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Soil tardigrades from the Antarctic Peninsula with a description of a new species and some remarks on the genus *Ramajendas* (Eutardigrada: Isohypsibiidae)

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ABSTRACT. In thirteen (mostly soil) mixed samples, collected from nine localities on the Antarctic continent and some of the neighbouring islands, 788 specimens and 32 eggs of tardigrades were found. In total, five species were identified: *Acutuncus antarcticus, Echiniscus jenningsi*, *Diphascon* (*D.*) *victoriae*, *Hypsibius dujardini* and *Ramajendas dastychi* sp. nov. *A. antarcticus* was the most abundant (nearly 90% of all specimens) and was the prevailing taxon found in the majority of locations. *R. dastychi* sp. nov. is the fourth species described in the exclusively Antarctic/sub-Antarctic genus. The new species differs from all other congeners by the presence of four gibbosities on the caudo-dorsal cuticle (configuration II:2–2) and also by some morphometric characters. In this paper we also briefly discuss the taxonomy and zoogeography of the genus *Ramajendas*.

Introduction

The Antarctic is divided into biogeographic zones that correspond to different climatic regions: the sub-Antarctic zone (small groups of islands scattered in the Southern Ocean), the maritime Antarctic (west coast of the Antarctic Peninsula and neighbouring archipelagos), and finally the continental Antarctic (east and south part of the Antarctic Peninsula and the Antarctic continent) (Ochyra 1998).

The phylum Tardigrada consist currently of ca. 1000 species inhabiting terrestrial and aquatic (freshwater and marine) environments throughout the world (Degma and others 2012). Tardigrades are known as one of the most extreme-climate resistant animals in the world, thus it is not surprising that they are also found in the Antarctic. Studies on Antarctic tardigrades were initiated in the beginning of the 20th century and progressed very slowly up to the 1990s and 2000s when more research on the subject was conducted. Currently, nearly fifty tardigrade species have been reported from the Antarctic region, mostly from islands in the maritime Antarctic (Convey and McInnes 2005).

In this study we report five species from the Antarctic, including one new species that belongs to a southern hemisphere genus *Ramajendas* Pilato and Binda, 1990. *Ramajendas* differs from other genera of the family Isohypsibiidae by having the *Ramajendas*-type claws (that is with very long and node-like curved primary branches of external claws). So far, the genus has comprised three species. The first species was described by Ramazzotti in 1972 as *Hypsibius* (*Isohypsibius*) *renaudi* from the Kerguelen Islands (sub-Antarctic) and was later transferred to the genus *Isohypsibius* Thulin, 1928 by Ramazzotti and Maucci (1983). In 1990 Pilato and Binda erected the genus *Ramajendas*, described a new species *R. frigidus* from Victoria Land (continental Antarctica) and also transferred *Isohypsibius renaudi* to the new genus. The last species, until now, *R. heatwolei* was described by Miller and others in 1995 from Macquarie Island (sub-Antarctic).

Four other species found in this study, *Acutuncus antarcticus* (Richters, 1904), *Echiniscus jenningsi* Dastych, 1984, *Diphascon* (*D.*) *victoriae* Pilato and Binda, 1999, *Hypsibius dujardini* (Doyère, 1840), have been already reported from the Antarctic region in the past (Convey and McInnes 2005).

Material and methods

Thirteen mostly soil samples were collected from nine localities on the Antarctic continent and some of the

neighbouring islands, during the 14th Ukrainian Antarctic Expedition between the 22 February and the 28 March 2010 by the second author (for more details see below). Samples were collected and examined for tardigrades using standard methods (Dastych 1980). After extractions, animals were mounted on microscope slides in Hoyer's medium. All specimens were examined, measured and photographed using Phase Contrast Microscopy (PCM). In total 788 specimens and 32 eggs were examined.

All measurements are given in micrometers [μm]. Structures were measured only if their orientation was suitable. Body length was measured from the anterior extremity to the end of the body, excluding the hind legs. Buccal tube length and the level of the stylet support insertion point were measured according to Pilato (1981). Buccal tube width was measured as the external diameter at the level of the stylet support insertion point. Claws were measured according Beasley and others (2008). 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). Configuration of cuticular gibbosities is denoted according to Michalczyk and Kaczmarek (2010).

Species were identified using keys in Fontoura and Pilato (2007), Kaczmarek and others (2011), Ramazzotti and Maucci (1983), and original descriptions (Dastych 1984; Miller and others 1995, 2005; Pilato and Binda 1990, 1997, 1999). Tardigrade taxonomy is presented according to Marley and others (2011) and Degma and others (2012).

Raw data underlying the description of *Ramajendas dastychi* sp. nov. are deposited in the Tardigrada register (Michalczyk and Kaczmarek in press) under http://www.tardigrada.net/register/0007.htm

Sampling localities

1. 65°10′22″S; 64°05′22″W, *ca.* 1 m asl: Antarctic Peninsula, Graham Coast, Kiev Peninsula, sea coast at the foot of south-western slopes of Mount Scott, moist coarse sand, 5 m inland (2 samples, slide codes: Mt Scott, sampling 1 and 2).
2. 65°14′54″S; 64°04′48″W, *ca.* 11 m asl: Antarctic Peninsula, Graham Coast, Kiev Peninsula, northern coast of Waddington Bay, sea coast at the foot of western slopes of Mount Mill, near an old British emergency hut, huge moss field on a rocky hill, as if at the bottom of a temporary stream (1 sample, slide code: VRA02).
3. 65°09′56″S; 64°08′47″W, *ca.* 9 m asl: west of Antarctic Peninsula, Wilhelm Archipelago, northern part of Petermann Island, lichen from rock (1 sample, slide code: 870/1).
4. 65°14′02″S; 64°09′43″W, *ca.* 2 m asl: west of Antarctic Peninsula, Wilhelm Archipelago, Yalour Islands, fine soil from the bed of a dry stream (2 samples, slide codes: V04, sampling 1 and 2).
5. 65°15′04″S; 64°14′34″W, *ca.* 5 m asl: west of Antarctic Peninsula, Wilhelm Archipelago, Argentine Islands, eastern coast of Galindez Island**,** soil (1 sample, slide code: V12 sampling 1).
6. 65°15′09″S; 64°15′44″W, *ca.* 5 m asl: west of Antarctic Peninsula, Wilhelm Archipelago, Argentine Islands, northern part of Skua Island, wet sand and stones with organic remnants at the glacier foot (2 samples, slide codes: VS03, sampling 1 and 2).
7. 65°14′13″S; 64°18′18″W, *ca.* 5 m asl: west of Antarctic Peninsula, Wilhelm Archipelago, Argentine Islands, North-east Barchans Islands, relatively wet coarse sand among pebbles (2 samples, slide codes: V0201 sampling 1 and V0203).
8. 65°10′45″S; 64°29′32″W, *ca.* 5 m asl: west of Antarctic Peninsula, Wilhelm Archipelago, Roca Islands, Locator Island, very fine soil (1 sample, slide code: MR, Middle Roca sampling 1).
9. 65°11′49″S; 64°32′19″W, *ca.* 5 m asl: west of Antarctic Peninsula, Wilhelm Archipelago, Cruls Islands, fine soil among rocks in an inlet of the island (1 sample, slide code: Crulz sampling 2).

Results

In total we found 788 tardigrade specimens and 32 eggs. Definitely the most abundant species was *Acutuncus antarcticus* (684 specimens, 87% of all records). Moreover, we found: *Echiniscus jenningsi*, *Diphascon* (*D.*) *victoriae*, *Hypsibius dujardini* and *Ramajendas dastychi* sp. nov. (see below for more details).

In two samples we also found thirteen specimens belonging to the *Macrobiotus harmsworthi* group, however, given that we have not found their eggs, we were unable to identify them to the species level. In order to avoid potential misinterpretations, we decided not to report here these incomplete identifications.

Taxonomic Account

Phylum: Tardigrada (Spallanzani, 1777); **Class:** Heterotardigrada Marcus, 1927; **Superfamily:** Echiniscoidea Richters, 1926; **Family:** Echiniscidae Thulin, 1928; **Genus:** *Echiniscus* C.A.S. Schultze, 1840.

***Echiniscus jenningsi* Dastych, 1984; Number of specimens:** 1 individual; **Localities: 1**

Remarks: Even though we found only a single specimen, we were confident in the identification as it corresponded perfectly to the original description by Dastych (1984). The species belongs to the *bigranulatus* group (*sensu* Michalczyk and Kaczmarek 2006, 2007). It is a southern hemisphere species limited to Antarctic Regions. It was previously known only from the Antarctic Peninsula and some of Antarctic Islands (McInnes 1994).

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Table 1. Measurements [in μm] and *pt* values of selected morphological structures of *Ramajendas dastychi* sp. nov. mounted in Hoyer's medium (N – number of specimens/structures measured, RANGE refers to the smallest and the largest structure among all measured specimens; SD – standard deviation, ? – trait oriented unsuitably for measurement).

		RANGE						MEAN		SD		Holotype	
CHARACTER	N	μm			*pt*			μm	*pt*	μm	*pt*	μm	*pt*
Body length	15	230	–	480	*807*	–	*1040*	403	*954*	63	*71*	370	*883*
Buccal tube													
Buccal tube length	15	28.5	–	47.7		–		42.0	–	4.4	–	41.9	–
Stylet support insertion point	15	19.7	–	32.1	*66.1*	–	*69.3*	28.4	*67.8*	2.8	*0.8*	28.6	*68.3*
Buccal tube external width	14	3.0	–	5.4	*10.2*	–	*12.3*	4.7	*11.2*	0.6	*0.7*	4.7	*11.2*
Buccal tube internal width	14	1.7	–	3.5	*6.0*	–	*8.3*	2.8	*6.7*	0.4	*0.8*	3.3	*7.9*
Placoid lengths													
Macroplacoid 1	15	4.3	–	7.3	*15.0*	–	*16.2*	6.5	*15.5*	0.7	*0.4*	6.8	*16.2*
Macroplacoid 2	15	3.4	–	5.4	*9.8*	–	*12.3*	4.7	*11.2*	0.6	*0.8*	4.1	*9.8*
Macroplacoid row	15	8.7	–	15.1	*29.4*	–	*31.7*	12.8	*30.4*	1.5	*0.7*	12.4	*29.6*
Claw 1 lengths													
External base	11	8.3	–	13.9	*28.2*	–	*32.0*	12.4	*30.2*	1.6	*1.2*	12.9	*30.8*
External primary branch	10	20.3	–	32.3	*59.1*	–	*71.9*	27.6	*67.3*	3.9	*4.7*	27.0	*64.4*
External secondary branch	11	8.7	–	14.1	*26.2*	–	*31.8*	12.3	*29.9*	1.7	*1.8*	12.3	*29.4*
Internal base	5	5.9	–	10.1	*20.5*	–	*23.3*	8.4	*21.3*	1.6	*1.2*	8.6	*20.5*
Internal primary branch	5	8.6	–	14.0	*26.3*	–	*32.3*	11.4	*29.0*	2.0	*2.3*	11.0	*26.3*
Internal secondary branch	5	5.4	–	12.0	*18.9*	–	*27.7*	9.3	*23.4*	2.4	*3.2*	9.3	*22.2*
Claw 2 lengths													
External base	14	8.3	–	16.0	*29.1*	–	*35.9*	14.0	*33.1*	2.0	*2.1*	13.8	*32.9*
External primary branch	14	20.2	–	35.0	*67.4*	–	*79.2*	30.6	*72.7*	3.9	*3.7*	28.5	*68.0*
External secondary branch	14	9.3	–	15.5	*28.8*	–	*35.7*	13.6	*32.4*	1.7	*1.9*	13.3	*31.7*
Internal base	10	7.4	–	12.1	*22.9*	–	*27.7*	10.7	*25.6*	1.5	*1.5*	9.6	*22.9*
Internal primary branch	6	8.6	–	16.7	*24.6*	–	*35.0*	13.1	*31.6*	3.2	*3.8*	?	*?*
Internal secondary branch	11	7.7	–	13.4	*24.4*	–	*31.0*	11.6	*27.6*	1.7	*2.0*	12.0	*28.6*
Claw 3 lengths													
External base	14	8.9	–	16.2	*30.4*	–	*36.6*	14.0	*33.7*	1.9	*1.9*	14.0	*33.4*
External primary branch	14	21.0	–	35.3	*66.3*	–	*78.7*	30.7	*73.8*	3.9	*4.0*	29.2	*69.7*
External secondary branch	14	9.6	–	15.9	*29.5*	–	*36.1*	13.7	*32.9*	1.5	*1.6*	13.7	*32.7*
Internal base	13	6.8	–	12.1	*20.7*	–	*27.5*	10.4	*24.8*	1.6	*2.2*	9.4	*22.4*
Internal primary branch	12	8.4	–	16.0	*25.4*	–	*36.4*	12.7	*30.7*	2.2	*3.2*	11.5	*27.4*
Internal secondary branch	13	7.5	–	14.5	*24.5*	–	*32.3*	11.5	*27.5*	1.8	*2.4*	10.5	*25.1*
Claw 4 lengths													
Anterior base	0		?			*?*		?	*?*	?	*?*	?	*?*
Anterior primary branch	0		?			*?*		?	*?*	?	*?*	?	*?*
Anterior secondary branch	0		?			*?*		?	*?*	?	*?*	?	*?*
Posterior base	3	9.0	–	16.8	*31.6*	–	*40.3*	14.0	*35.7*	4.4	*4.4*	?	*?*
Posterior primary branch	3	24.2	–	40.0	*83.9*	–	*90.8*	33.6	*86.5*	8.3	*3.8*	?	*?*
Posterior secondary branch	3	9.4	–	16.0	*33.0*	–	*38.6*	13.7	*35.0*	3.7	*3.1*	?	*?*

Class: Eutardigrada Richters, 1926; **Order:** Parachela Schuster, Nelson, Grigarick and Christensen, 1980; **Superfamily:** Hypsibioidea Pilato, 1969 (in Marley and others 2011); **Family:** Hypsibiidae Pilato, 1969; **Subfamily:** Diphasconinae Dastych, 1992**; Genus:** *Diphascon* (*Diphascon*) Plate, 1888.

***Diphascon (Diphascon) victoriae* Pilato and Binda, 1999; Number of specimens:** 3 individuals;
Localities: 6.

Remarks: Although we found only three specimens, we were confident in identifying them to *D.* (*D.*) *victoriae* because they corresponded perfectly to the original description by Pilato and Binda (1999) and were also successfully identified with the key by Fontoura and Pilato (2007). The species belongs to the *pingue* group and has been previously known only from its *locus typicus* in the Victoria Land (Pilato and Binda 1999).

Subfamily: Hypsibiinae Pilato, 1969**; Genus:** *Acutuncus* Pilato and Binda, 1997.

***Acutuncus antarcticus* (Richters, 1904) Number of specimens:** 684 individuals and 17 eggs. **Localities:** 1, 3–4 and 6–9.
Remarks: The most abundant and prevalent species in the studied material. It was present in 10 of 13 examined samples (from 7 of 9 localities). Formally placed in *Hypsibius*, the species was often misidentified, but work by Dastych (1991) corrected/amended most of the records and Pilato and Binda (1997) reassigned the species to the current genus. *A. antarcticus* is widely distributed in fellfield and freshwater habitats in the Antarctic, but

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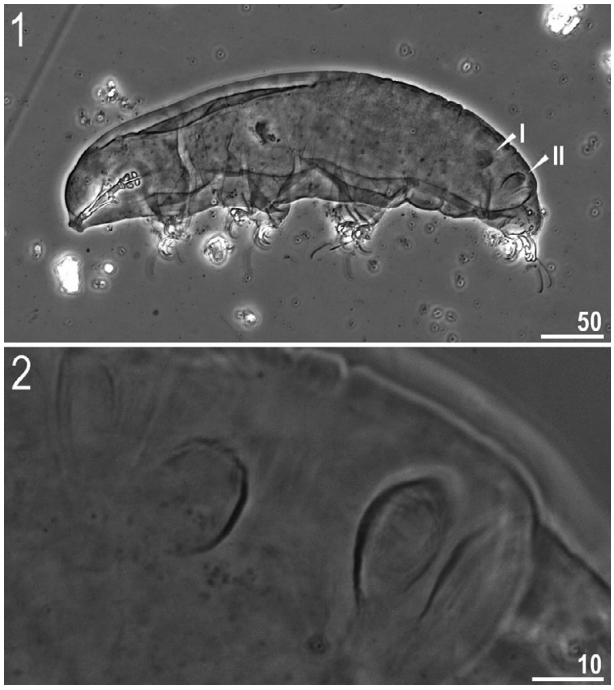
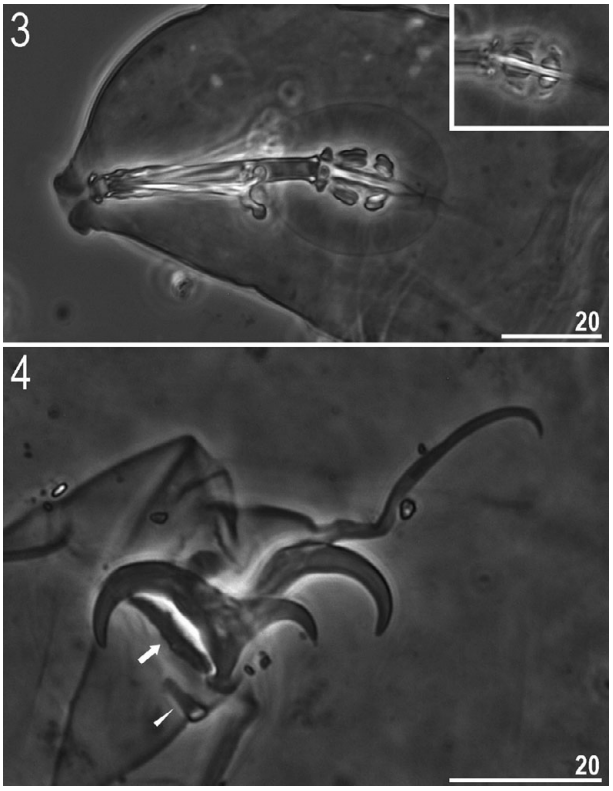


Figs. 1–2. *Ramajendas dastychi* sp. nov.: 1 habitus (holotype, lateral view, arrowheads indicate the two rows of gibbosities); 2 caudo-dorsal cuticle with gibbosities (holotype).



Figs. 3–4. *Ramajendas dastychi* sp. nov.: 3 buccal apparatus (holotype, lateral view, the insert shows placoids in the frontal view); 4 claws III (paratype, arrow indicates the short longitudinal bar and the arrowhead shows a portion of the thin transverse bar).

was also reported from South Shetlands, South Orkneys, South Sandwich Island and South Georgia. All records from the northern hemisphere are probably misidentifications (Dastych 1991).

Genus: *Hypsibius* Ehrenberg, 1848.

***Hypsibius dujardini* (Doyère, 1840); Number of specimens:** 6 individuals; **Localities:** 2.
Remarks: Considered cosmopolitan in the past, it is most likely a complex of very similar (possibly also cryptic) species found throughout the world. Because of a poor original description *Hypsibius dujardini sensu stricto* urgently needs a modern redescription. Nevertheless, the examined specimens correspond perfectly with the original description and the *H. dujardini* characters proposed by Miller and others (2005).

Superfamily: Isohypsibioidea Marley, McInnes and Sands, 2011; **Family:** Isohypsibiidae Marley, McInnes and Sands, 2011; **Genus:** *Ramajendas* Pilato and Binda, 1990.

***Ramajendas dastychi* sp. nov.** (Table 1, Fig. 1–4); **Localities:** 3, 5, 8; **Type material:** Holotype and 67 paratypes (56 individuals and 11 eggs)**; Type locality:** 65°09′56″S; 64°08′47″W, ca. 9 m asl: west of Antarctic Peninsula, Wilhelm Archipelago, northern part of Petermann Island, lichen from rock (1 sample, slide code: 870/1); **Additional material:** samples 5 (23 specimens and 4 eggs) and 8 (1 specimen).
Description (measurements in Table 1): Body white/colourless (after preparation) (Fig. 1). Eyes present (also after preparation). Cuticle smooth, but with two transverse rows of gibbosities in the caudo-dorsal part of the body (two gibbosities in each row, configuration II:2–2) (Figs. 1–2). In some individuals gibbosities are well defined whereas in others weakly developed and therefore difficult to identify under PCM. Legs without gibbosities.

Mouth antero-ventral. Peribuccal lamellae and papulae absent. Bucco-pharyngeal apparatus of the *Isohypsibius* type (Fig. 3). Oral cavity armature absent or not visible under PCM. Buccal tube without the ventral lamina and with a single delicate posterior bend (visible in lateral view only). Two rod-shaped macroplacoids in the pharyngeal bulb present. The first macroplacoid (with a central constriction) distinctly longer than the second (with a subtle posterior constriction). Microplacoid and septulum absent (Fig. 3).

Claws of the *Ramajendas* type with extremely elongated and flexible primary branches of external claws (Fig. 4). Primary branches of claws with thin, but well visible accessory points ending at the highest point of the primary branch. Smooth lunules (Fig. 4) present on all legs, but distinctly larger under external claws. A single long and thin transverse (sometimes sinusoidal in shape) cuticular bar is present under internal and external claws I–III. Additionally, a short thick longitudinal (often with

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Table 2. Distribution of the species from genus *Ramajendas* based on current literature (1 – Ramazzotti (1972); 2 – Jennings (1976a); 3 – Jennings (1976b); 4 – Dastych (1994); 5 – Usher and Dastych (1987); 6 – Pilato and Binda (1990); 7 – Miller and others (1995); 8 – Miller and others (1996); 9 – present study; * – see remarks and discussion).

Species	Localities	Citations	Remarks
R. dastychi sp. nov.	**Maritime Antarctica:** Petermann Island (Type locality), Gallindez and Locator Islands	**9**	Known only from the *locus typicus* and two other islands in neighbourhood.
*R. frigidus**	**Continental Antarctic:** Victoria Land (Crater Cirque, Kay Island); Windmill Islands (Bailey Peninsula)	6, 8	Only confirmed localities are presented here (see comments on *R. renaudi* and the discussion).
R. heatwolei	**sub-Antarctic:** Macquarie Island	7	Known only from the *locus typicus*.
*R. renaudi**	**sub-Antarctic:** Kerguelen Islands **Maritime Antarctic:** Alexander Island; Antarctic Peninsula, Astrolabe Island; Dion Islands (Emperor Is., Courtier Is.); Graham Land (Brewster Is); Macquarie Bay (Avian Island, Horseshoe Island, Barbara Island); Palmer Archipelago (Brabant Is., Torgersen Is.); South Orkney Islands (Signy Is., Monroe Is.); South Sandwich Islands (Candlemas Is.); South Shetlands Islands (King George Is., Deception Is.); Thumb Rock	1, 2, 3, 4, 5, 6	As it was earlier suggested by Pilato and Binda (1990) and subsequently confirmed by Miller *et al.* (1995), some records of this species may in fact belong to *R. frigidus*. However, this needs to be verified with the original material. For the time being, we assumed that *R. renaudi* has a wide geographic range but some records need to be verified.

irregular edges) bar is present near internal claws I–III (Fig. 4).

Eggs (3–7) smooth, deposited in exuvium.

Differential diagnosis

Until now only three species were described in the genus *Ramajendas*. The new species differs from all others by the presence of gibbosities on the dorso-caudal cuticle. Additionally, *R. dastychi* sp. nov. differs specifically from:

R. frigidus Pilato and Binda, 1990 by shorter placoids (I: (7.5 [*19.6*]) and II (5.3 [*13.9*])) in *R. frigidus* (specimen 330 long) *vs.* (I: (6.0 [*15.2*]) and II (4.0 [*10.2*]) in the new species (specimen 336 long)), and by a shorter placoid row (13.1 [*34.3*] in *R. frigidus* (specimen 330 long) *vs.* 11.6 [*29.4*] in the new species (specimen 336 long)).

R. heatwolei Miller and others, 1995 by the absence of cuticular sculpturing, stylet supports inserted in a more caudal position (*pt ca. 63.6* in *R. heatwolei vs. 66.1–69.3* in the new species), and by a slightly larger body size (187–275 in *R. heatwolei vs.* 230–480 in the new species).

R. renaudi (Ramazzotti, 1972) by slightly shorter buccal tube (42.8 in *R. renaudi* (specimen 330 long) *vs.* 39.4 in the new species (specimen 336 long)), shorter placoids (I: (9.8 [*22.9*]) and II (7.3 [*17.0*])) in *R. renaudi* (specimen 330 long) *vs.* (I: (6.0 [*15.2*]) and II (4.0 [*10.2*]) in the new species (specimen 336 long)), and by a shorter placoid row (18.2 [*42.4*] in *R. renaudi* (specimen 330 long) *vs.* 11.6 [*29.4*] in the new species (specimen 336 long)).

We dedicate this species to a distinguished Polish tardigradologist, Dr. Hieronymus Dastych.

Type depositories: Holotype and 80 paratypes are deposited in the Department of Animal Taxonomy and Ecology at the Adam Mickiewicz University (Poznań, Poland).

Discussion

Distribution of the genus *Ramajendas* is limited to the Antarctic, maritime Antarctic and sub-Antarctic regions. Ecologically, *Ramajendas* has been most often reported from interstitial habitats near or on the beach. The nominal species, *Ramajendas renaudi*, appears to have a relatively wide distribution, for example it is known from the Kerguelen Islands (*locus typicus*), many islands in the maritime Antarctic and also from the Antarctic Peninsula. *R. frigidus* has been reported only from Victoria Land and the Windmill Islands (Convey and McInnes 2005). Finally, *R. heatwolei* is known from its type locality (Macquarie Island) and *R. dastychi* sp. nov., from its type locality (Petermann Island) and immediate environs (Locator and Gallindez Islands) (see also Table 2 and the comments below).

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The relatively wide geographic range of *R. renaudi* is potentially an artefact caused by the unique claw morphology of this species. Unique and obvious traits are probably a primary cause of underestimation of species numbers which in turn result in overestimation of species ranges. This is so, because when such traits are present, researchers seem not to pay sufficient attention to other morphological and morphometric details and variation. As a consequence, multiple taxa are likely to be attributed to a single species. This phenomenon has been described in other tardigrade taxa such as *Milnesium*, *Paramacrobiotus* or the *Macrobiotus hufelandi* and *harmsworthi* groups (for example Michalczyk and others 2012a, 2012b). Thus, it would not be surprising if older records of specimens with *Ramajendas*-type claws were classified as *R. renaudi* without further insights into potential differences in morphometry. As it was shown by Pilato and Binda (1990), some species of the genus *Ramajendas* have a very similar morphology but are clearly different in terms of morphometry. Pilato and Binda (1990) additionally suggested that some specimens reported by Dastych (1984) as *Hypsibius renaudi*, and by Jennings (1976a, 1967b) as *Hypsibius* (*Isohypsibius*) *renaudi*, may in fact belong to *R. frigidus*. Another possibility is that these maritime Antarctic *R. frigidus*/*R. renaudi* individuals represent a number of as yet undescribed species that could be differentiated only by morphometric characters. Thus, based on the limited available data and the above mentioned concerns, we should conclude that the exact geographic ranges of *R. frigidus* and *R. renaudi* cannot be currently described beyond the type locales, although they may indeed be wider.

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