 86.8/4), 31 paratypes and 9 exuvia (including two with eggs) (slides numbers: 86.7/1, 86.7/2, 86.7/3, 86.7/5, 86.8/1, 86.8/2, 86.8/3, 86.8/4, 86.8/6, 86.8/7, 86.8/11), deposited in the Department of Animal Taxonomy and Ecology at Adam Mickiewicz University, Poznań, Poland.

**Type locality.** — 77°00′ N 15°32′ E, ca. 7 m. asl, Svalbard Archipelago, Spitsbergen, Hornsund, Ariekammen area, coll. K. Wojczulanis–Jakubas and D. Jakubas.

**Etymology.** — Karen means Katarzyna in Norwegian. I dedicate this new species to Katarzyna Wojczulanis–Jakubas from the University of Gdańsk (Poland) who collected the material.

**Description** (measurements in Table 1). — Body colourless or slightly yellow (also after preparation) (Fig. 1a–b). Eyes were present in 75% of the examined specimens (mounted in Hoyer medium). Dorso-lateral cuticle, including outer portions of legs IV, covered with a faint reticular pattern. The reticulum with irregular ridges, mesh 1.4–3.1 in diameter (Fig. 1c–d). Diameter of reticular mesh slightly increasing towards caudal end of the body. Ventral cuticle smooth (*i.e.* without sculpturing).

Mouth antero-ventral. Peribuccal lamellae absent. Oral cavity armature comprises a single ventral and dorsal band of small round teeth in the posterior portion of the oral cavity (barely visible in some specimens) (Fig. 2a, insert). Bucco-pharyngeal apparatus of the *Isohypsibius* type. Buccal tube without the ventral lamina. Buccal tube walls very thick along the entire length. Pharyngeal bulb with apophyses, with three rod-shaped macroplacoids, all without constrictions. Macroplacoid length sequence 1<2<3, microplacoid and septulum absent (Fig. 2a).

Claws of the *Isohypsibius* type (Figs 2b–c, 3a–c), similar in shape and size on all legs, sometimes with a very wide basal part. External claws I–III and posterior claws IV slightly larger than internal claws I–III and anterior claws IV, respectively (see Remarks). All primary branches with accessory points, but those on the internal and external claws I–III are usually very poorly visible. The primary branches of anterior and posterior claws IV with better developed and visible accessory points (Fig. 2a). Small smooth lunules present on legs, but sometimes very hard to observe (Figs 2b–c, 3b) or invisible (Fig. 3a, c), especially on internal claws (see Remarks) (Fig. 2c). Oblique cuticular bars near the bases of claws I–III present (Fig. 3c).

Smooth eggs (3–4 in number) deposited in the exuvium (Fig. 1b).

**Remarks.** — In the majority of specimens instead of proper lunules only widened claw bases can be observed. Usually lunules are evident on the external claws and rather poorly visible on the internal claws (Fig. 2b–c). Sometimes they are vis-
ible only on the external claws (Figs 2b, 3b). Presence of lunules in this species could be confused, I have not observed lunules on the anterior claws IV which certainly are absent (Fig. 3a). Presence of lunules is variable character in *I. karenae* sp. n. Thus, this species could have lunules on internal and external claw I–III, but sometimes they are invisible.

In two paratypes small, short cuticular thickenings are present on outer (lateral) side of legs I–III. They are most likely folds of cuticle and they are under proper cuticular bars.
Table 1

Measurements and $pt$ values of selected morphological structures of fifteen specimens (including the holotype) from the type population of *Isohypsibius karenae* sp. n.: N – number of specimens or structures measured; Range – the smallest and the largest structure found among all specimens measured, SD – standard deviation; $pt$ – ratio of the length of a given structure to the length of the buccal tube, expressed as a percentage; ? – structure oriented unsuitably for measurement.

<table>
<thead>
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<th>Character</th>
<th>N</th>
<th>Range</th>
<th>Mean</th>
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<td>36.2</td>
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<td>26.6–36.6</td>
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<td>30.0–42.3</td>
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<td>27.1–45.3</td>
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</table>
Accessory points at primary branches are usually poorly visible, such similarity is known from the description of *I. ladogensis* Tumanov, 2003. In one specimen the primary anterior branch is slightly longer than the primary posterior branch.

Fig. 2. *Isohypsibius karenæ* sp. n.: a, buccal apparatus (the insert shows the oral cavity armature composed of a single ventral band of small teeth, holotype); b, external claw I with a well developed lunule (holotype); c, claw III with visible lunules (paratype). Scale in μm.

Accessory points at primary branches are usually poorly visible, such similarity is known from the description of *I. ladogensis* Tumanov, 2003. In one specimen the primary anterior branch is slightly longer than the primary posterior branch.
Differential diagnosis. — Isohypsibius karenae sp. n. has been found in samples collected from a very wet habitat located near small water bodies. Thus, like many of its congeners, it is potentially a hygrophilous terrestrial or an aquatic species (copepods were also found in the samples). The new species should be therefore compared with both aquatic and terrestrial species of the genus Isohypsibius with sculptured dorsal cuticle.


- I. asper, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species vs. hemispherical thickenings in I. asper) and by the presence of cuticular bars under claws I–III;
- I. baldii, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species vs. reticular pattern composed of small tubercles in I. baldii), a different macroplacoid length sequence (1<2<3 in the new species vs. 2<1<3 in I. baldii), the presence of cuticular bars under claws I–III, presence of oral cavity armature, and by a wider buccal tube (2.4–4.3 μm in the new species vs. ca. 1.6–1.8 μm in I. baldii);
- I. baldiioides, by a different type of cuticular sculpturing in caudal region (reticular pattern with irregular polygons of various size in the new species vs. reticular pattern composed of small partially fused tubercles in I. baldiioides), the absence of ventral sculpture, a different macroplacoid length sequence (1<2<3 in the new species vs. 2<1<3 in I. baldiioides), different oral cavity armature and by a lower pt ratio of the stylet support insertion point (63.8–67.3 in the new species vs. 67.5–73.8 in I. baldiioides);
- I. granulifer granulifer, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species vs. irregularly distributed thickenings in I. g. granulifer), absence of granulation on the legs, a longer buccal tube (32.2 μm in the new species (specimen 230 μm long) vs. 26 μm in I. g. granulifer (specimen 230 μm long)), and by the presence of cuticular bars under claws I–III;
- I. granulifer koreanensis, by absence of ventral sculpture, a different type of dorsal cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species vs. irregularly distributed granules in I. g. koreanensis), absence of granulation on the legs, the shape of macroplacoids (rods in the new species vs. round macroplacoids in I. g. koreanensis), and by the presence of cuticular bars under claws I–III;
A new species of Tardigrada from Svalbard

Fig. 3. *Isohypsibius karenæ* sp. n.: a, claws IV with not developed lunules (paratype), anterior claw with well visible accessory point; b, anterior claw IV with a lunule (holotype); c, cuticular bar under claws III (paratype). Scale in μm.
I. kotovae, by the presence of cuticular bars under claws I–III, presence of oral cavity armature, a lower pt ratio of the stylet support insertion point (63.8–67.3 in the new species vs. 67.2–68.8 in I. kotovae), and by presence of eyes;

I. ladogensis, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species vs. indistinct granules fused laterally which form reticulate pattern in I. ladogensis), a different macroplacoid length sequence (1<2<3 in the new species vs. 2<1<3 in I. ladogensis), and by different oral cavity armature;

I. laevis, by the presence of cuticular bars under claws I–III and the presence of lunules (but see the Remarks above) (McInnes pers. comm.);

I. marii, by a different macroplacoid length sequence (1<2<3 in the new species vs. 2<1<3 in I. marii), a thicker buccal tube (2.1 μm internal and 4.1 μm external diameter in the new species (specimen 395 μm in length) vs. 5.2 μm internal and 5.9 μm external diameter in I. marii (specimen 483 μm in length)), a lower pt ratio of the stylet support insertion point (63.8 in the new species (specimen 395 μm in length) vs. 67.1 in I. marii (specimen 483 μm in length)) (Bertolani pers. comm.), and by the presence of lunules (but see also Remarks above);

I. monoicus, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species vs. wrinkles in I. monoicus), the shape of macroplacoids (rods in the new species vs. granules in I. monoicus), and by the presence of cuticular bars under claws I–III;

I. pushkini, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species vs. reticular pattern with granules connected by thin, winding ridges), a different macroplacoid length sequence (1<2<3 in the new species vs. 2<1<3 in I. pushkini), presence of lunules on internal claws (but see the Remarks above), and by the slightly smaller third macroplacoid (4.4 μm in the new species (specimen 395 μm long) vs. 5.5 μm in I. pushkini (specimen 405 μm long));

I. tubereticulatus, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species vs. reticular pattern with granules connected by thin, winding ridges in I. tubereticulatus), a different macroplacoid length sequence (1<2<3 in the new species vs. 2<1<3 in I. tubereticulatus) the absence of a constriction in the third macroplacoid, the presence of cuticular bars under claws I–III, and by a lower pt ratio of the stylet support insertion point (63.8–67.3 in the new species vs. 67.2–69.5 in I. tubereticulatus).

Additionally, by having three macroplacoids in the pharynx and a sculptured dorsal cuticle the new species is most similar to the following terrestrial species of the genus Isohypsibius: I. brulloi Pilato et Pennisi, 1976, I. irregibilis Biserov, 1992, I. kenodontis Kendall-Fite et Nelson, 1996, I. liae X. Li et L. Wang, 2006, I. palmai Pilato, 1996, and I. yunnanensis Yang, 2002, but differs in particular from:
I. brulloi, by absence of ventral sculpture, a longer buccal tube (32.2 μm in the new species (specimen 230 μm in length) vs. 25 μm in I. brulloi (specimen 230 μm in length)), a different macroplacoid length sequence (1<2<3 in the new species vs. 2<1<3 in I. brulloi), presence of oral cavity armature, and by the presence of eyes;

I. irregibilis, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species vs. wrinkled cuticle in I. irregibilis), a different macroplacoid length sequence (1<2<3 in the new species vs. 2<1<3 in I. irregibilis), a lower pt ratio of the stylet support insertion point (63.8–67.3 in the new species vs. 67.8–75.0 in I. irregibilis), and by the lack of a projections on the primary branches on legs I–IV;

I. kenodontis, by a different type of cuticular sculpture (reticular pattern with irregular polygons of various size in the new species vs. partially fused granules in I. kenodontis), a different macroplacoid length sequence (1<2<3 in the new species vs. 2<1<3 in I. kenodontis), the presence of cuticular bars under claws I–III, and by the presence of the oral cavity armature;

I. liae, by the absence of cuticular undulations, absence of ventral sculpture, a different shape of macroplacoids (rods in the new species vs. granules in I. liae), a longer buccal tube (32.2 μm in the new species (specimen 230 μm in length) vs. 26.1 μm in I. liae (specimen 232 μm in length)), and by the presence of eyes;

I. palmai, by a different macroplacoid length sequence (1<2<3 in the new species vs. 1=2=3 in I. palmai), a different oral cavity armature (a ventral band of small round teeth in the new species vs. three transverse ventral and three dorsal ridges in I. palmai), a lower pt ratio of the stylet support insertion point (66.8 in the new species (specimen 230 μm in length) vs. 73.0 in I. palmai (specimen 222 μm in length)) and by the presence of eyes;

I. yunnanensis, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species vs. poriform sculpture in I. yunnanensis) and by the presence of lunules (but see also Remarks above).

Superfamily Macrobiotoidea Thulin, 1928 in Marley et al. 2011
Family Macrobiotidae Thulin, 1928
Genus Macrobius C.A.S. Schultze, 1834
Macrobius crenulatus Richters, 1904

Material examined. — 2 specimens + 1 exuvium.

Discussion

Recent systematic revision (Marley et al. 2011), placed the genus *Isohypsibius* Thulin, 1928 within the family Isohypsibiidae, along with *Doryphoribius* Pilato, 1969, *Thulinius* Bertolani, 2003, *Pseudobiotus* Nelson, 1980, *Halobiotus* Kristensen, 1982, *Ramajendas* Pilato et Binda, 1990, and *Eremobiotus* Biserov, 1992. *Isohypsibius* comprises 131 valid species (Degma et al. 2012); though further work is required on many older descriptions as characters defining the more recently described genera may have been missed or omitted from the original descriptions. For example the presence/absence of the ventral lamina on the buccal tube, a distinguishing character of *Doryphoribius*, was omitted in older descriptions as *D. vietnamensis* (Iharos, 1969) and *D. zyxiglobus* (Horning, Schuster et Grigarick, 1978) (Beasley et al. 2006 and Claxton et al. 2010, respectively). Similarly, the presence of twelve peribuccal lamellae around the mouth in *Thulinius*, or 30 peribuccal lamellae of *Pseudobiotus*, can be difficult to identify in poorly fixed specimens (e.g. *Thulinius augusti* (Murray, 1907), *Thulinius saltursus* (Schuster, Tofner et Grigarick, 1978), *Thulinius itoi* (Tsurusaki, 1980) or *Pseudobiotus kathmanae* Nelson, Marley et Bertolani, 1999 (Bertolani et al. 1999; Nelson et al. 1999; Marley et al. 2008; Kaczmarek and Michalczyk 2009; Kaczmarek et al. 2010). It is therefore important to provide as detailed descriptions as possible for new *Isohypsibius* species, with clear statements on the absence of the above mentioned characters as ventral lamina and peribuccal lamellae.

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