Preliminary description of tardigrade species diversity and distribution pattern around coastal Syowa Station and inland Sør Rondane Mountains, Dronning Maud Land, East Antarctica.

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Abstract

Tardigrades are important members of the simple terrestrial ecosystems in the extreme environments in Antarctica. This study provides a baseline description of tardigrade species diversity and distribution pattern within the terrestrial and lake environments of the coastal regions around Syowa Station and the neighbouring inland Sør Rondane Mountains, Dronning Maud Land. We combined data obtained from new and previously described collections and updated data available in the existing literature. We recorded five tardigrade species, three of which (*Echiniscus pseudowendti* Dastych 1984, *Hebesuncus ryani* Dastych and Harris 1994, *Pseudechiniscus* sp.) have not previously been reported from the area, increasing the total recorded tardigrade diversity for this region of continental Antarctica to nine species. The results of our study indicate that tardigrades have been and are major components of the lake environment community in continental Antarctica, with *Acutuncus antarcticus* Richters 1904 the most common and dominant species. Our data confirm that the tardigrade species diversity in the vicinity of Syowa Station is very low, and suggest potential relationships between individual tardigrade species and terrestrial moss species and depth in freshwater ecosystems.

Keywords: Tardigrades, Antarctica, species diversity, distribution pattern, freshwater lakes, mosses

Introduction

Terrestrial ecosystems in Antarctica are considered relatively simple, comprising a limited flora of bryophytes, lichens, algae, and cyanobacteria, and an invertebrate fauna of micro-arthropods, nematodes, tardigrades, rotifers, and protozoans (Convey 2013). The severe environmental conditions of continental Antarctica and very limited extent of ice-free ground result in very low floral and faunal diversity (Adams et al. 2006; Cannone et al. 2013). Tardigrades are important members of the simple faunal assemblages found in these extreme environments.

The greater number of research stations and relative ease of access to the Antarctic Peninsula (maritime Antarctica) has resulted in a reasonably well documented knowledge of tardigrade communities, which is in marked contrast to those of continental Antarctica (Convey and McInnes 2005). Reports of tardigrades from Dronning Maud Land in continental Antarctica include studies of inland nunataks (Dastych and Drummond 1996; Dastych and Harris 1994; Sohlenius et al. 1995; 1996; 2004; Sohlenius and Boström 2005), the vicinity of Syowa Station (Morikawa 1962; Sudzuki 1964), and a biogeographic study of the coastal regions and neighbouring inland sites at the Sør Rondane Mountains (Utsugi and Ohyama 1989). Two tardigrade taxa (*Acutuncus* sp. and *Macrobiotus* sp.) were recently reported in a biogeographic study (Czechowski et al. 2012) using molecular operational taxonomic units (MOTUs) to explore Sør Rondane Mountains invertebrate diversity. A molecular and morphological study of *Acutuncus antarcticus* Richters 1904 obtained from terrestrial moss in the vicinity of Syowa Station has also been completed recently (Kagoshima et al. 2013). These limited studies suggest that the regional tardigrade diversity is under-reported and, as is often the case with these cryptic and under-researched groups (Adams et al. 2006), requires updating in the light of current taxonomic knowledge.

Reports of tardigrades from lake and other freshwater environments in the continental Antarctic are even more restricted. Lake ecosystems hold some of the most diverse vegetation on the Antarctic continent (Quesada et al. 2008). The absence of physiological stresses such as freezing and desiccation in the lake environment allows the growth and accumulation of vegetation on lake beds consisting of various mat-forming cyanobacteria, algae and aquatic mosses (Imura et al. 2003; Priddle and Dartnall 1978; Quesada et al. 2008; Sabbe et al. 2004). Lakes and ponds in even the most extreme and otherwise biologically barren locations host well-developed cyanobacterial mats (Hodgson et al. 2010). Maritime Antarctic lakes can support a high diversity of tardigrades, largely due to the absence of macrofaunal competitors and predators (McInnes and Pugh 1999). In continental Antarctica a small number of tardigrade species have been reported from a pond in Dronning Maud Land (Morikawa 1962), a small lake in Victoria Land (Binda and Pilato 2000) and lakes and ponds in the Pensacola Mountains (Hodgson et al. 2010). A study of sediment cores collected from Enderby Land lakes confirmed that at least three species had been present throughout the Holocene (Gibson et al. 2007), and a recent molecular study of eukaryotic phylotypes indicated the presence of two tardigrade species in aquatic mosses in Hotoke-Ike Lake in the vicinity of Syowa Station (Nakai et al. 2012). In these freshwater ecosystems in continental Antarctica, where tardigrades can be abundant and even dominant, understanding of ecosystem structure and function requires more detailed knowledge of species diversity and distribution.

The objective of this study was to provide a baseline description of tardigrade species diversity and distribution pattern within the terrestrial and lake environments of the coastal regions around Syowa Station and the neighbouring inland Sør Rondane Mountains, Dronning Maud Land, East Antarctica. We combine data obtained from new and previously described collections and update data available in the existing literature

Materials and Methods

During the 49th Japanese Antarctic Research Expedition (JARE 49) summer operation between December 2007 and February 2008, nine terrestrial moss samples (approximately 5 cm^3 each) were collected from Sinnan Iwa (67°57'S, 44°34'E), East Ongul Island (69°28′S 39°39′E), Langhovde (69°14'S, 39°44'E), Skarvsnes (69°28′S 39°39′E), and Skallen (69°40′S 39°25′E) (Fig. 1a-c), and stored in sealed plastic containers at 4°C. Twenty-four benthic samples were collected from five freshwater lakes (Fig. 1d) in Skarvsnes using a glove sampler (Ekman-Birge type, RIGO). The benthic samples were collected from three separate depths in four of the lakes, and 12 depths at Naga-Ike Lake, in order to examine any association of depth with tardigrade diversity and distribution. These samples were placed into 2.5ml tubes, stored at -70 °C and returned frozen to Japan. During the JARE 53 summer operation in January 2012, three additional terrestrial moss samples (approximately 5 cm^3 each) were collected from the Sør Rondane Mountains (72°00'S, 24°00'E) (Fig. 1b).

Terrestrial moss samples were placed into individual Petri dishes to which water was added, and then left at room temperature (approximately 20°C) for 2 - 3 h. Frozen samples from lakes were first thawed at 3°C for 24 h, before being placed into individual Petri dishes and water added. All the samples were disaggregated with tweezers in the Petri dish and then examined under a dissection microscope. Tardigrades were isolated and mounted on slides in Faure's solution, then identified under a phase-contrast microscope. Terrestrial moss samples were identified under the light microscope following Ochyra et al. (2008).

Results

Three species of tardigrade, *A. antarcticus*, *Echiniscus pseudowendti* Dastych 1984, *Hebesuncus ryani* Dastych and Harris 1994, were identified from the terrestrial moss samples (Table 1; Fig. S1a-c). Four moss species, *Bryum argenteum, B. pseudotriquetrum*, *Ceratodon purpureus*, *Coscinodon lawianus*, were present in these samples, and tardigrades were extracted in good numbers from 25% of the samples examined, with only a single species being obtained from any given moss.

Three species of tardigrade, *A. antarcticus*, *Diphascon (Diphascon) langhovdense* Sudzuki 1964, *Pseudechiniscus* sp., were obtained from the phyto-benthos samples of the Skarvsnes lakes (Table 2; Fig. S1d, e). *A. antarcticus* was present in all the lakes sampled and, although we did not quantify the abundance, there was variation in the numbers present in each sample. *Diphascon (D.) langhovdense* was only obtained from Naga-Ike Lake at 0.8m depth, and in lower numbers than *A. antarcticus* from the same sample. The *Pseudechiniscus* sp. (suillis-group) found in the present study is significantly different from the congener reported by Utsugi and Ohyama (1989) in terms of the size and density of its dorsal granulation (Fig. S1e). Only one individual, an adult female, of *Pseudechiniscus* sp. was obtained from Tsubaki-Ike Lake.

Discussion

Within the environs of Syowa Station six tardigrade species have previously been reported from the terrestrial environment and two from fresh waters (Table 3). In this study we recorded five tardigrade species, three of which (*Echiniscus pseudowendti*, *Hebesuncus ryani*, *Pseudechiniscus* sp.) have not previously been reported from the area. *E. pseudowendti* was first described in Enderby Land (Dastych 1984) and is considered to be restricted to continental Antarctica (Convey and McInnes 2005). *H. ryani* was first reported from inland nunataks in western Dronning Maud Land (Dastych and Harris 1994) and has since been found at further inland nunatak sites in Dronning Maud Land and Ellsworth Land (Convey and McInnes 2005; Sohlenius et al. 1995; 2004; Sohlenius and Boström 2005). With only a single specimen of *Pseudechiniscus* sp. available in the current study it is inappropriate to describe this as a new species until further material becomes available. The more common species identified here (*A. antarcticus* and *D. (D.) langhovdense*) have been reported from different nunataks within Dronning Maud Land (Dastych and Drummond 1996; Sohlenius et al. 1995; Sohlenius and Boström 2005). All the species found in this study have only been reported from the Antarctic, and increase the total recorded for this region of continental Antarctica to nine species.

Miller et al. (1996) reported strong associations between *A. antarcticus* and *Bryum* spp. mosses in ice-free regions of the Windmill Islands near Casey Station, East Antarctica. These two taxa again occurred together here, although the overall number of samples available precludes any categorical conclusion of relationship between tardigrade and moss species. Whereas mosses are perhaps the easiest to sample and appear to provide more favourable habitats for Antarctic tardigrades (e.g. Miller et al. 1996), further investigations should also include a wider range of habitat types (e.g. soils, algae, lichens) to fully understand any associations between tardigrades and different terrestrial habitats.

In the Skarvsnes lakes we obtained tardigrades, mainly *A. antarcticus*, across the sampling depth gradient (Table 2). Although present in all the lakes studied, the species diversity was much lower than that reported from maritime Antarctic lakes (McInnes 1995). In Hotoke Ike Lake in Skarvsnes unique, tall pillar-like colonies of aquatic mosses with epiphytic algae and cyanobacteria occur (Imura et al. 1999). A metagenomic study of these moss pillars (Nakai et al. 2012) reported the presence of tardigrades throughout the outer surface. Their study found tardigrade 18S rRNA sequences with close homology to known *A. antarcticus* and Northern Hemisphere *Diphascon* (*Diphascon*) *pingue* Marcus 1936 sequences. Previous studies have identified, *A. antarcticus* and *D. (D.) ongulensis* Morikawa 1962 from a pond in East Ongul Island (Morikawa 1962), and eggs and exuviae of *A. antarcticus*, *Macrobiotus blocki* Dastych 1984, and *Minibiotus weinerorum* Dastych 1984 from Holocene sediment cores from Enderby Land lakes (Gibson et al. 2007). These results indicate that tardigrades have been and are major components of the lake environment community in continental Antarctica, with *A. antarcticus* the most common and dominant species (e.g. Dastych and Drummond 1996; Dougherty and Harris 1963; Dougherty 1964; Murray 1910). The data obtained from Naga-Ike Lake suggest a potential relationship between tardigrade species and depth. *A. antarcticus* was present from the shallows to a depth of about 8.8m, while *D. (D.) langhovdense* occurred only at shallower sites. *Pseudoechiniscus* sp. found in Tsubaki-Ike Lake was also obtained at a shallow depth. *Pseudechiniscus* species are more commonly associated with terrestrial rather than aquatic habitats (Ramazzotti and Maucci 1983) and, as only a single individual was found, it is possible that individuals are blown or washed into the lake margins.

A. antarcticus is known to be one of the most widespread Antarctic tardigrade species and is present on sub-Antarctic islands, and in both maritime and continental

Antarctica (McInnes 1995). In the present study, *A. antarcticus* was abundant in the moss *B. argenteum* in Langhovde, and was found throughout the freshwater lakes studied in Skarvsnes, confirming reports that it occurs in both terrestrial and lake environments in the vicinity of Syowa Station (Kagoshima et al. 2013; Morikawa 1962; Sudzuki 1964; Utsugi and Ohyama 1989). Our samples collected at Abi-Ike Lake, which had been frozen at -70°C for over five years before analysis, included a number of live individuals. These individuals were able to develop and deposit eggs on culture media, clearly demonstrating considerable freeze tolerance ability in this species.

The results of our study confirm that the tardigrade species diversity in the vicinity of Syowa Station is very low, as earlier studies have suggested (Morikawa 1962; Sudzuki 1964; Utsugi and Ohyama 1989). However, we report three species not previously known for the region, increasing the recorded diversity. In the relatively simple terrestrial and freshwater ecosystems of continental Antarctica, typified by the environs of coastal Syowa Station and the neighbouring inland Sør Rondane Mountains, tardigrades are an important component of the biota. Biodiversity in Antarctica is currently under threat in association with the climate and other environmental change trends occurring in some parts of Antarctica (Quayle et al. 2002; Turner et al. 2009) together with the increasing risk of non-native species introduction into the continent (Chown et al. 2012). Further more detailed studies, with greater replication and sampling of a wider variety of habitats, are urgently required to be able to understand tardigrade species diversity and distribution patterns, and provide a baseline for identifying future changes.

Acknowledgements

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References

Adams B, Bardgett RD, Ayres E, Wall DH, Aislabie J, Bamforth S, Bargagli R, Cary C, Cavacini P, Connell L, Convey P, Fell J, Frati F, Hogg I, Newsham N, O'Donnell A, Russell N, Seppelt R, Stevens MI (2006) Diversity and distribution of Victoria Land biota. Soil Biol Biochem 38:3003–3018

Binda MG, Pilato G (2000) *Diphascon* (*Adropion*) *tricuspidatum*, a new species of eutardigrade from Antarctica. Polar Biol 23:75–76

Cannone N, Convey P, Guglielmin M (2013) Diversity trends of bryophytes in continental Antarctica. Polar Biol 36:259–271

Chown SL, Huiskes AHL, Gremmen NJM, Lee JE, Terauds A, Crosbie K, Frenot Y, Hughes KA, Imura S, Kiefer K, Lebouvier M, Raymond B, Tsujimoto M, Ware C, Vijver B, Bergstrom DM Continent-wide risk assessment for the establishment of nonindigenous species in Antarctica (2012) Proc Natl Acad Sci 109: 4938-4943

Convey P (2013) Antarctic Ecosystems. In Levin SA (ed) Encyclopedia of biodiversity, 2nd edn. vol 1. Elsevier, San Diego, pp 179–1888

Convey P, McInnes SJ (2005) Exceptional tardigrade–dominated ecosystems in Ellsworth Land, Antarctica. Ecology 86:519–527

Czechowski P, Sands CJ, Adams BJ, D'Haese CA, Gibson JAE, McInnes SJ, Stevens MI (2012) Antarctic Tardigrada: a first step in understanding molecular operational taxonomic units (MOTUs) and biogeography of cryptic meiofauna. Invertebr Syst 26:526–538

Dastych H (1984) The tardigrada from Antarctic with descriptions of several new species. Acta Zool Cracov 27:377–436Dastych H (1991) Redescription of *Hypsibius antarcticus* (Richters, 1904), with some notes on *Hypsibius arcticus* (Murray, 1907)

(Tardigrada). Mitt. Hamb. Zool. Mus. Inst. 88:141–159

Dastych H (2002/2003) *Diphascon langhovdense* (Sudzuki, 1964) stat. nov., a new taxonomic status for the semi-terrestrial tardigrade (Tardigrada). Acta Biol. Benrodis 12:19–25

Dastych H, Drummond AE (1996) Notes on limnic water–bears (Tardigrada) from the Robertskollen nunataks, Dronning Maud Land, Antarctica. Entomol Mitt 12: 111–117

Dastych H, Harris JM (1994) A new species of the genus *Hebesuncus* from the Antarctic nunataks, Dronning Maud Land (Tardigrada). Entomol Mitt 11:139–145

Dougherty EC (1964) Cultivation and nutrition of the micrometazoa. II. An antarctic strain of the tardigrade *Hypsibius arcticus* (Murray 1907) Marcus 1928, Trans. Am.Microsc. Soc. 83(1): 7-11.

Dougherty EC, Harris LG (1963) Antarctic micrometazoa: freshwater species in the McMurdo Sound area, Science 140: 497-498.Gibson JAE, Comer L, Agius JT, McInnes SJ, Marley NJ (2007) Tardigrade eggs and exuviae in Antarctic lake sediments: insights into Holocene dynamics and origins of the fauna. J Limnol 66(s1):65–71

Hodgson D, Convey P, Verleyen E, Vyverman W, McInnes S, Sands CS, Fernández–Carazo R, Wilmotte A (2010) Observations on the limnology and biology of the Dufek Massif, Transantarctic Mountains 82° South. Polar Science 4:197–214

Imura S, Bando T, Saito S, Seto K, Kanda H (1999) Benthic moss pillars in Antarctic lakes. Polar Biol 22:137–140

Imura S, Bando T, Seto K, Ohtani S, Kudoh S, Kanda H (2003) Distribution of aquatic mosses in the Sôya Coast region. Polar Biosci16:1–10

Kagoshima H, Imura S, Suzuki AC (2013) Molecular and morphological analysis of an Antarctic tardigrade, *Acutuncus antarcticus*. J Limnol 72(s1):15–23

McInnes SJ, Pugh PJA (1999) Zonation in Antarctic lake–dwelling benthic meiofauna, with emphasis on the Tardigrada. Zool Anz 238:283–288

McInnes SJ (1995) Tardigrades from Signy Island, South Orkney Islands, with particular reference to freshwater species. J Nat Hist 29:1419–1445

Miller WR, Miller JD, Heatwole HF (1996) Tardigrades of the Australian Antarctic Territories: the Windmill Islands, East Antarctica. Zool J Linn Soc 116:175–184

Morikawa K (1962) Notes on some Tardigrada from the Antarctic region. Biol Res Jap Ant Res Exp 17:3–6

Murray J (1910) Tardigrada, British Antarctic Expedition 1907-9, Reports on the Scientific Investigations, Vol. 1 Biology (Part V), 83-187 (plates 14-21).Nakai R, Abe T, Baba T, Imura S, Kagoshima H, Kanda H, Kohara Y, Koi A, Niki H, Yanagihara K, Naganuma T (2012) Eukaryotic phylotypes in aquatic moss pillars inhabiting a freshwater lake in East Antarctica, based on 18S rRNA gene analysis. Polar Biol 35:1495–1504

Ochyra R, Lewis Smith RI, Bednarek-Ochyra H (2008) The illustrated moss flora of Antarctica. Cambridge University Press, Cambridge

Priddle J, Dartnall HJG (1978) Biology of an Antarctic aquatic moss community. Freshw Biol 8:469–480

Quayle WC, Peck LS, Peat H, Ellis-Evans JC, Richard Harrigan P (2002) Extreme Responses to Climate Change in Antarctic Lakes. Science 295:645

Quesada A, Fernandez-Valiente E, Hawes I, Howard-Williams C (2008) Benthic primary production in polar lakes and rivers. In: Vincent WF, Laybourn–Parry J (eds) Polar lakes and rivers. Oxford University Press, Oxford, pp 179–196

Ramazzotti G, Maucci W (1983) Il phylum Tardigrada. Mem. Istit. Ital. Idrobiol. 41:1–1012

Sabbe K, Hodgson DA, Verleyen E, Taton A, Wilmotte A, Vanhoutte K, Vyverman W (2004) Salinity, depth and the structure and composition of microbial mats in continental Antarctic lakes. Freshw Biol 49:296–319

Sohlenius B, Boström S (2005) The geographic distribution of metazoan microfauna on East Antarctic nunataks. Polar Biol 28:439–448

Sohlenius B, Boström S, Hirschfelder A (1995) Nematodes, rotifers and tardigrades from nunataks in Dronning Maud Land, East Antarctica. Polar Biol 15:51–56

Sohlenius B, Boström S, Hirschfelder A (1996) Distribution patterns of microfauna (nematodes, rotifers and tardigrades) on nunataks in Dronning Maud Land, East Antarctica. Polar Biol 16:191–200

Sohlenius B, Boström S, Jönsson IK (2004) Occurrence of nematodes, tardigrades and rotifers on ice–free areas in East Antarctica. Pedobiologia 48:395–408

Sudzuki M (1964) On the microfauna of the Antarctic region. 1. Moss-water community at Langhovde. JARE Sci Rep 19:1–41

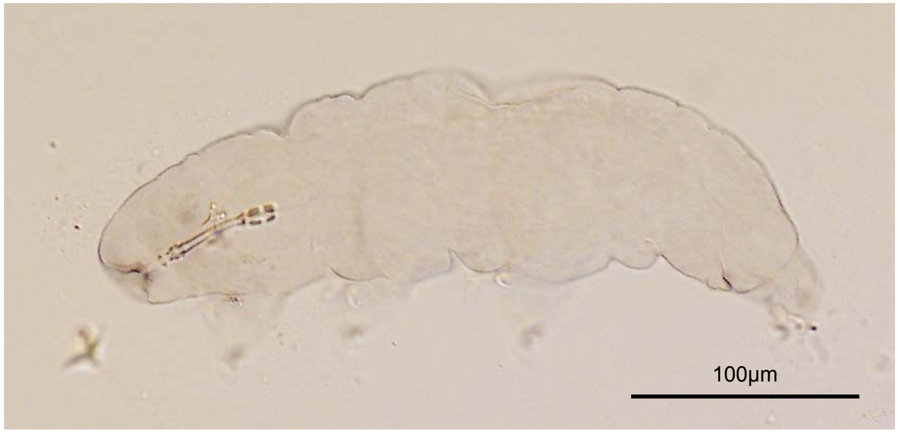
Turner J, Bindchadler R, Convey P, Di Prisco G, Fahrbach E, Gutt J, Hodgson D, Mayewski P, Summerhayes C (eds) (2009) Antarctic Climate Change and the Environment. Scientific Committee on Antarctic Research, Cambridge

Utsugi K, Ohyama Y (1989) Antarctic Tardigrada. Proc NIPR Symp Polar Biol 2:190–197

Figure captions

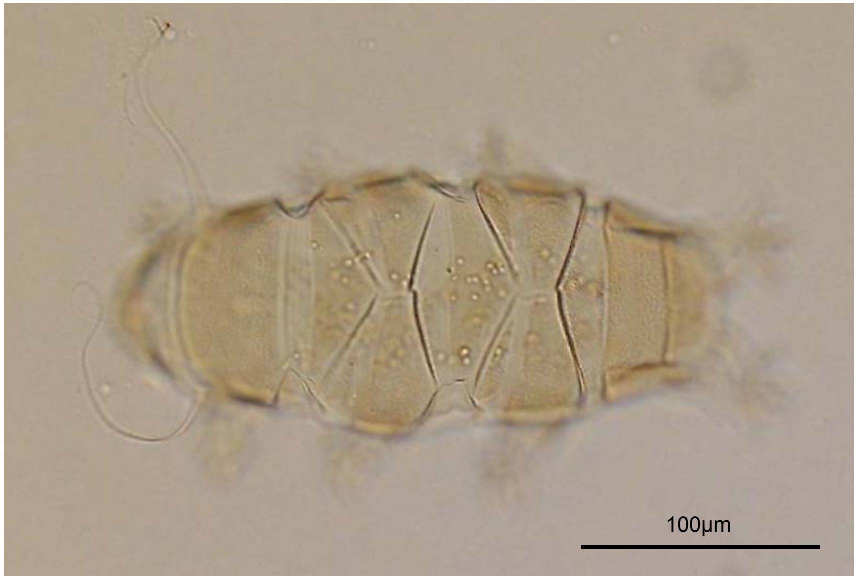
Fig. 1a A Map of Antarctica showing region of the study sites; **1b** The study sites showing Sinnan Iwa, Sôya coastal region, and the Sør Rondane Mountains. Black areas represent ice-free areas; **1c** Detail of the Sôya coastal region; **1d** Locations of Skarvsnes lakes. a: Abi-Ike Lake, b: Ayame-Ike Lake, c: Maruyama-Ike Lake, d: Naga-Ike Lake, e: Tsubaki-Ike Lake.

Fig. S1a



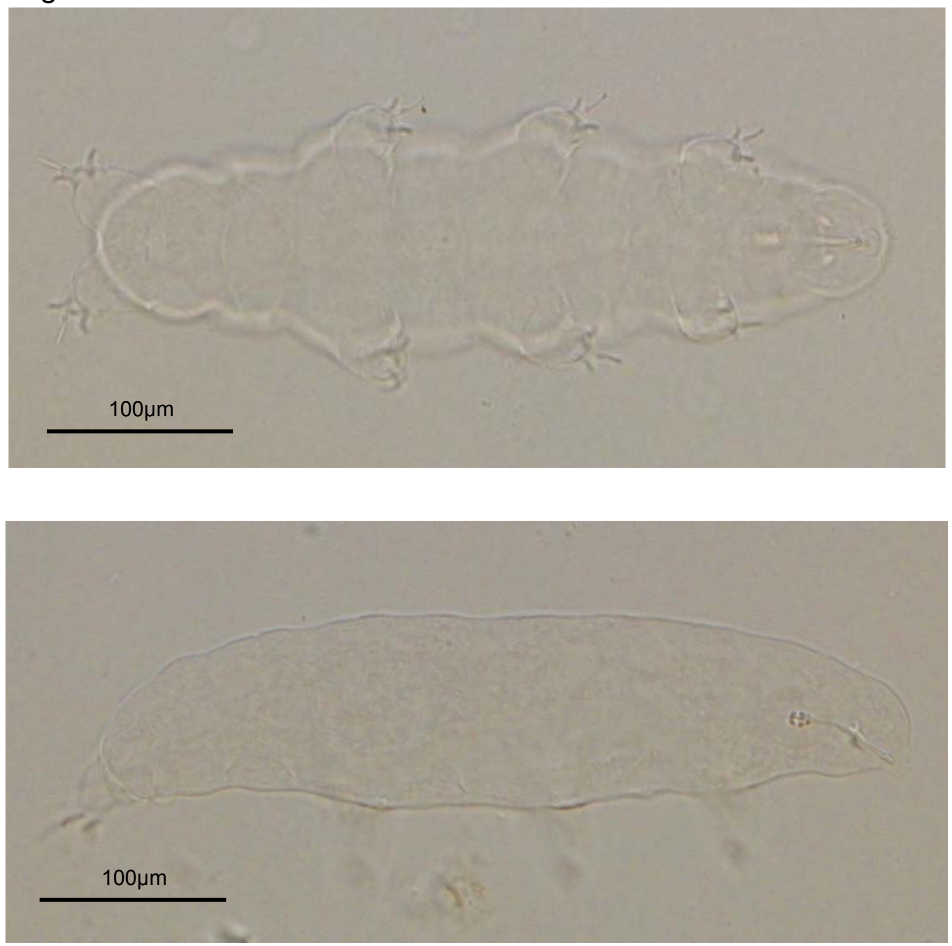
Acutuncus antarcticus (Richters 1904)
Specimens conform to the descriptions in Pilato & Binda (1997)

Fig. S1b



Echiniscus pseudowendti Dastych 1984
Specimens conform to the description in Dastych (1984)

Fig. S1c

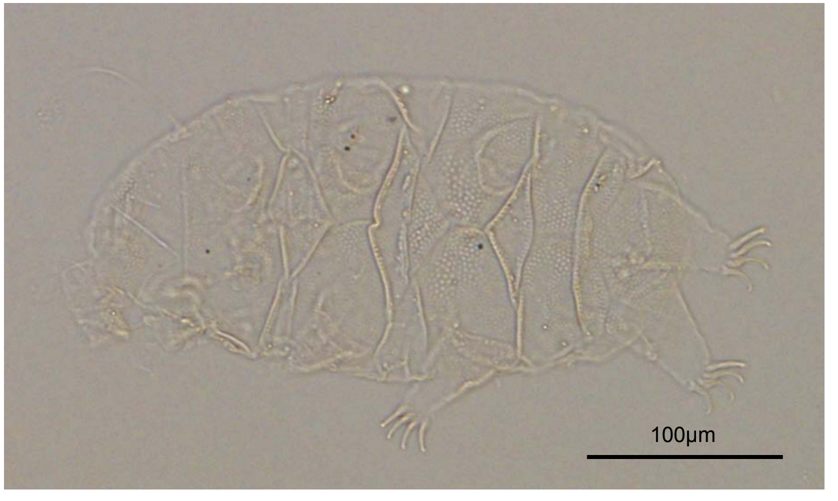


Hebesuncus ryani Dastych and Harris 1994
Specimens conform to the description in Dastych and Harris (1994)

Fig. S1d

Diphascon (D.) langhovdense (Sudzuki 1964)
Specimens conform to the description in Dastych (2002/2003)

Fig. S1e



Pseudechiniscus sp. (*suillus group*)
Single specimen, adult, female. Size and density of its dorsal granulation differs from the congener reported by Utsugi and Ohyama (1989)

Supplementary Figure captions

Fig. S1a Specimen of *Acutuncus antarcticus* (Richters 1904); **S1b** Specimen of *Echiniscus pseudowendti* Dastych 1984; **S1c** Specimen of *Hebesuncus ryani* Dastych and Harris 1994; **S1d** Specimen of *Diphascon (D.) langhovdense* (Sudzuki 1964); **S1e** Specimen of *Pseudechiniscus* sp. (*suillus group*)

References for the Supplementary Figures

Pilato G. Binda MG (1997) *Acutuncus*, a new genus of Hypsibiidae (Eutardigrada). Entomol. Mitt. Zool. Mus. Hamburg. 12(155), 159–162.

Dastych H (2002/2003) *Diphascon langhovdense* (Sudzuki, 1964) stat. nov., a new taxonomic status for the semi-terrestrial tardigrade (Tardigrada). Acta Biol. Benrodis 12, 19–25.

Dastych, H. (1984) The Tardigrada from Antarctic with descriptions of several new species, *Acta Zoologica Cracoviensia*, 27(19), 377–436

Dastych H, Harris JM (1994) A new species of the genus *Hebesuncus* from the Antarctic nunataks, Dronning Maud Land (Tardigrada). Entomol Mitt 11, 139–145

Utsugi K, Ohyama Y (1989) Antarctic Tardigrada. Proc NIPR Symp Polar Biol 2:190–197