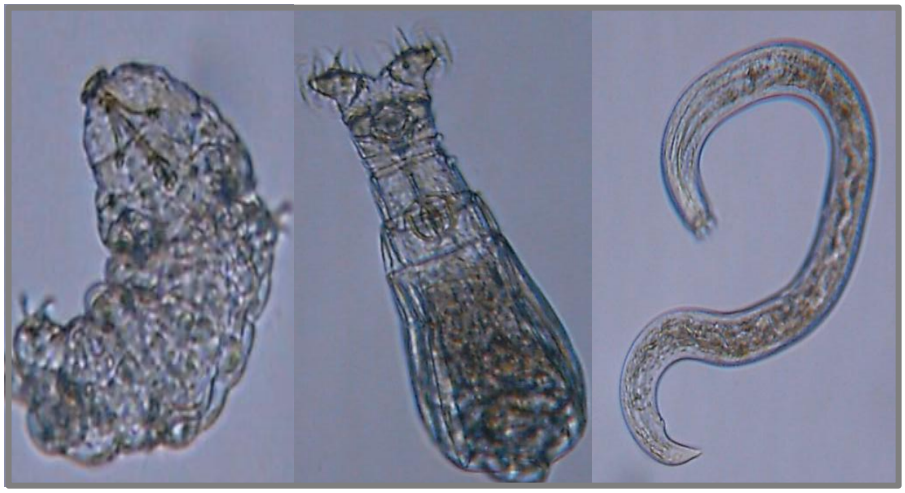
Diversity and Distribution of Limno-Terrestrial Microfauna from Antarctica



ALEJANDRO VELASCO-CASTRILLÓN

Presented for the Degree of Doctor of Philosophy

School of Earth and Environmental Sciences

The University of Adelaide, South Australia

May 2014

This page has been left blank intentionally

TABLE OF CONTENTS

ABSTRACT

Antarctic terrestrial life has been described as some of the simplest on Earth. The terrestrial animals that have survived the harsh Antarctic environment are composed mostly of microfauna, such as rotifers, tardigrades and nematodes. Numerous studies have hypothesised about the lack of diversity, but few have examined this using empirical data. Molecular studies have been shown to be useful in determining relationships among populations, delineating species boundaries, dispersal patterns, and biogeographic connectivity. However, such studies of these ecologically-important animals are still limited because original taxonomic work has not been revised broadly across Antarctica. It is apparent that species diagnoses are difficult in many cases due to the minute size and conservative morphology of these animals. Here I compile a species diversity list from the microfaunal groups (Chapter I), and also examine morphological and molecular (using the mitochondrial cytochrome *c* oxidase I gene) data from 371 nematodes (Chapter III), 438 tardigrades and 526 bdelloid rotifers (Chapter IV). These data suggest that a molecular strategy is vital to discern among cryptic species and to delineate species boundaries for microfaunal groups from Antarctica compared to the sub-Antarctic and global distributions. Sequence comparisons showed local endemic and widespread distributed species, even beyond the Antarctic continent. Those widespread species and the wider range of habitats in which they were found may reflect the ability to withstand environmental stresses. Correlations of soil geochemistry and environmental variables were also established with abundance and distribution data for sites as far as 2000 km from Framnes Mountains (67.78° S- 62.79° E) to Bailey Peninsula (66.28º S-110.54º E) in East Antarctica. These data reveal bdelloid rotifers as the most diverse and widespread group inhabiting a broader range of habitats followed by tardigrades and nematodes. In this study I have uncovered potential new species as well as revealing abiotic habitat requirements and distribution levels for Antarctic limno-terrestrial microfauna. Such information is vital in future conservation and land management plans, and in identifying new putative species and detecting exotic introductions. By using the current knowledge on microfaunal diversity together with the species delimited and the distributional records presented in this study, it will help to better understand biogeography and to provide information on the species mobility in short and long term climatic changes.

DECLARATION

I, Alejandro Velasco Castrillón certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I acknowledge that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

_____

Alejandro Velasco Castrillón

ACKNOWLEDGEMENTS

First of all I would like to thank my supervisors Dr Mark Stevens, Prof Andrew Austin and Prof Steve Cooper. You have all helped in your own way. I would like to express my enormous gratitude to my main supervisor Mark Stevens, who offered me the opportunity to undertake this amazing Antarctic research under his supervision. Thank you, Mark, for sharing with me your wealth of knowledge on Antarctic matters and for giving the privilege of going to one of the most amazing places that this planet (still) has to offer. Thank you for always being supportive and accessible and for the endless time you spent reading drafts, and assisting in the editing and submission of manuscripts. Most of all, thank you for your good character, willingness to make this work possible, and positive attitude all throughout this long journey.

I am extremely grateful to all the funding bodies which made this research possible. Special thanks to the University of Adelaide, South Australian Museum and the Australian Antarctic Division. I also want to thank all the people who collaborated with me during the field trip in Antarctica. Special thanks to Mark Schultz for experiencing the frozen continent with me, assisting in sample collection and giving me valuable feedback on the manuscripts before submission. My gratitude goes to all the personnel at the Australian Antarctic Division, the Aurora Australis and the Antarctic Research Stations with special thanks to the field assistants who showed me the most remote and hidden places in the Antarctic..

I would like to thank every single person who assisted me throughout my lab work at the Waite campus. Special thanks to Federica Colombo for all the hours spent looking down the microscope and sorting out micro-invertebrates. Many thanks to Rebecca Stonor, María Manjarrez, Pauline Glocke and Tanya Matic for providing guidance at the lab and for helping me dealing with hundreds of soil bags. I am also grateful to Dr Kerrie Davies for her taxonomic expertise on nematodes and her intellectual input.

I would also like to pass on my sincere appreciation of the external collaborators who contributed to this work. To Dr John Gibson for showing me tardigrades and rotifers for the first time, providing literature and revising manuscripts; to Dr Byron Adams for providing

sequences and extraction protocols; and to Dr Tim Page for providing rotifer sequences. I am enormously grateful for the access to laboratory installations and soil samples given to me by the staff of the British Antarctic Survey; for Chester Sands making possible my two visits to England, giving me access to soil samples, providing sequences, and sharing his invaluable knowledge on molecular matters; and for Dr Sandra McInnes, who provided sequences and expertise in tardigrades. I am also very grateful to Dr Allan Green, Dr Leopoldo Sancho and Dr María Arróniz-Crespo from the Complutense University of Madrid for welcoming me to their lab and providing their precious time, soil samples, and a positive attitude.

I would like to thank Prof Andrew Austin's lab group for their support and feedback. Special thanks to Javid, Vanesa, Kym, Kate, Rachel, Gary, Michelle, Rebecca, Simon and Paul. I must specially thank my incredible friends Javid and Vanesa who provide me with knowledge, laughter, positive energy and always a smile in their faces which made my PhD live much more enjoyable. I will never forget you my friends. I would also like to thank my bicycle who kept my mental sanity, and of course my Colombian buddies Camilo and Fernando for sharing their University time with me and enjoying coffees, lunches and lots of happiness.

I owe special thanks to my mother, for supporting me in every possible way to come to Australia, coping with my absence, and encouraging me to pursue my studies even in a faraway destination. I am forever grateful to you Lore, not only for all you have given me and for raising me excessively well, but also for gifting me with unimaginable good looks and indescribable talent. Last, and obviously not least, thanks to you Jilarita for your loving support and for understanding my lack of free time and my University priorities.

Notes on chapter format

This thesis is a combination of conventional and publication formats, therefore Chapters have been formatted in different styles. Chapter I is a review paper submitted to the journal *Polar Biology* and thus follows the journal format. Chapter II is a research article published in the journal *PLoS ONE*, and follows precisely the formatting required for the journal. Chapter III is also a research article published in the journal *Soil Biology and Biochemistry* and thus follows the style of the journal. Chapter IV compiles two research manuscripts (rotifer and tardigrade). The rotifer manuscript has been accepted in the journal *Biodiversity*, and the tardigrade manuscript has been submitted to the journal *Invertebrate Systematics*. The style used in Chapter IV follows the guidelines required by the journal *Biodiversity*. Chapter V is the General Discussion of thesis and it has not been submitted to any journal.

The format of this thesis complies with the outlined in 'Specifications for Thesis' supplied by the University of Adelaide Graduate Centre: http://www.adelaide.edu.au/graduatecentre/program-rules/docs/specifications_thesis_2013.pdf.

Statements declaring Co-Author contributions preludes each chapter published, accepted or submitted for publication.

CHAPTER I

(GENERAL INTRODUCTION)

A review of current Antarctic limno-terrestrial microfauna

Alejandro Velasco-Castrillón[a,*], John A. E. Gibson[b] and Mark I. Stevens[a,c]

[a] Australian Centre for Evolutionary Biology and Biodiversity, School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, South Australia 5005, Australia

[b] Institute of Marine and Antarctic Studies, University of Tasmania, Private Bag 129, Hobart, Tasmania 7001, Australia.

[c] South Australian Museum, GPO Box 234, Adelaide, South Australia 5000, Australia

[*]Corresponding author: Alejandro Velasco-Castrillón. Australian Centre for Evolutionary Biology and Biodiversity, School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, South Australia 5005, Australia. E-mail: macalusio@yahoo.com

This chapter has been accepted and formatted in a style required by the journal *Polar Biology*

Statement of authorship

Title of Paper	A review of current Antarctic limno-terrestrial microfauna
Publication Status	Accepted for publication
Publication Details	This chapter was submitted as a review article to *Polar Biology* Velasco-Castrillón A., Gibson, J.A.E., and Stevens, M. I. A review of current Antarctic limno-terrestrial microfauna. *Polar Biology*

Author Contributions

By signing the Statement of authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Alejandro Velasco Castrillón		
Contribution to the Paper	Performed literature review on the current stage of knowledge on Antarctic soil invertebrates, produced tables and wrote manuscript.		
Signature		Date	11/11/2013

Name of Co-Author	John A E Gibson		
Contribution to the Paper	Provided literature and critically revised manuscript		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	21/10/2013

Name of Co-Author	Mark I Stevens		
Contribution to the Paper	Supervised the content of the review and critically revised manuscript		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	09/10/2013

Abstract Antarctic arthropods (mites and springtails) have been the subject of numerous studies. However, by far the most diverse and numerically dominant fauna in Antarctica are the limno-terrestrial microfauna (tardigrades, rotifers and nematodes). Although they have been the focus of several studies there remains uncertainty of the actual number of species in Antarctica. Inadequate sampling and conserved morphology are the main cause of misclassification of species and underestimation of this diversity. Most species' distributional records are dominated by proximity to research stations or limited opportunistic collections, and therefore an absence of records for a species may also be a consequence of the limitations of sampling. Limitations in fundamental knowledge of how many species are present and how widespread they are prevents any meaningful analyses that have been applied more generally to the arthropods within Antarctica, such as exploring ancient origins (at least pre-Last Glacial Maximum) and tracking colonisation routes from glacial refugia. In this review, we list published species names and where possible the distribution of microfaunal (tardigrade, rotifer and nematode) species reported for Antarctica. Our current state of knowledge of Antarctic records (south of 60°S) includes 28 bdelloid rotifers, 66 monogonont rotifers, 59 tardigrades and 68 nematodes. In light of the difficulties in working with microfauna across such geographical scales, we emphasise the need for molecular markers to help understand the 'true levels' of diversity, and suggest future directions for Antarctic biodiversity assessment and species discovery.

Keywords Tardigrada, Rotifera, Nematoda, DNA barcoding, Antarctic Conservation Biogeographic Regions (ACBR)

Introduction

Antarctica has one of the most extreme and challenging environments on the planet, experiencing prolonged winters, freezing temperature and lack of liquid water. It spans nearly 30° of latitude (61°–90°S), and covers an area of 14 million km^2 with only 0.3% of its total area remaining ice and snow-free year-round (British Antarctic Survey 2004). It

has been isolated from the other southern continents for around 28 million years by the Southern Ocean (Lawver et al. 1998), since the opening of the South Tasman Rise (32My) and the Drake Passage (28My) (Lawver and Gahagan 2003). It has also been covered in a permanent ice sheet for ~34 My (Tripati et al. 2005) and has experienced more than 10 major glacial cycles over the last million years (Hays et al. 1976). Despite this, life has managed to survive. Some of the Antarctic terrestrial arthropods consist of likely descendants of ancestors present in Gondwanan times that have diversified in ice-free isolated locations, such as nunataks, since the completion of glaciation in the late Miocene (~21–11 Mya) (Marshall and Pugh 1996; McInnes and Pugh 1998**;** Stevens and Hogg 2003**;** Stevens et al. 2006a). In the case of Antarctic lakes, few studies have dealt with their continuous presence since the break-up of Gondwana. De Smet and Gibson (2008) suggested survival of rotifers in freshwater environments since the Last Glacial Maximum (LGM). Nowadays, it is well accepted that several Antarctic localities have remained ice-free throughout the LGM (e.g. Convey and Stevens 2007; Convey et al. 2008, 2009) and some likely to have been ice-free for much longer. Continental regions such as Dronning Maud Land (Marshall and Pugh 1996), Antarctic Peninsula (Pugh and Convey 2000), southern Victoria Land (Stevens and Hogg 2003, 2006b), and coastal areas (Burgess et al. 1994; Gore et al. 2001; Hodgson et al. 2001) have been suitable for the long-term survival of terrestrial life in ice-free refugia (Cromer et al. 2006; Convey and Stevens 2007) with many terrestrial habitats becoming available for colonisation from refuges within the current inter-glacial period (<17,000 years) (Stevens and Hogg 2003).

The Antarctic limno-terrestrial microfauna is fragmented, patchily distributed, and taxonomically restricted, and mostly comprises rotifers, tardigrades and nematodes (e.g. Wharton 2003; Sohlenius et al. 2004; Huiskes et al. 2006; Sohlenius and Boström 2005, 2008). Microfaunal communities have commonly been associated with habitats rich in organic material (algae, moss or lichen), in the vicinity of bird colonies (e.g. Sohlenius et al. 2004; Sohlenius and Boström 2005; Wall 2007), or in lakes or melt pools (e.g. Kirjanova 1958; Suren 1990; Dartnall 2000; De Smet and Gibson 2008; Andrássy and Gibson 2007). The limno-terrestrial microfauna form a vital component of the food web, playing an essential function in soil ecosystem processes, mainly in recycling nutrients and

processes of decomposition (Sands et al. 2008). Today fewer than 550 non-marine invertebrate species have been identified from Antarctica (Adams et al. 2006; Convey et al. 2008, 2009). Most of these are endemic (58%) and can be defined as continental (>25%) or maritime (>29%), with only 3% of species having a pan-Antarctic distribution (Pugh and Convey 2008). Diversity is greatest for the microfauna (rotifers, tardigrades and nematodes) (e.g. Dastych 1984; Andrássy 1998; Convey and McInnes 2005; Adams et al. 2006; Sohlenius and Boström 2008), followed by arthropods, particularly springtails (Collembola) and mites (Acari) (e.g. Hogg and Stevens 2002; Stevens and Hogg 2006b; Sinclair and Stevens 2006). Given these basic statistics it is surprising that the arthropods have received a disproportionate amount of attention and that there is no single study that provides a complete list of diversity and distribution for the Antarctic microfaunal species of the Phyla Rotifera, Tardigrada and Nematoda. Such an important synopsis of the microfauna may have been seen as a difficult task when it is widely regarded that identification to morpho-species of these minute microfauna are often difficult given the lack of distinctive morphological features (e.g. Andrássy 1998; Floyd et al. 2002; Robeson et al. 2009) resulting in misclassification and underestimation of diversity (Adams et al. 2006; Fontaneto et al. 2009; Stevens et al. 2011).

In order to assess microfaunal diversity in Antarctica (south of 60°S) we have used, for continental Antarctica, the sectors: Maud, Enderby, Wilkes, Scott, Byrd and Ronne (see Pugh 1993). We have also included the Antarctic Peninsula (AP), and the maritime Antarctica (west of AP, and the sub-Antarctic islands of South Orkney and South Shetland; Fig 1).The selection of these largely empirical sectors has also been adopted by other studies (e.g. McInnes and Pugh 1998; Convey and McInnes 2005; Pugh and Convey 2008) but do not represent the bioregions as defined by Terauds et al. (2012). The aim here is to compile the current state of knowledge of Antarctic limno-terrestrial microfaunal diversity and distribution based on morphology of rotifers, tardigrades and nematodes (collectively referred to in this review as microfauna) from continental and maritime Antarctica. We then discuss potential dispersal mechanisms and the need to establish diversity by combining molecular methods. We conclude with suggestions for future directions for Antarctic biodiversity assessment and species discovery.

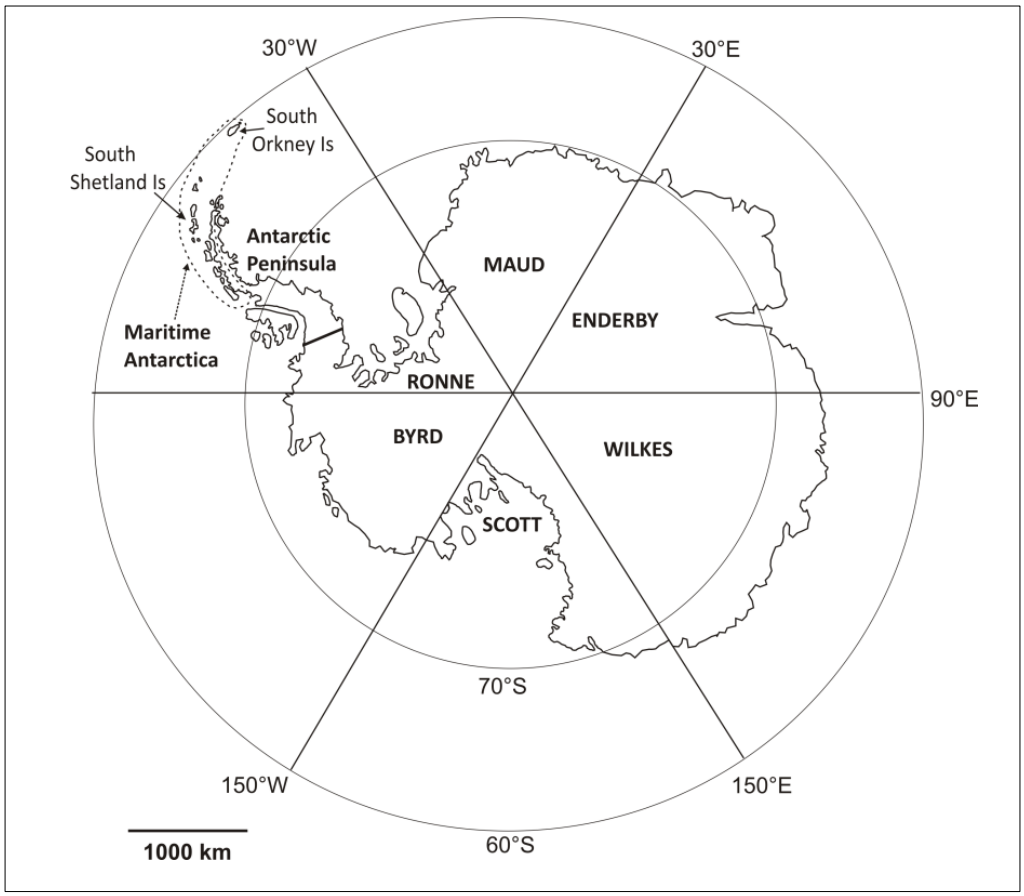


Fig 1. Map of Antarctica showing the Antarctic Peninsula, maritime Antarctica and the eight sectors of continental Antarctica

Current state of knowledge

Microfauna community

Tardigrada

The Phylum Tardigrada is divided into three Classes (Heterotardigrada, Mesotardigrada and Eutardigrada), which comprise a total of ~800 species of freshwater, terrestrial and marine tardigrades worldwide (McInnes and Pugh 1998). Most of the limno-terrestrial forms belong to the class Eutardigrada, and to some extent the Heterotardigrada (which

also include marine forms) (Kinchin 1994). To date, 64 species of tardigrades have been reported for Antarctica and sub-Antarctic islands (including records north of 60°S; McInnes and Pugh 2007), although no species list was included in their work. In the present review we list 59 records of Antarctic tardigrades (south of 60°S) from 30 references and compiled a species distribution list for all named Antarctic tardigrades (Table 1). Records for continental Antarctica include 42 species, while for maritime Antarctica 36 species are reported (19 shared species). We found no records for Byrd sector and only three records for Ronne sector, reflecting a probable lack of studies in these areas. The most widespread tardigrades in Antarctica are the pan-Antarctic species *Acutuncus antarcticus* Binda & Pilato, 2000 and *Milnesium tardigradum* Doyère, 1840 (Table 1). Misidentifications and species synonyms have been included in the online Supporting Information (Appendix 1: Table S1).

Table 1 (following page). Literature source: (1) Adams et al. 2006, (2) Binda and Pilato 2000, (3) Convey and McInnes 2005, (4) Dastych 1984, (5) Dastych 1989, (6) Dastych 1991, (7) Dastych 2003, (8) Dastych and Harris 1995, (9) Dastych and McInnes 1994, (10) Dastych et al. 1990, (11) Gibson et al. 2007, (12) Janiec 1996, (13) McInnes 1995, (13b) McInnes 2010, (14) Miller and Heatwole 1995, (15) Miller et al. 1988, (16) Miller et al. 1994, (17) Miller et al. 1996, (18) Murray 1910, (19) Pilato and Binda 1999, (20) Pilato et al. 2012, (21) Rounsevell and Horne 1986, (22) Smykla et al. 2012, (23) Sohlenius and Boström 2005, (24) Sohlenius et al. 1995, (25) Sohlenius et al. 2004, (26) Tumanov 2006, (27) Utsugi and Ohyama 1993, (28) Utsugi and Ohyama 1991, (e1) Australian Antarctic Data Centre (https://data.aad.gov.au/aadc/biodiversity/search_taxon.cfm).

Table 1. List of Tardigrada species recorded from the Antarctic and their regional distributions. The numbers in each column refer to reference (see below the table). Acronyms: Antarctic Peninsula (AP), South Shetland and South Orkney Islands (SS-SO). Those references in parenthesis indicate possible misidentifications

Tardigrade species / Sectors	Continental Antarctica					AP - Maritime Antarctica	
	Maud	Enderby	Wilkes	Scott	Ronne	AP	SS-SO
Class Heterotardigrada							
Echiniscus corrugicaudatus McInnes, 2010					13b		
Echiniscus jenningsi Dastych, 1984		14,e1				3,5	4,3,13
Echiniscus kerguelensis Richters, 1904		(27,28)					(27)
Echiniscus pseudowendti Dastych, 1984	24	4				e1	
Echiniscus punctus (McInnes, 1995)							3,13
Testechiniscus meridionalis Murray, 1906							4,3,13
Oreella mollis Murray, 1910							3
Pseudoechiniscus cf. *suillus* (Ehrenberg, 1853)		4,16	5, 17				4,3,13
Pseudoechiniscus novaezeelandiae Richters, 1903		21, 15, 16					
Class Eutardigrada							
Acutuncus antarcticus (Binda & Pilato, 2000)	23,24,25	21, 27,28,11,14,16,6	5, 17	1,22,6		3	3,12,27
Amphibolus volubilus Durante Pasa & Maucci, 1975							(27)
Dactylobiotus cf. *ambiguus* (Murray, 1907)						11	3,12,13
Hexapodibius boothi Dastych & McInnes, 1994							3,9
Diphascon ongulensis Morikawa, 1962		27					
Diphascon (Adropion) greveni Dastych, 1984						3	3,12,13
Diphascon (Adropion) maucci Dastych & McInnes, 1996							3
Diphascon (Adropion) tricuspidatum Binda & Pilato, 2000				1, 2			
Diphascon (Diphascon) alpinum Murray, 1906							(27)
Diphascon (Diphascon) dastychi Pilato & Binda, 1999				1, 19			
Diphascon (Diphascon) higginsi Binda, 1971							(27)
Diphascon (Diphascon) langhovdensis Sudzuki, 1964	23, 24	7					3
Diphascon (Diphascon) mirabilis Dastych, 1984							3,12
Diphascon (Diphascon) pingue ('Variety A') Marcus, 1936			17			3,5	3
Diphascon (Diphascon) pingue ('Variety B') Marcus, 1936	19					5	4
Diphascon (Diphascon) polare Pilato & Binda, 1999				1, 19			
Diphascon (Diphascon) victoriae Pilato & Binda, 1999				1, 19			
Diphascon (Diphascon?) puniceum Jennings, 1971	e1	15					3,13
Diphascon sanae Dastych, Ryan & Watkins, 1990	10	14,e1			3	3	
Hebesuncus mollispinus Pilato, McInnes & Lisi, 2012						20	
Hebesuncus ryani Dastych & Harris, 1994	23, 25				3	3	
Hebesuncus schusteri (Dastych, 1984)	24	4				3	3
Hypsibius allisoni Horning, Schuster & Grigarick, 1978		15					
Hypsibius (Diphascon) scoticus Murray, 1905				(1)			
Hypsibius cf. *convergens* (Urbanowicz, 1925)				(1)			
Hypsibius cf. *dujardini* (Doyère, 1840)						3	3,12,13
Hypsibius cf. *mertoni simoizumii* (Sudzuki, 1964)			e1	1			
Isohypsibius asper Murray, 1905						e1	27,3,12,13
Isohypsibius improvisus Dastych, 1984		4					4
Isohypsibius laevis (McInnes, 1995)							3,13
Isohypsibius papillifer Murray, 1905							27,3,12,13
Isohypsibius saracenus Pilato, 1973		(27)					
Macrobiotus blocki Dastych, 1984	23, 24	4,11,14					
Macrobiotus cf. *hufelandi* (Schultze, 1833)	23		e1			3,5	
Macrobiotus cf. *polaris* (Murray, 1910)				1,18			
Macrobiotus harmsworthi coronatus (Utsugi, 1991)		(27,28)					
Macrobiotus harmsworthi (Barros, 1942)	e1						(27)
Macrobiotus krynauwi Dastych & Harris, 1995	23, 25,8						12,13
Macrobiotus meridionalis Richters, 1909				22			
Macrobiotus montanus Murray, 1910		(27)					
Macrobiotus mottai Binda & Pilato, 1994				1			
Macrobiotus polaris Dougherty & Harris, 1963				1			
Minibiotus stuckenbergi (Dastych, Ryan & Watkins, 1990)	3, 10	14,e1					
Minibiotus vinciguerrae Binda & Pilato, 1992				1			
Minibiotus weinerorum (Dastych, 1984)		4,11,16					
Ramajendas frigidus Pilato & Binda, 1990			17	1			
Ramajendas renaudi Ramazzotti, 1972						3,4	3,12
Ramazzottius cf. *oberhäuseri* (Doyère, 1840)	e1	e1		1		3,e1	
Milnesium antarcticum Tumanov, 2006				22			26
Milnesium cf. *tardigradum (*Doyère, 1840)	23, 24	16			3	3	3

Rotifera

The Phylum Rotifera includes the Classes Bdelloidea, Monogononta and Seisonidea; with the former two being most common in Antarctica. Segers (2007) listed 92 rotifer species and assigned them to 'Antarctica' (including sub-Antarctic islands north of 55°S) but without specifying geographic regions. We confirmed, from other references, the presence of 63 of those species (44 monogononts and 19 bdelloids) listed by Segers (2007) to occur in continental and/or maritime Antarctica (south of 60°S) (see Table 2a, 2b). Most records in the literature correspond to the widely known Antarctic endemic *Philodina gregaria* Murray, 1910, which has been reported from across Antarctica. Frequently found with *P. gregaria* is another endemic Antarctic rotifer *Adineta grandis* Murray, 1910 and two cosmopolitan species *Epiphanes senta* Müller, 1773 and *Cephalodella catellina* Müller, 1786. All four species are usually found in bodies of water that remain frozen in the winter, and have a circumpolar distribution; similar to other cosmopolitan species from terrestrial habitats (*Adineta gracilis* Janson, 1893) and lake habitats (*Collotheca ornata cornuta* Dobie, 1849 and *Lepadella patella* Müller, 1773) (Dartnall 1983). We have compiled a distribution list of Antarctic limno-terrestrial rotifers that includes 66 monogonont and 28 bdelloid species from 24 different reference sources (Table 2a, 2b). Species records reported by Segers (2007) for Antarctica that were not confirmed by other references can be found in Appendix 1: Table S2. For a list of species synonyms refer to Appendix 1: Table S3 in the Supporting Information files.

Table 2a – 2b (following page). Records from Antarctic Peninsula (AP) include Palmer sector and Graham sector. References from South Shetland and South Orkney Islands (SS-SO) are shown combined. Literature source: (1) Dartnall and Hollowday 1985, (2) Dartnall 1983, (3) Dartnall 2005, (4) Dartnall 2000, (5) Dartnall 1995, (6) De Smet and Gibson 2008, (7) Donner 1972, (8) Dougherty and Harris 1963, (9) Fontaneto et al. 2008, (10) Hansson et al. 2012, (10b), Janiec 1996, (11) Murray 1910, (12) Opalinski 1972, (13) Suren 1990, (14) Segers 2007, (15) Smykla et al. 2010 (16) Sohlenius and Boström 2005, (17) Sohlenius et al. 1995, (18) Sohlenius et al. 1996, (19) Sudzuki 1979, (20) Sudzuki 1988, (21) Vincent and James 1996, (22) Webster-Brown et al. 2010, (e1) Australian Antarctic Data Centre (https://data.aad.gov.au/aadc/biodiversity/search_taxon.cfm).

Table 2a. List of Monogononta (Rotifera) species recorded from the Antarctic and their regional distributions. The numbers in each column refer to reference. Acronyms: Antarctic Peninsula (AP), South Shetland and South Orkney Islands (SS-SO)

Rotifer species / Sectors	Antarctica	Continental Antarctica				AP - Maritime Antarctica	
	(unspecified)	Maud	Enderby	Wilkes	Scott	AP	SS-SO
Class Monogononta							
Brachionus angularis Gosse, 1851					10		
Brachionus bidentatus bidentatus Anderson, 1889*	14					10,e1	e1
Brachionus bidentatus inermis Rousselet, 1906						10	
Brachionus calyciflorus Pallas, 1766	14			10,e1			
Brachionus havanaensis trahea Murray, 1913						10,e1	10,e1
Brachionus quadridentatus quadridentatus Hermann, 1783*	14	10		10,e1			
Brachionus urceolaris urceolaris Müller, 1773*	14				10	10	10
Cephalodella auriculata Müller, 1773	14						1,e1
Cephalodella catellina Müller, 1786	14				2,1	e1	1,2,10b,e1
Cephalodella forficata (Ehrenberg, 1832)	14					e1	1,10b,e1
Cephalodella gibba (Ehrenberg, 1830)	14					e1	1,2,e1
Cephalodella megalocephala (Glascott, 1893)	14						1,e1
Cephalodella sterea (Gosse, 1887)	14		5				
Cephalodella tenuior (Goose, 1886)	14					e1	
Cephalodella ventripes angustior Donner, 1950			5				
Collotheca gracilipes Edmonson, 1939							1,e1
Collotheca ornata cornuta (Dobie, 1849)	14		4,5,e1	2,e1	2,1	e1	1,2,e1
Colurella colurus colurus (Ehrenberg, 1830)	14					e1	10b,e1
Colurella colurus compressa (Lucks, 1912)	14						1,e1
Dicranophorus permollis giganthea Dartnall & Hollowday, 1985					1	e1	1,e1
Dicranophorus uncinatus (Milne, 1886)							1,e1
Encentrum brevifulcrum Dartnall, 1997	14		4				
Encentrum forcipatum Dartnall, 1997	14		4,e1				
Encentrum mustela Milne, 1885	14		4,5,e1	e1		e1	1,10b,e1
Encentrum permolle Gosse, 1886							e1
Encentrum salinum Dartnall, 1997	14		4				
Encentrum spatiatum Wulfert, 1936			4,5,e1				
Encentrum uncinatum (Milne, 1886)	14					e1	e1
Eosphora najas (Ehrenberg, 1832)							1,2,e1
Epiphanes senta Müller, 1773	14		4,5,2,e1	2,6,e1	1,2,13,e1	e1	1,2,10b,e1
Euchlanis dilatata dilatata Ehrenberg, 1832	14						1,e1
Euchlanis dilatata parva Rousselet, 1832							e1
Kellicottia longispina (Kellicott, 1879)					10		
Keratella americana Carlin, 1943	14					10,20,e1	10,20,e1
Keratella cochlearis Gosse, 1851	14		4,10		10	10,20,e1	10,20,e1
Keratella quadrata Müller, 1786					10		
Keratella valga (Ehrenberg, 1834)	14					e1	e1
Lecane lunaris (Ehrenberg, 1832)	14					e1	1,2,e1
Lepadella acuminata (Ehrenberg, 1834)	14		5	e1			
Lepadella elliptica (Turner, 1892)	14		e1				
Lepadella intermedia Dartnall & Hollowday, 1985	14						1,e1
Lepadella patella Müller, 1773	14		2,4,5,e1	2,6,e1		e1	2,10b,e1
Lepadella patella oblonga Ehrenberg, 1834	14						1,e1
Lepadella rhomboides signiensis Dartnall & Hollowday, 1985	14						1,e1
Lepadella triptera (Ehrenberg, 1832)	14				10		10,1,e1
Lindia torulosa antarctica Dartnall & Hollowday, 1985			4				
Lindia torulosa Dujardin, 1841	14		e1			e1	
Notholca foliacea (Ehrenberg, 1838)					10		
Notholca jugosa Gosse, 1887			10				
Notholca salina Focke, 1961	14					10,e1	1,10,10b,e1
Notholca verae Kutikova, 1958	14		2,e1	10,2,6,e1			2
Notholca walterkostei de Paggi, 1982	14					10,e1	1,10,10b,e1
Notholca walterkostei reducta Dartnall & Hollowday, 1985	14						1,10,e1
Paradicranophorus sordidus Donner, 1968	14		e1				
Proales reinhardti (Ehrenberg, 1834)			4,6				
Ptygura crystallina (Ehrenberg, 1834)	14		4,5,e1	e1			1,e1
Ptygura melicerta (Ehrenberg, 1832)	14						1,2,e1
Resticula gelida (Harring & Myers, 1922)	14		4,5,e1	e1		e1	1,2,10b,e1
Resticula nyssa (Harring & Myers, 1924)						e1	10b,e1
Rhinoglena fertoeensis (Varga, 1929)				6,e1			
Rhinoglena kutikovae De Smet, 2007				6			
Scaridium bostjani Daems & Dumont, 1974							1,2,e1
Scaridium longicaudum Müller, 1786	14						e1
Trichocerca brachyura (Gosse, 1851)	14						1,e1
Trichocerca rattus globosa Dartnall & Hollowday, 1985							1,e1
Trichocerca rattus Müller, 1776	14						e1

Table 2b. List of Bdelloidea (Rotifera) species recorded from the Antarctic and their regional distributions. The numbers in each column refer to reference. Acronyms: Antarctic Peninsula (AP), South Shetland and South Orkney Islands (SS-SO)

Rotifer species / Sectors	Antarctica (unspecified)	Continental Antarctica				AP - Maritime Antarctica	
		Maud	Enderby	Wilkes	Scott	AP	SS-SO
Class Bdelloidea							
Adineta barbata Janson, 1893	14	16,17,18,e1	4		1,8,11	e1	1,e1
Adineta gracilis Janson, 1893	14	16, 17,18,e1	2	2,e1	1,2,11	e1	1,2,9,e1
Adineta grandis Murray, 1910	14	e1	4,5,e1	2,3,12,e1	1,8,11,13,15,e1	e1	1,2,9,e1
Adineta longicornis Murray, 1906	14				11	e1	
Adineta steineri Bartoš, 1951	14	16,17,18,e1					
Adineta vaga vaga (Davis, 1873)*	14	16, 17,18,e1			1,11,22	e1	
Habrotrocha angularis (Murray, 1910)	14				1,8,11	e1	
Habrotrocha constricta (Dujardin, 1841)	14	16,17,18,e1	4,5,e1	3,e1	1,7,8,15,22	e1	1,e1
Habrotrocha elusa elusa Milne, 1916*	14	16,17,18,e1		e1			
Habrotrocha gulosa Milne, 1916				17,19,e1			
Habrotrocha tridens Milne, 1886	14	16,17,18,e1				e1	
Macrotrachela ambigua Donner, 1965		16,18,e1					
Macrotrachela concinna (Bryce, 1912)	14						1,e1
Macrotrachela constricta Milne, 1886					11	e1	
Macrotrachela insolita De Koning, 1947	14	16,17,18,e1		17,19,e1	1,7,15	e1	
Macrotrachela habita (Bryce, 1894)	14	16,17,18,e1			1,8,11	e1	
Macrotrachela libera Donner, 1949		16,17,18,e1					
Macrotrachela cf. *ligulata* Haigh, 1965		16,18,e1					
Macrotrachela nixa Donner, 1962	14	16,18,e1		17,19,e1			
Macrotrachela quadricornifera quadricornifera Milne, 1886*	14		4,e1				
Macrotrachela timida Milne, 1916		16,17,18,e1					
Mniobia russeola (Zelinka, 1891)	14		4,e1				
Mniobia symbiotica (Zelinka, 1886)		16,17,18,e1					
Otostephanos torquatus (Bryce, 1913)		16,18,e1					
Philodina alata Murray, 1910	14		10	6,10,e1	1,8,10,11,21,22,e1	e1	
Philodina antarctica Murray, 1910	14				1,8,11,22,e1	e1	
Philodina gregaria Murray, 1910	14	e1	4,5,e1	2,3,12,e1	1,2,7,8,11,13,21,22	1,e1	2,1,e1
Rotaria rotatoria (Pallas, 1766)					15		

Nematoda

Nematodes are usually associated with rotifers and tardigrades and generally found in areas where moss, lichens or algae are present (e.g Timm 1971; Sohlenius et al. 2004; Velasco-Castrillón et al., 2014). Some species (*Plectus frigophilus* Kirjanova, 1958; *Halomonhystera* spp) have also been recorded from Antarctic lakes (Kirjanova 1958; Andrássy and Gibson 2007) or in highly organic soils adjacent to bird colonies, for example *Panagrolaimus* (Sohlenius 1989; Sinclair 2001). According to Wharton (2003) nematodes are the most diverse and abundant invertebrates in both the maritime and continental Antarctic regions. The Phylum includes the Class Dorylaimia, Enoplia and Chromadoria (Meldal et al. 2007); which according to Andrássy (2008) are represented by 54 species from Antarctica, 32 in the maritime region and 22 from continental Antarctica.

In the present review we list 68 species for Antarctica (Table 3). We identified 34 species occurring in continental Antarctica and 37 species in maritime Antarctica (see Velasco-Castrillón and Stevens 2014). Of particular interest is the geographical overlap of three species (*Plectus murrayi* Yeates, 1970; *Pl. frigophilus* and *Teratocephalus tilbrooki* Maslen, 1979). *Plectus murrayi* and *Pl. frigophilus* (commonly known for continental Antarctica) were represented by unconfirmed records for maritime Antarctica. While *T. tilbrooki* known from maritime Antarctica (Andrássy 1998) was reported for continental Antarctica (Table 3). Unfortunately no morphological or molecular data were provided in these studies. The overlap of *Pl. murrayi* with other species could be a result of the difficulties encountered in the identification of *Plectus* species and especially of those lacking males (see Boström 2005). Species synonyms have been included in Supporting Information (Appendix 1: Table S4).

Table 3 (following page). References followed by (*) indicate marine inhabitants. Literature source: (1) Adams et al. 2006, (2) Adams et al. 2007, (3) Andrássy 1981, (4) Andrássy 1998 (5) Andrássy 2006, (6) Andrássy 2008a, (7) Andrássy 2008b, (8) Andrássy and Gibson 2007, (9) Boström 1995, (10) Boström 1996, (10b) Boström 2005, (11) Boström et al. 2010, (12) Courtright et al. 2000, (13) Freckman and Virginia 1997, (14) Gagarin 2009, (14b) Ghosh et al. 2005, (15) Heyns 1994, (15b) Holovachov and Boström 2006, (16) Kirjanova 1958, (17) Kito and Ohyama 2008, (18) Kito et al. 1991, (19) Kito et al. 1996, (20) Maslen and Convey 2006, (21) Bohra et al. 2010, (22) Nedelchev and Peneva 2000, (23) Rounsevell and Horne 1986, (24) Ryss et al. 2005, (25) Shishida and Ohyama 1986, (26) Sinclair 2001, (27) Sohlenius et al. 1995, (28) Sohlenius et al. 1996, (29) Timm 1971, (30) Yeates 1970, (31) Yeates 1979, (32) Ingole and Parulekar 1993, (33) Verlecar et al. 1996, (34) Maslen 1979, (35) Maslen 1981, (36) Spaull 1973a, (37) Spaull 1973b.

Table 3. List of Nematoda species recorded from the Antarctic and their regional distributions. The numbers in each column refer to reference (see below the table)

Nematode species / Sectors	Continental Antarctica				Maritime Antarctica
	Maud	Enderby	Wilkes	Scott	
Class Chromadorea					
Acrobeloides arctowskii Holovachov & Boström 2006					15b
Aglenchus agricola (de Man, 1884) Andrássy, 1954	24				
Antarctenchus motililus Ghosh, Chatterjee, Mitra, De, 2005	14b				
Antarctenchus hooperi Spaull, 1972					4,34,35,36,37
Aphelenchoides haguei Maslen, 1979					4,20,35
Aphelenchoides vaughani Maslen, 1979					4,20,35
Apratylenchoides joenssoni Ryss *et al.* 2005	24				
Ceratoplectus armatus (Butschli, 1873) Andrássy, 1984					4,20,34,36
Chiloplacoides antarcticus Heyns, 1994	15				
Chiloplectus masleni Boström, 1997	4,10				
Cuticularia firmata Andrássy, 1998					4
Ditylenchus parcevivens Andrássy, 1998					4
Dolichorhabditis tereticorpus Kito & Ohyama, 2008			17		
Eumonhystera vulgaris (de Man, 1880) Andrássy, 1981					4,20,3
Geomonhystera antarcticola Andrássy, 1998				1,4,29	
Geomonhystera villosa (Butschli, 1873) Andrássy, 1981					4,20,34,35
Halomonhystera antarctica (Cobb, 1914) Andrássy, 2006				5*	
Halomonhystera continentalis Andrássy, 2006		5,8			
Halomonhystera disjuncta (Bastian, 1865) Andrássy, 2006					5*
Halomonhystera glaciei (Blome & Riemann, 1999) Andrássy, 2006					5*
Halomonhystera halophila Andrássy, 2006		5,8			
Halomonhystera uniformis (Cobb, 1914) Andrássy, 2006				5*	
Helicotylenchus diagonicus Perry in Perry, Dariling & Thorne, 1959	21				
Helicotylenchus dihystera (Cobb, 1893) Sher, 1961	21				
Helicotylenchus exallus Sher, 1966	21				
Hypodontolaimus antarcticus Andrássy & Gibson, 2007		8			
Laimaphelenchus helicosoma (Maslen, 1979) Peneva & Chipev, 1999					4,20,35
Panagrolaimus davidi Timm, 1971				1,26,29,34	
Panagrolaimus magnivulvatus Boström, 1995	4,27,28,9				
Paratylenchus nanus Coob, 1923	24				
Plectus antarcticus de Man, 1904					4,20,34,35,36,37
Plectus belgicae de Man 1904					4,20
Plectus frigophilus Kirjanova, 1958		8,25, 18	16,31	1,4,29,34	20
Plectus insolens Andrássy, 1998					4
Plectus meridianus Andrássy, 1998					4
Plectus murrayi Yeates, 1970	4,27,28,9	4,23,25,18	7,16	1,4,13,29,30	20
Plectus telekii Mulk & Coomans, 1978	21				
Plectus tolerans Andrássy, 1998					4,20
Pratylenchus andinus Lordello, Zamith & Boock, 1961	24				
Rhabditis krylovi Tsalolikhin, 1989					4
Rotylenchus capensis Van den Berg & Heyns, 1974	4				
Scottnema lindsayae Timm, 1971		25, 2		1,2,4,11,12,13,26,29	
Teratocephalus pseudolirellus Maslen, 1979					20
Teratocephalus rugosus Maslen, 1979					20,35
Teratocephalus tilbrooki Maslen, 1979	32,33				4,20,35
Tylenchorhynchus maximus Allen, 1955	24				
Class Enoplea					
Amblydorylaimus isokaryon (Loof, 1975) Andrássy, 1998					4,34
Calcaridorylaimus signatus (Loof, 1975) Andrássy, 1986					4,20,34
Coomansus gerlachei (de Man, 1904) Jairajpuri & Khan, 1977					4,20
Enchodelus signyensis Loof, 1975					4,20,34,35
Eudorylaimus antarcticus (Steiner, 1916) Yeates, 1970				1,4,6,13,29	
Eudorylaimus coniceps Loof, 1975					4,20,34,35
Eudorylaimus glacialis Andrássy, 1998		6		1,6,30	
Eudorylaimus nudicaudatus Heyns, 1993	4,6				
Eudorylaimus pseudocarteri Loof, 1975					4,20,34,35
Eudorylaimus quintus Andrássy, 2008		6		6	
Eudorylaimus sabulophilus Tijepkema, Ferris & Ferris, 1971	21				
Eudorylaimus sextus Andrássy, 2008		6			
Eudorylaimus shirasei Kito, Shishida & Ohyama, 1996	10b	4,6,19		1	
Eudorylaimus spauli Loof, 1975					4,20,34,35
Eudorylaimus verrucosus Loof, 1975					4,20,34,35
Eutobrilus antarcticus Tsalolikhin, 1981			4,14		
Mesodorylaimus antarcticus Nedelchev & Peneva, 2000					22
Mesodorylaimus chipevi Nedelchev & Peneva, 2000					22
Mesodorylaimus imperator Loof, 1975					4,20,34
Mesodorylaimus masleni Nedelchev & Peneva, 2000					22
Paramphidelus antarcticus Tsalolikhin, 1989					4
Rhyssocolpus paradoxus (Loof, 1975) Andrássy, 1986					4,20,34,35

Microfaunal dispersal and occurrence

Information on dispersal of Antarctic invertebrates results from casual observations from arthropod collections, which have received comparatively more work in Antarctica (see Convey et al. 2008, 2009). It is believed that air currents are one potential mode of passive dispersal (Greenslade et al. 1999; Muñoz et al. 2004; Miller and Heatwole 1995; Nkem et al. 2006; Hawes et al. 2007). This method of transport may not be as successful for arthropods (springtails, mites, dipterans) due to a high risk of desiccation and an apparent lack of a anhydrobiotic dispersal stage (see Marshall and Pugh 1996). Other possible dispersal mechanisms are birds (Stevens and Hogg 2002), bubbles carried in water currents (Rounsevell and Horne 1986) or on floating-materials in melt-water streams (Moore 2002, Sinclair and Stevens 2006). For nematodes, tardigrades and rotifers, with a specialised dispersal life-stage, a far greater potential for dispersal via wind and water has been suggested (Stevens and Hogg 2006a). However, long-range dispersal (inter-oceanic), even during the anhydrobiotic phase, has been questioned by McInnes and Pugh (1998). Dispersal by human activities has also been reported in the literature, particularly for the sub-Antarctic islands and Maritime Antarctica (e.g. Burn 1984; Greenslade and Wise 1984; Rounsevell and Horne 1986).

Records of species in some areas could be relicts from a warmer pre-Pleistocene period in Antarctica (McInnes and Pugh 1998), descendants of more recent arrivals from outside the continent (Sohlenius et al. 2004), or simply the result of misidentification (McInnes 1995; Czechowski et al. 2012). Successful colonisation requires suitable conditions for the propagules to survive, establish and reproduce (Miller et al. 1994). Given the isolation of ice-free habitats, we would expect a very low probability of colonisation and presence of habitat patches lacking microfauna (Sohlenius et al. 2004). For slow, more gradual changes (climate and environmental change) dispersal to new areas of suitable habitat may be possible provided that the rate of change does not exceed their dispersal ability to find a new alternative habitat. At a larger scale (hundreds of kilometres), the rate of change may occur in conjunction with other changes (soil formation, vegetation growth), although long distance dispersal between habitats may be

limited (Wise 1967; Hogg and Stevens 2002; Stevens and Hogg 2002). Furthermore, several studies have suggested that the time since the last glaciation has been insufficient for successful colonisation of favourable habitats by soil taxa (Convey and Block 1996; Convey and Stevens 2007; Convey et al. 2008), and this is supported by recent data for arthropods (Stevens et al. 2006a; Stevens and Hogg 2006a). Accordingly, the natural dispersal of animals, other than local, seems unlikely to provide an adequate response to any environmental change. Long-term patterns can be useful in determining whether taxa are capable of migrating over large distances, whether they have persisted over long-term environmental change, or if they are the result of exotic introductions either by natural (passive) or anthropogenic means. Such analyses for the microfauna is, however, currently limited until accurate widespread data for species identifications can lead to informed diversity and distributions.

Establishing diversity and distribution

Rotifera, Tardigrada, and Nematoda are the most abundant and diverse microfaunal groups in the Antarctic region, but even greater levels of cryptic diversity are expected. Studies on the arthropods (Collembola and Acari) (e.g. Stevens et al. 2006b) have revealed that several new genetic entities (species) are present in the Antarctic and on sub-Antarctic islands, and this has also been found for the microfauna (Fontaneto et al. 2008; Sands et al. 2008; Czechowski et al. 2012). The species diversity of these ecologically-important animals is still unresolved because taxonomic work has been dominated by arthropods (Greenslade and Wise 1984; Greenslade 1995; Stevens et al. 2006b). However, it is apparent that species diagnosis is difficult in many cases due to the conservative morphology of the microfauna (e.g. Andrássy 1998; Floyd et al. 2002; Robeson et al. 2009).

Molecular studies are needed to delineate species boundaries and dispersal patterns (e.g. Stevens et al. 2006b; Sands et al. 2008; Torricelli et al. 2010). It will then be possible to make accurate assessments of the patterns and processes of biodiversity of the

microfauna, which will further our knowledge of the evolutionary history throughout the Southern Hemisphere (Convey and Stevens 2007; Convey et al. 2008). These studies are now beginning to explain the significance of glacial events in determining patterns of species' distribution and genetic diversity for terrestrial communities in Antarctica (Courtright et al. 2000; Frati et al. 2001; Stevens and Hogg 2006a). They have revealed that some taxa of little dispersal capability, have large-scale biogeographic distributions across Antarctica and the sub-Antarctic islands (e.g. Convey and McInnes 2005; Stevens and Hogg 2006a; Czechowski et al. 2012). Collectively, these studies have revealed a significant effect of glacial and sea-ice barriers to examine the mobility and gene flow of Antarctic taxa across fragmented landscapes over evolutionary time-scales.

Future directions in biodiversity assessment and species discovery in Antarctica

With increased access to molecular techniques (Hebert et al. 2003) the diversity of Antarctic invertebrates and the association between organisms and environments can now be estimated to levels previously unimaginable (Peck et al. 2005; Ji et al. 2013). Molecular techniques can be used to test hypotheses related to connectivity (i.e. gene flow) and reveal phylogeographic processes that have moulded the pattern of genetic diversity among populations, as well as their evolutionary history and relationships to other among taxa (Stevens and Hogg 2006a). The usefulness of the mitochondrial cytochrome *c* oxidase I (COI) gene as a DNA-barcode to determine sequence divergence among invertebrates and discern among morphologically similar (cryptic) species is now well established (e.g. Hebert et al. 2003; Stevens and Hogg 2003; Stevens et al. 2006a). COI records can now be found for Antarctic arthropods (e.g. Stevens and Hogg 2003; Stevens and Hogg 2006a; Stevens et al. 2006a) and collectively have revealed patterns of recolonisation from glacial refugia that show far greater diversity than known previously. Much of the success of these data has been due to capturing most of the geographical range for species. Comparatively, molecular data for the microfauna from Antarctica are limited to tardigrades (Sands et al. 2008; Czechowski et al. 2012) and more recently nematodes (Velasco-Castrillón and Stevens 2014). Unfortunately these studies have tended to have very restricted sample

sizes and/or geographic coverage limiting the use of such data beyond diversity and systematics. Despite this limitation, these few studies have already revealed greater diversity in Antarctica than has been previously recognised. With an increasing attention of microfauna outside continental Antarctica on bdelloid rotifers (Fontaneto at al. 2008) and nematodes (e.g. Blouin 2000; Derycke et al. 2010; Prosser et al. 2013) the potential for examining the distribution of microfauna throughout Antarctica and its neighbours will provide one of the most comprehensive datasets for any group of organisms across the continent.

Rotifera, Nematoda and Tardigrada are critical microfaunal groups given their role in nutrient recycling and their importance in Antarctic limno-terrestrial ecosystems. Unfortunately, we are in our infancy in our understanding of these ecosystems in Antarctica and we highlight below three areas that are fundamental in providing information on diversity, distributional range and type of habitats in which microfauna are found; information that is critical for future conservation and land management, and in detecting new species and species introductions.

(1) Molecular techniques need to be applied to the identification of species. Most of the Antarctic microfauna to date are limited to morphological assessments and past molecular studies have shown that this has not accurately reflected the biodiversity present, particularly where wide species ranges have been reported. This is fundamental information necessary for understanding and managing sustainable biodiversity as well as detecting exotic introductions.
(2) Sampling in Antarctica has tended to ignore information linked to abiotic (e.g. soil chemistry, mineral analyses, and other environmental) data which are important in comparisons of microfaunal communities (i.e. are the same communities occurring in similar habitats) and can also be used in predictive modelling of Antarctic biodiversity and habitat requirements.
(3) Recently, biotic data have been assessed for large regions of Antarctica in an attempt to determine Antarctic bioregions using GIS systems; Antarctic Conservation Biogeographic Regions (ACBR) (after Terauds et al. 2012). This is

an important step forward, but only with the inclusion of phylogenically informed biodiversity will we be able to have accurate ACBRs. The implementation of the current knowledge on microfaunal diversity (as shown in this review) with genetic lineages identified by phylogenetic studies combined with abiotic data will help to better delineate ACBRs.

Acknowledgements

We thank Dieter Piepenburg for editorial comments and two anonymous reviewers. In particular we thank Dr Sven Boström for providing a thorough review of the nematodes and Dr Sandra McInnes for assisting with the tardigrades. We are grateful to the University of Adelaide (http://www.sciences.adelaide.edu.au/) for a PhD scholarship to AVC and the South Australian Museum Mawson Trust for providing funding for the Sir Douglas Mawson Doctoral Scholarship (http://www.samuseum.sa.gov.au/). This study was partially supported and funded by the Australian Antarctic Division (http://www.antarctica.gov.au/) Project (ASAC 2355 to MIS).

References

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

Adams BJ, Wall DH, Gozel U, Dillman AR, Chaston JM, Hogg ID (2007) The southernmost worm, *Scottnema lindsayae* (Nematoda): diversity, dispersal and ecological stability. Polar Biol 30:809–815

Andrássy I (1981) Revision of the order Monhysterida (Nematoda) inhabiting soil and inland waters. Opusc Zool Budapest 17–18:13–47

Andrássy I (1998) Nematodes in the Sixth Continent. J Nematode Morph Syst 1:107–186

Andrássy I (2006) *Halomonhystera*, a new genus distinct from *Geomonhystera* Andrássy, 1981 (Nematoda: Monhysteridae). Meiofauna Mar 15:11–24

Andrássy I (2008a) *Eudorylaimus* species (Nematoda: Dorylaimida) of continental Antarctica. J Nematode Morph Syst 11:49–66

Andrássy I (2008b) On the male of the Antarctic nematode species, *Plectus murrayi* Yeates, 1970. J Nematode Morph Syst 11:87–89

Andrássy I, Gibson J (2007) Nematodes from saline and freshwater lakes of the Vestfold Hills, East Antarctica, including the description of *Hypodontolaimus antarcticus* sp. n. Polar Biol 30:669–678

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

Blouin M (2000) Brief communication. Neutrality tests on mtDNA: unusual results from nematodes. J Hered 91:156–158

Bohra P, Sanyal AK, Hussain A, Mitra B (2010) Five new records of nematodes from East Antarctica. J Threatened Taxa 2:974–977

Boström S (1995) Populations of *Plectus acuminatus* Bastian, 1865 and *Panagrolaimus magnivulvatus* n. sp. (Nematoda) from nunatakks in Dronning Maud Land, East Antarctica. Fundam Appl Nematol 18:25–34

Boström S (1997) *Chiloplectus masleni* sp. nov. and variability in populations of *Plectus acuminatus* Bastian 1865 (Nematoda: Plectidae) from the nunatak Basen, Vestfjella, Dronning Maud Land, East Antarctica. Polar Biol 17:74–80

Boström S (2005) Nematodes from Schirmacher Oasis, Dronning Maud Land, East Antarctica. Russ J Nematol 13:43–54

Boström S, Holovachov O, Nadler S (2011) Description of *Scottnema lindsayae* Timm, 1971 (Rhabditida: Cephalobidae) from Taylor Valley, Antarctica and its phylogenetic relationship. Polar Biol 34:1–12

British Antarctic Survey, 2004. Antarctica, 1:10,000,000 scale map. British Antarctic Survey, Cambridge

Burgess JS, Spate AP, Shevlin J (1994) The onset of deglaciation in the Larsemann Hills, Eastern Antarctica. Antarct Sci 6:491–495

Burn AJ (1984) Life cycle strategies in two Antarctic Collembola. Oecologia 64:223–229

Convey P, Block W (1996) Antarctic Diptera: Ecology, physiology and distribution. Eur J Entomol 93:1–13

Convey P, Gibson JAE, Hillenbrand CD, Hodgson DA, Pugh PJA, Smellie JL, Stevens MI (2008) Antarctic terrestrial life – challenging the history of the frozen continent? Biol Rev 83:103–117

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

Convey P, Stevens MI (2007) Antarctic biodiversity. Science 317:1877–1878.

Convey P, Stevens MI, Hodgson DA, Smellie JL, Hillenbrand CD, Barnes DKA, Clarke A, Pugh PJA, Linse K, Cary SC (2009) Exploring biological constraints on the glacial history of Antarctica. Quatern Sci Rev 28:3035–3048

Courtright EM, Wall DH, Virginia RA, Frisse LM, Vida JT, Thomas WK (2000) Nuclear and mitochondrial DNA sequence diversity in the Antarctic nematode *Scottnema lindsayae*. J Nematol 32:143–153

Cromer L, Gibson JAE, Swadling KM, Hodgson DA (2006) Evidence for a lacustrine faunal refuge in the Larsemann Hills, East Antarctica, during the Last Glacial Maximum. J Biogeogr 33:1314–1323

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 Systematics 26:526–538

Dartnall HJG (1983) Rotifers of the Antarctic and Subantarctic. Hydrobiologia 104:57–60

Dartnall HJG (1995) Rotifers, and other aquatic invertebrates, from the Larsemann Hills, Antarctica. Pap Proc Roy SocTasmania 129:17–23

Dartnall HJG (2000) A limnological reconnaissance of the Vestfold Hills. ANARE Rep 141:1–53

Dartnall HJG (2005) Freshwater invertebrates of subantarctic South Georgia. J Nat Hist 39:3321–3342

Dartnall HJG, Hollowday ED (1985) Antarctic Rotifers. Br Antarct Surv Sci Rep 100: 1–46, Cambridge

Dastych H (1984) The Tardigrada from Antarctic with descriptions of several new species. Acta Zool Cracov 27:377–436

Dastych H (1989) An annotated list of Tardigrada from the Antarctic. Entomol Mitt Zool Mus Hamburg 9:249–257

Dastych 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, Harris JM (1995) A new species of the genus *Macrobiotus* from inland nunataks in western Dronning Maud Land, continental Antarctica (Tardigrada). Entomol Mitt Zool Mus Hamburg 11:175–182

Dastych H, McInnes S (1994) *Hexapodibius boothi* sp. n., a new species of semi-terrestrial tardigrade from the Maritime Antarctic. Entomol Mitt Zool Mus Hamburg 11:111–117

Dastych H, Ryan PG, Watkins BP (1990) Notes on Tardigrada from western Dronning Maud Land (Antarctica) with a description of two new species. Entomol Mitt Zool Mus Hamburg 10:57–66

De Smet WH, Gibson JA (2008) *Rhinoglena kutikovae* n. sp. (Rotifera: Monogononta: Epiphanidae) from the Bunger Hills, East Antarctica: a probable relict species that survived Quaternary glaciations on the continent. Polar Biol 31:595–603

Derycke S, Vanaverbeke J, Rigaux A, Backeljau T, Moens T (2010) Exploring the Use of Cytochrome Oxidase c Subunit 1 (COI) for DNA Barcoding of Free-Living Marine Nematodes. PLoS ONE 5:e13716

Donner J (1972) Report on the finding of Rotifera (Rotatoria) from Antarctica. Polskie Arch Hydrobiol 19:251–252

Floyd R, Abebe E, Papert A, Blaxter M (2002) Molecular barcodes for soil nematode identification. Mol Ecol 11:839–850

Fontaneto D, Barraclough TG, Chen K, Ricci C, Herniou EA (2008) Molecular evidence for broad-scale distributions in bdelloid rotifers: everything is not everywhere but most things are very widespread. Mol Ecol 17:3136–3146

Fontaneto D, Kaya M, Herniou EA, Barraclough TG (2009) Extreme levels of hidden diversity in microscopic animals (Rotifera) revealed by DNA taxonomy. Mol Phylogen Evol 53:182–189

Frati F, Spinsanti G, Dallai R (2001) Genetic variation of mtCOII gene sequences in the collembolan Isotoma klovstadi from Victoria Land, Antarctica: evidence for population differentiation. Polar Biol 24:934–940

Freckman DW, Virginia RA (1997) Low-diversity Antarctic soil nematode communities: Distribution and response to disturbance. Ecology 78:363–369

Gagarin VG (2009) A revision of the genus *Eutobrilus* Tsalolikhin, 1981 (Nematoda, Triplonchida). Inl Wat Biol 2:205–212

Ghosh SC, Chatterjee A, Mitra B, De J. (2005) *Antarctenchus motililus* sp. n. (Nematoda: Tylenchida) from Schirmacher Oasis, East Antarctica. J Interacademica 9:367–371

Gibson JAE, Cromer 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 (Suppl. 1):65–71

Gore DB, Rhodes EJ, Augustinus PC, Leishman MR, Colhoun EA, Rees-Jones J (2001) Bunger Hills, East Antarctica: Ice free at the Last Glacial Maximum. Geology 29:1103–1106

Greenslade P (1995) Collembola from the Scotia Arc and Antarctic Peninsula including descriptions of two new species and notes on biogeography. Pol Pismo Entomol 64:305–319

Greenslade P, Farrow RA, Smith JMB (1999) Long distance migration of insects to a subantarctic island. J Biogeogr 26:1161–1167

Greenslade P, Wise KAJ (1984) Additions to the collembolan fauna of the Antarctic. Trans R Soc Aust 108:203–205

Hansson LA, Hylander S, Dartnall HJG, Lidström S, Svensson JE (2012) High zooplankton diversity in the extreme environments of the McMurdo Dry Valley lakes, Antarctica. Antarct Sci 24:131–138

Hawes TC, Worland MR, Convey P, Bale JS (2007) Aerial dispersal of springtails on the Antarctic Peninsula: implications for local distribution and demography. Antarct Sci 19:3–10

Hays JD, Imbrie J, Shackleton NJ (1976) Variations in the Earth's Orbit: Pacemaker of the Ice Ages. Science 194:1121–1132

Hebert PDN, Ratnasingham S, deWaard JR (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc R Soc Lond (Suppl) 270:S96–S99

Heyns J (1994) *Chiloplacoides antarcticus* n. gen., n. sp. from western Dronning Maud Land, Antarctica (Nematoda : Cephalobidae). Fundam Appl Nematol 17:333–338

Hodgson DA, Noon PE, Vyverman W, Bryant CL, Gore DB, Appleby P, Gilmour M, Verleyen E, Sabbe K, Jones VJ, Ellis-Evans JC, Wood PB (2001) Were the Larsemann Hills ice-free through the Last Glacial Maximum? Antarct Sci 13:440–454

Hogg ID, Stevens MI (2002) Soil fauna of Antarctic coastal landscapes. In: Beyer L, Bolter M (eds) Geoecology of Antarctic ice-free coastal landscapes. Ecological Studies 154:265–282

Holovachov O, Boström S (2006) Description of *Acrobeloides arctowskii* sp. n. (Rhabditida: Cephalobidae) from King George Island, Antarctica. Russ J Nematol 14:51–56

Hughes KA, Convey P, Huiskes AHL (2014) Global Movement and Homogenisation of Biota: Challenges to the Environmental Management of Antarctica? In: Tin T et al. (eds) Antarctic Futures. Springer Science, Dordrecht, pp 113–137

Huiskes AHL, Convey P, Bergstrom DM (2006) Trends in Antarctic Terrestrial and Limnetic Ecosystems: Antarctica as a Global Indicator. In: Bergstrom DM, Convey P, Huiskes AHL (eds) Trends in Antarctic Terrestrial and Limnetic Ecosystems. Springer, pp 1–13

Ingole BS, Parulekar AH (1993) Limnology of freshwater lakes of Schirmacher Oasis, East Antarctica. Proc Indian Natl Sci Acad B 59:589–600

Janiec K (1996) The comparison of freshwater invertebrates of Spitsbergen (Arctic) and King George Island (Antarctic). Pol Polar Res 17:173-202

Ji Y et al (2013) Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. Ecol Letters 16:1245–1257

Kinchin IM (1994) The Biology of Tardigrades. Portland Press Ltd, London, pp 186

Kirjanova ES (1958) Antarctic specimens of freshwater nematodes of the genus *Plectus* Bastian (Nematoda, Plectidae). Sov Antarct Exped Inf Bull 3:101–103

Kito K, Ohyama Y (2008) Rhabditid nematodes found from a rocky coast contaminated with treated waste water of Casey Station in East Antarctica, with a description of a new species of *Dolichorhabditis* Andrássy, 1983 (Nematoda: Rhabditidae). Zootaxa 1850:43–52

Kito K, Shishida Y, Ohyama Y (1991) *Plectus antarcticus* de Man, 1904 and *P. frigophilus* Kirjanova, 1958 (Nematoda: Plectidae), with emphasis on the male, from the Soya Coast, East Antarctica. Nematologica 37:252–262

Kito K, Shishida Y, Ohyama Y (1996) New species of the genus *Eudorylaimus* Andrassy, 1959 (Nematoda: Qudsianematidae) from East Antarctica. Polar Biol 16:163–169

Lawver LA, Gahagan LM (2003) Evolution of Cenozoic seaways in the circum-Antarctic region. Palaeogeogr, Palaeoclim, Palaeoecol 198:11–37

Lawver LA, Gahagan LM, Dalziel IWD (1998) A tight fit-Early Mesozoic Gondwana, a plate reconstruction perspective. Mem Natl Inst Polar Res Spec 53:214–229

Marshall DJ, Pugh PJA (1996) Origin of the inland Acari of Continental Antarctica, with particular reference to Dronning Maud Land. Zool J Linn Soc 118:101–118

Maslen NR, Convey P (2006) Nematode diversity and distribution in the southern maritime Antarctic—clues to history? Soil Biol Biochem 38:3141–3151

McInnes SJ (1995) Taxonomy and ecology of tardigrades from Antarctic lakes. M Phil, Open University:248

McInnes SJ (2010) *Echiniscus corrugicaudatus* (Heterotardigrada; Echiniscidae) a new species from Ellsworth Land, Antarctica. Polar Biol 33:59–70

McInnes SJ, Pugh PJA (1998) Biogeography of limno-terrestrial Tardigrada, with particular reference to the Antarctic fauna. J Biogeogr 25:31–36

McInnes SJ, Pugh PJA (2007) An attempt to revisit the global biogeography of limno-terrestrial Tardigrada. J Limnol 66:90–96

Meldal BHM, Debenham NJ, De Ley P, De Ley IT, Vanfleteren JR, Vierstraete AR, Bert W, Borgonie G, Moens T, Tyler PA, Austen MC, Blaxter ML, Rogers AD, Lambshead PJD (2007) An improved molecular phylogeny of the Nematoda with special emphasis on marine taxa. Mol Phylogen Evol 42:622–636

Miller JD, Horne P, Heatwole H, Miller WR, Bridges L (1988) A survey of the terrestrial Tardigrada of the Vestfold Hills, Antarctica. Hydrobiologia 165:197–208

Miller WR, Heatwole H (1995) Tardigrades of the Australian Antarctic Territories: the Mawson Coast, East Antarctica. Invertebr Biol 114:27–38

Miller WR, Heatwole H, Pidgeon RWJ, Gardiner GR (1994) Tardigrades of the Australian Antarctic Territories: the Larsemann Hills, East Antarctica. Trans Am Microsc Soc 113:142–160

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

Moore PD (2002) Biogeography: Springboards for springtails. Nature 418:381–381

Muñoz J, Felicisimo AM, Cabezas F, Burgaz AR, Martinez I (2004) Wind as a long-distance dispersal vehicle in the Southern Hemisphere. Science 304:1144–1147

Murray J (1910) Part III. Antarctic Rotifera. British Antarctic Expedition 1907–9, under the command of Sir EH Shackleton, cvo Reports on the scientific investigations 1:41–65

Nedelchev S, Peneva V (2000) Description of three new species of the genus *Mesodorylaimus* Andrássy, 1959 (Nematoda: Dorylaimidae) from Livingston Island, Antarctica, with notes on *M. imperator* Loof, 1975. Russ J Nematol 8:161–172

Nkem JN, Wall DH, Virginia RA, Barrett JE, Broos EJ, Porazinska DL, Adams BJ (2006) Wind dispersal of soil invertebrates in the McMurdo Dry Valleys, Antarctica. Polar Biol 29:346–352

Opalinski K (1972) Freshwater fauna and flora in Haswell island(Queen Mary Land, Eastern Antarctica). Pol Arch Hydrobiol 19:377–381

Peck LS (2005) Prospects for survival in the Southern Ocean: vulnerability of benthic species to temperature change. Antarct Sci 17:497–507

Peneva V, Chipev N (1999) *Laimaphelenchus helicosoma* (Maslen, 1979) n. comb. (Nematoda: Aphelenchida) from the Livingston Island (the Antarctic). Bulg Antarct Res 2:57–61

Pilato G, Binda MG (1999) Three new species of *Diphascon* of the *pingue* group (Eutardigrada, Hypsibiidae) from Antarctica. Polar Biol 21:335–342

Pilato G, McInnes SJ, Lisi O (2012) *Hebesuncus mollispinus (*Eutardigrada, Hypsibiidae), a new species from maritime Antarctica. Zootaxa 3446:60–68

Prosser SWJ, Velarde-Aguilar MG, León-Règagnon V, Hebert PDN (2013) Advancing nematode barcoding: A primer cocktail for the cytochrome c oxidase subunit I gene from vertebrate parasitic nematodes. Mol Ecol Resour 13:1108–1115

Pugh PJA (1993) A synonymic catalogue of the Acari from Antarctica, the sub-Antarctic Islands and the Southern Ocean. J Nat Hist 27:323–421

Pugh PJA, Convey P (2000) Scotia Arc Acari: antiquity and origin. Zool J Linn Soc 130:309–328

Pugh PJA, Convey P (2008) Surviving out in the cold: Antarctic endemic invertebrates and their refugia. J Biogeogr 35:2176–2186

Robeson MS, Costello EK, Freeman KR, Whiting J, Adams B, Martin AP, Schmidt SK (2009) Environmental DNA sequencing primers for eutardigrades and bdelloid rotifers. BMC Ecol 9:25

Rounsevell DE, Horne PA (1986) Terrestrial, parasitic and introduced invertebrates of the Vestfold Hills. In: Pickard J (ed) Antarctic Oasis. Terrestrial environments and history of the Vestfold Hills. Academic Press Australia, Sydney, pp 309–331

Ryss A, Boström S, Sohlenius B (2005) Tylenchid nematodes found on the nunatak Basen, East Antarctica. Ann Zool 55:315–324

Sands CJ, Convey P, Linse K, McInnes SJ (2008) Assessing meiofaunal variation among individuals utilising morphological and molecular approaches: an example using the Tardigrada. BMC Ecol 8:7

Segers H (2007) Annotated checklist of the rotifers (Phylum Rotifera), with notes on nomenclature, taxonomy and distribution. Zootaxa 1564:1–104

Shishida Y, Ohyama Y (1986) A note on the terrestrial nematodes around Syowa station, Antarctica (extended abstract). Mem Natl Inst Polar Res, Spec Issue 44:259–260

Sinclair BJ (2001) On the distribution of terrestrial invertebrates at Cape Bird, Ross Island, Antarctica. Polar Biol 24:394–400

Sinclair BJ, Stevens MI (2006) Terrestrial microarthropods of Victoria Land and Queen Maud Mountains, Antarctica: Implications of climate change. Soil Biol Biochem 38:3158–3170

Smykla J, Porazinska DL, Iakovenko N, Janko K, Weiner WM, Niedbala W, Drewnik M (2010) Studies on Antarctic soil invertebrates: Preliminary data on rotifers (Rotatoria), with notes on other taxa froPolar Biolm Edmonson Point (Northern Victoria Land, Continental Antarctic). Acta Soc Zool Bohem 74:135–140

Smykla J, Iakovenko N, Devetter M, Kaczmarek Ł (2012) Diversity and distribution of tardigrades in soils of Edmonson Point (Northern Victoria Land, continental Antarctica). Czech Polar Reports 2:61–70

Sohlenius B (1989) Interactions between two species of *Panagrolaimus* in agar cultures. Nematologica 34:208–217

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

Sohlenius B, Boström S (2008) Species diversity and random distribution of microfauna in extremely isolated habitable patches on Antarctic nunataks. Polar Biol 31:817–825

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 KI (2004) Occurrence of nematodes, tardigrades and rotifers on ice-free areas in East Antarctica. Pedobiologia 48:395–408

Stevens MI, Fjellberg A, Greenslade P, Hogg ID, Sunnucks P (2006b) Redescription of the Antarctic springtail *Desoria klovstadi* using morphological and molecular evidence. Polar Biol 29:820–830

Stevens MI, Greenslade P, Hogg ID, Sunnucks P (2006a) Southern Hemisphere springtails: Could Any Have Survived Glaciation of Antarctica? Mol Biol Evol 23:874–882

Stevens MI, Hogg ID (2002) Expanded distributional records of Collembola and Acari in southern Victoria Land, Antarctica. Pedobiologia 46:485–495

Stevens MI, Hogg ID (2003) Long-term isolation and recent range expansion from glacial refugia revealed for the endemic springtail *Gomphiocephalus hodgsoni* from Victoria Land, Antarctica. Mol Ecol 12:2357–2369

Stevens MI, Hogg ID (2006a) Contrasting levels of mitochondrial DNA variability between mites (Penthalodidae) and springtails (Hypogastruridae) from the Trans-Antarctic Mountains suggest long-term effects of glaciation and life history on substitution rates, and speciation processes. Soil Biol Biochem 38:3171–3180

Stevens MI, Hogg ID (2006b) The molecular ecology of Antarctic terrestrial and limnetic invertebrates and microbes. In: Bergstrom DM, Convey P, Huiskes AHL (eds) Trends in Antarctic Terrestrial and Limnetic Ecosystems. Springer, pp 177–192

Stevens MI, Porco D, D'Haese CA, Deharveng L (2011) Comment on "Taxonomy and the DNA barcoding enterprise" by Ebach (2011). Zootaxa 2838:85–88

Sudzuki M (1988) Comments on the antarctic Rotifera. Hydrobiologia 165:89–96

Suren A (1990) Microfauna associated with algal mats in melt ponds of the Ross Ice Shelf. Polar Biol 10:329–335

Terauds A, Chown SL, Morgan F, Peat HJ, Watts DJ, Keys H, Convey P, Bergstrom DM (2012) Conservation biogeography of the Antarctic. Divers Dist 18:726–741

Timm RW (1971) Antarctic Soil and Freshwater Nematodes from the McMurdo Sound Region. Proc Helminthol Soc Wash 38:42–52

Torricelli G, Carapelli A, Convey P, Nardi F, Boore JL, Frati F (2010) High divergence across the whole mitochondrial genome in the "pan-Antarctic" springtail *Friesea grisea*: Evidence for cryptic species? Gene 449:30–40

Tripati A, Backman J, Elderfield H, Ferretti P (2005) Eocene bipolar glaciation associated with global carbon cycle changes. Nature 436:341–346

Tumanov DV (2006) Five new species of the genus *Milnesium* (Tardigrada, Eutardigrada, Milnesiidae). Zootaxa 1122:1–23

Utsugi K, Ohyama Y (1991) Antarctic Tardigrada II. Molodezhnaya and Mt. Riiser-Larsen areas.Proc NIPR Symp Polar Biol 4:161–170

Utsugi K, Ohyama Y (1993) Antarctic Tardigrada III. Fildes Peninsula of King George Island. Proc NIPR Symp Polar Biol 6:139–151

Velasco-Castrillón A, Stevens MI (2014). Morphological and molecular diversity at a regional scale: A step closer to understanding Antarctic nematode biogeography. Soil Biol Biochem 70:272–284

Velasco-Castrillón A, Schultz MB, Colombo F, Gibson JAE, Davies KA, Austin AD, Stevens MI (2014). Distribution and diversity of microfauna from East Antarctica: assessing the link between biotic and abiotic factors. PLoS ONE 9:e87529

Verlecar XN, Dhargalkar VK, Matondkar SGP (1996) Ecobiological studies of the freshwater lakes at Schirmacher Oasis, Antarctica. Sci Rep: Twelfth Indian Exp Antarct, Techn Publ 10: 233–257

Vincent WF, James MR (1996) Biodiversity in extreme aquatic environments: lakes, ponds and streams of the Ross Sea sector, Antarctica. Biodivers Conserv 5:1451–1471

Wall DH (2007) Global change tipping points: above-and below-ground biotic interactions in a low diversity ecosystem. Phil Trans R Soc B 362:2291–2306

Webster-Brown J, Gall M, Gibson J, Wood S, Hawes I (2010) The biogeochemistry of meltwater habitats in the Darwin Glacier region (80° S), Victoria Land, Antarctica. Antarct Sci 22:646–661

Wharton DA (2003) The environmental physiology of Antarctic terrestrial nematodes: a review. J Comp Physiol B 173:621–628

Wise KAJ (1967) Collembola (springtails). Antarct Res Ser 10:123–148

Yeates GW (1970) Two Terrestrial Nematodes from the McMurdo Sound Region, Antarctica, with a Note on *Anaplectus arenicola* Killick, 1964. J Helminthol 44:27–34

Yeates GW (1979) Terrestrial nematodes from the Bunger Hills and Gaussberg, Antarctica. NZ J Zool 6:641–643

CHAPTER II

Distribution and diversity of soil microfauna from east Antarctica: assessing the link between biotic and abiotic factors

Alejandro Velasco-Castrillón[1*], Mark B. Schultz[2], Federica Colombo[3], John A. E. Gibson[4], Kerrie A. Davies[5], Andrew D. Austin[1] and Mark I. Stevens[1,6]

[1] *Australian Centre for Evolutionary Biology and Biodiversity, School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, South Australia 5005, Australia*

[2] *Department of Genetics, Bio21 Institute, The University of Melbourne, Parkville, Victoria, 3010, Australia*

[3] *Hawkesbury Institute for the Environment, University of Western Sydney, Richmond, NSW 2751, Australia*

[4] *Institute of Marine and Antarctic Studies, University of Tasmania, Private Bag 129, Hobart, Tasmania 7001, Australia*

[5] *Australian Centre for Evolutionary Biology and Biodiversity, School of Agriculture Food and Wine, The University of Adelaide, Urrbrae, Adelaide, South Australia 5064, Australia*

[6] *South Australian Museum, GPO Box 234, Adelaide, South Australia 5000, Australia*

[*] Corresponding author: Alejandro Velasco-Castrillón. E-mail: macalusio@yahoo.com

This chapter has been published and formatted in a style required by the journal *PLoS One*

Statement of authorship

Title of Paper	Distribution and diversity of soil microfauna from east Antarctica: assessing the link between biotic and abiotic factors
Publication Status	Published
Publication Details	This chapter was published as a research article Velasco-Castrillón, A., Schultz, M. B., Colombo, F., Gibson, J. A. E., Davies, K. A., Austin, A. D., and Stevens M. I. (2014). Distribution and diversity of soil microfauna from east Antarctica: assessing the link between biotic and abiotic factors. *PLoS One* 9:e87529

Author Contributions

By signing the Statement of authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Alejandro Velasco Castrillón		
Contribution to the Paper	Conceived and design the experiment, coordinated project, collected and sorted soil samples, conducted microfauna extraction and identification, performed statistical analyses and interpreted data, produced figures and tables, wrote the manuscript.		
Signature		Date	11/11/2013

Name of Co-Author	Mark B Schultz		
Contribution to the Paper	Collected soil samples, assisted in the statistical analyses, provided critical revision and final approval of the manuscript.		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	08/10/2013

Name of Co-Author	Federica Colombo		
Contribution to the Paper	Conducted soil sorting and microfuana extraction, revised and approved manuscript		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	8/10/2013

Name of Co-Author	John A E Gibson		
Contribution to the Paper	Conceived and design the experiment, conducted microfauna identification, critically revised and approved manuscript.		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	4/10/13

Name of Co-Author	Kerrie A Davis		
Contribution to the Paper	Conducted nematode identification and fixing of specimens, critically revised and approved manuscript.		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	03/10/2013

Name of Co-Author	Andrew D Austin		
Contribution to the Paper	Co-supervised project, critically revised and approved manuscript.		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	29/10/13

Name of Co-Author	Mark I Stevens		
Contribution to the Paper	Conceived and supervised the project, sought and won funding, assisted in data analyses, provided constant intellectual contribution, critically revised and approved manuscript.		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	09/10/2013

Preamble

Chapter II considers the microfauna phyla presented in Chapter I (Rotifera, Tardigrada, Nematoda and Arthropoda) as well as Protozoa. The main focus in this chapter is to correlate abiotic parameters (mainly soil geochemical parameters) with abundance and composition of microfauna collected from the sampling sites in Continental East Antarctica. The content of this chapter has been published in the journal *PLoS ONE*.

Abstract

Terrestrial life in Antarctica has been described as some of the simplest on the planet, and mainly confined to soil microfaunal communities. Studies have suggested that the lack of diversity is due to extreme environmental conditions and thought to be driven by abiotic factors. In this study we investigated soil taxa composition, abundance, and distribution in East Antarctica, and assessed correlations with soil geochemistry and environmental variables. We examined 109 soil samples from a wide range of ice-free habitats, spanning 2000 km from Framnes Mountains to Bailey Peninsula. Microfauna across all samples were patchily distributed, from complete absence of invertebrates to over 1600 specimens/gram of dry weight of soil (gdw), with highest microfauna abundance observed in samples with visible vegetation. Bdelloid rotifers were on average the most abundant organisms (44 specimens/gdw), followed by tardigrades (12 specimens/gdw), nematodes (3 specimens/gdw), ciliates (1.3 specimens/gdw), and mites (0.04 specimens/gdw). The most widespread taxa were rotifers, nematodes and tardigrades, found in 87%, 71% and 57% of sites sampled, respectively. Several soil geochemical parameters (phosphorus, salinity, organic carbon, moisture, NH_4^+, and NO_3^-) were correlated with microfaunal abundance and taxa composition. We found that taxa composition and abundance were mostly correlated with soil phosphorus, NO_3^- and salinity, and likely to be the result of soil properties and historic landscape formation and alteration, rather than the geographic region from which they were found. Studies focusing on Antarctic biodiversity must take into account soil geochemical and environmental factors that influence population and species heterogeneity.

Introduction

Desert ecosystems are often regarded as some of the simplest on Earth, in terms of trophic levels and biodiversity, when contrasted to temperate and tropical ecosystems [1]. In hot desert environments, soil microfaunal composition and diversity are linked to plant distribution and organic matter accumulation [2], with water as a potential determinant for species diversity [3,4]. Examination of hot and cold deserts, often lacking vascular plants and where water is a limiting factor, offers the opportunity to understand biotic interactions at multiple spatial scales, which are difficult to elucidate in less extreme environments that tend to have more intricate soil structures [2,5]. Organisms that survive in Antarctic (cold desert) refuges are constantly subjected to extreme abiotic stresses such as low temperatures, freeze-thaw cycles, available liquid water, high salt content, months of darkness, excessive solar radiation and nutrient and carbon restrictions [6,7,8,9]. Only those species with specific physiological adaptations have been able to survive under such extreme conditions, and this has been hypothesised as one of the main reasons for a depauperate soil microfaunal community [9,10,11]. The soil microfauna play an essential role in recycling nutrients and aiding decomposition, forming a vital component in Antarctic food webs [1,12]. Low diversity food webs found in these soils ensure that nutrient recycling and trophic level interactions are restricted to microbial and metazoan invertebrate communities [13,14]. The influence of soil geochemistry and physical properties on the presence and distribution of these communities has been increasingly recognised [15,16,17], with the main suggested drivers being organic carbon [1,7,18], conductivity [7,9], and availability of liquid water [17,19].

Even within ice-free areas, the distribution of microfaunal populations remains irregular and taxonomically limited [20,21]. It remains unclear if these populations are limited by edaphic factors, microclimatic conditions, vegetation, or topography (e.g. [22]), with more abundant and diverse communities usually occurring in connection with patches of moss, lichens, algae [9,11] and bird colonies [23,24]. Rotifers, nematodes, tardigrades, protozoans [25,26,27] and, to a lesser extent, mites and springtails [28] make up the invertebrate communities of soil microfauna in East Antarctica (EA). Invertebrates are patchily distributed in soil and vegetation in ice-free areas in coastal and continental

Antarctica and inland nunataks (exposed ridges or mountain peaks) [24,29,30,31,32]. Recent studies revealed that several Antarctic localities remained ice-free throughout the Last Glacial Maximum [9,33] and that many terrestrial habitats are likely to have only become available for colonisation from refuges within the current inter-glacial period (<17,000 years) [9,34]. However, there is compelling evidence that some regions are likely to have been ice-free for much longer and so it is likely that there exists an Antarctic terrestrial invertebrate fauna that consists of descendants from Gondwanan times. These have diversified in isolated ice-free locations since the completion of glaciation within the late Miocene (at approximately 21 to 11 Myr; e.g. [35,36]).

Studies of ice-free areas across Antarctica have shown variations in microfaunal composition according to location and habitat. Microfaunal abundance also shows seasonal variation with respect to abiotic factors. Higher moisture content in soil during summer, as a consequence of higher temperatures, has been associated to increase the growth of photosynthetic autotrophs, microbial and microfaunal species [37]. Vertical distribution of microfauna in the soil profile has also been recorded to be affected by seasonal changes, with temperature and food source as likely determining factors [15]. For example, nematodes have been identified as the most diverse and abundant invertebrate group from Victoria Land [38,39]; contrasting with results from Dronning Maud Land that have revealed rotifers, followed by tardigrades and nematodes as the most common taxa [11,40,41].When considering the diversity of microfauna in soil, competition should also be expected to influence community structure – some studies have identified nematodes as the top grazers [1], while others reported competition among nematodes, rotifers, tardigrades and ciliates, and in some cases tardigrades and mites preying on nematodes [42].

Given the diversity of nematodes, a number of studies have focused their attention on the identification of Antarctic species. A total of 22 nematode species for continental Antarctica have been recorded and at least 90% of them are endemic [43,44]. Some of the most common species recorded for the continent are the microbial feeders *Plectus murrayi*, *P. frigophilus* [27,45,46], *Scottnema lindsayae* [47], the omnivore genus *Eudorylaimus* [27,44,48], and the bacterial feeder *Panagrolaimus* [10,40]. Other nematodes, occurring in

lower abundance in EA, include the genus *Halomonhystera* [43,49], *Hypodontolaimus* [43], and *Dolichorhabditis* [50]. Studies of tardigrades in EA have recorded 18 species [51,52,53,54], belonging to three Orders (Apochela, Parachela and Echiniscoidea). The Order Parachela includes 15 species in ten genera; and the remaining two Orders are represented by the tardigrade genera *Echiniscus* and *Pseudoechiniscus* (Echiniscoidea) and the predatory species *Milnesium tardigradum* (Apochela). For rotifers, the Classes Bdelloidea and Monogononta have been reported for the Antarctic continent [55,56], with Bdelloidea being the most widespread and abundant invertebrate group for EA soils [40,57], with 22 species belonging to the genera *Adineta, Habrotrocha, Macrotrachela, Mniobia, Otostephanos* and *Philodina* (e.g. [41,56,58,59]). Unfortunately, most studies have limited spatial coverage (often opportunistic) and low sampling sizes to gauge if the true biodiversity is accurately represented in current records (Table 1). In particular, some areas in EA have revealed a lower than expected diversity; with the recorded species down to nine nematode, seven bdelloid rotifer, and 15 tardigrade species. More comprehensive studies covering not only the diversity but also taking into account the environmental micro-habitats will provide data that can allow more robust comparisons at broad geographic scales.

In this study, we investigate environmental variables, soil geochemistry, and abundance and diversity of soil microfauna from different habitat types in East Antarctic regions; from Holme Bay (67.60ºS – 62.87º E) and Framnes Mountains (67.78°S– 62.79°E) to Bailey Peninsula (66.28º S-110.54ºE). To the best of our knowledge this is the first single study that correlates biotic and abiotic parameters for an area spanning more than 2,000 km from any region in Antarctica. Other works have focused on diversity at a much smaller scale (e.g. [11,31,60]) including those that have considered abiotic variables for other Antarctic regions [9,13,17,61]. We examine four key questions: (1) Do abiotic variables differ significantly among the sample sites?; (2) If abiotic variables differ among sites, which variables best correlate taxa composition among sites (and to what extent); (3) Is microfaunal abundance affected by soil geochemistry and other abiotic variables; and (4) Is the occurrence of taxa correlated with the presence of other taxa?

Table 1. Diversity list for nematodes, bdelloid rotifers and tardigrades from East Antarctica showing previous record from the sampled regions. The list includes taxa (nematodes, bdelloid rotifers and tardigrades) reported in the literature for the regions: Mawson Station – Framnes Mtns (MS-FM), Vestfold Hills – Larsemann Hills (VH-LH), and Casey Station (CS, including the Windmill Islands). New records for the regions obtained in this study are indicated by 'nr' (new records are based on absence of published literature for the designated region). Symbol '?' means uncertainty for the record. Numbers indicate reference source (as in Reference list)

	MS-FM	VH-LH	CS
NEMATODA			
Order Rhabditida			
Dolichorhabditis tereticorpus Kito & Ohyama, 2008	-	-	50
Scottnema lindsayae Timm, 1971	nr	nr	-
Order Plectida			
Plectus frigophilus Kirjanova, 1958	nr	43	nr
Plectus murrayi Yeates, 1970	nr	60	72
Order Dorylaimida			
Eudorylaimus glacialis Andrássy, 1998	44	-	-
Eudorylaimus quintus Andrássy, 2008	-	44	-
Eudorylaimus sextus Andrássy, 2008	-	44	-
Order Monhysterida			
Halomonhystera continentalis Andrássy, 2006	-	49, 43	-
Halomonhystera halophila Andrássy, 2006	-	49, 43	-
Order Desmodorida			
Hypodontolaimus antarcticus Andrássy & Gibson, 2007	-	43	-
Order Panagrolaimida (family cf. Panagrolaimidae)	nr?	nr?	
ROTIFERA			
Order Bdelloidea			
Adineta barbata Janson, 1983	-	56	*
Adineta grandis Murray, 1910	*	56, 55	58
Habrotrocha constricta Dujardin, 1841	*	56, 55	58
Macrotrachela quadricornifera Milne, 1886	-	56	-
Mniobia russeola (Zelinka, 1891)	-	56	-
Philodina gregaria Murrayi, 1910	*	56, 55	58
Philodina alata	*	*	-
TARDIGRADA			
Order Parachela			
Acutuncus antarcticus (Binda & Pilato, 2000)	53	60, 83	54
Diphascon chilenense (Sudzuki, 1964)	-	83	54
Diphascon (Diphascon) pingue Marcus, 1936	-	-	54
Diphascon (Diphascon?) puniceum Jennings, 1971	*	29	-
Diphascon sanae Dastych, Ryan & Watkins, 1990	53	-	-
Hypsibius allisoni Horning, Schuster & Grigarick, 1978	-	29	-
Macrobiotus blocki Dastych, 1984	53	-	-
Macrobiotus furciger Murray, 1907	*	29	-
Macrobiotus weinerorum Dastych, 1984	-	83	-
Minibiotus stuckenbergi Dastych, Ryan & Watkins, 1990	53	-	-
Ramajendas frigidus Pilato & Binda, 1990	-	-	54
Order Apochela			
Milnesium cf. *tardigradum* Doyere, 1840	53	83	-
Order Echiniscoidea			
Echiniscus jenningsi Dastych, 1984	53	-	-
Pseudechiniscus cf. *suillus*	-	83	54
Pseudoechiniscus novaezeelandiae Richters, 1903	-	60, 29, 83	-

* Previously reported by John Gibson (unpublished data)

Materials and Methods

Sampling sites

All field activities and sampling in Antarctica was undertaken with permits granted by the Australian Antarctic Division (Australian Federal Government, Department of Sustainability, Environment, Water, Population and Communities)**.** Samples returned to Australia under required quarantine protocols with permits granted by Australian Quarantine Inspection Service (AQIS, Australian Federal Government, Department of Agriculture, Fisheries and Forestry). Under these permitted guidelines, sampling in EA was conducted during the 2009-2010 austral summer from Casey Station on 24 December 2009, and from all other locations from 14 January 2010 to 4 March 2010 (for sampling sites refer to Appendix 2: Table S1). Sampling locations were distributed over ten arbitrarily defined regions ranging from 67º-69º S to 62º-110º E with elevations ranging from 0 m to 490 m (Fig. 1). A total of 109 samples from ice-free areas were collected from ten regions: Casey Station (CS), Vestfold Hills (VH), Larsemann Hills-Broknes Peninsula (BP), Larsemann Hills-Stornes Peninsula (SP), Larsemann islands (L-Isl), Hop Island (HI), Mather Peninsula (MP), Sansom Island (SI), Framnes Mountains (FM) and Mawson Station (MS; Table 2). Sites were selected to represent a diversity of habitats with the intent of capturing a wide diversity of microinvertebrates; habitat types included visible vegetation (moss, cyanobacteria or algae), bird colonies and/or water bodies, and dry soils to semi-dry soil with no apparent vegetation. Soil samples (each 500 g – 800 g wet weight) were ~10 cm in surface area and ~10 cm deep (depth varied depending on the terrain); soil core samples were excavated using a metal trowel which was carefully cleaned to avoid cross contamination. The top 10 cm were sampled as earlier studies have shown that throughout the summer season the majority of Antarctic soil microfauna inhabit this layer [15]. Samples were thoroughly mixed and kept in sterile 42 fl. oz. Whirl-pak® bags inside insulated containers while in the field and maintained at -20°C during storage and transit.

Table 2. Geographic location and type of samples collected from ten regions across East Antarctica.

REGION	Coordinates		Area sampled (km)	Elev (m)	Sample content			
	South	East			Soil-Gravel	Soil-al-cy	Soil-Moss	Total Samples
Casey Station CS)	66.28º	110.52º-110.54º	1 x 1.5	4-44	4	1	9	14
Vestfold Hills (VH)	68.48º-68.60º	77.87º-78.51º	17 x 20	4-66	11	5	6	22
Broknes Peninsula (BP)	69.38º-69.4º	76.32º-76.40º	3.5 x 2	0-69	13	1	0	14
Stornes Peninsula (SP)	69.37º-69.43º	75.99º-76.14º	6 x 1	4-59	4	1	4	9
Larsemann Islands (L-Isl)	69.36º-69.41º	76º-76.14º	7 x 0.1*	21-27	6	0	5	11
Hop Island (HI)	68.82º-68.83º	77.68º-77.73º	2 x 2	10-36	10	5	1	16
Mather Peninsula (MP)	68.85º-68.86º	77.93º-77.94º	1 x 1	44-80	1	1	4	6
Sansom Island (SI)	69.71º	73.75º	0.2 x 0.2	15-20	0	0	3	3
Mawson Station (MS)	67.60º	62.86º-62.87º	0.6 x 0.8	4-24	2	2	2	6
Framnes Mountains (FM)	67.77º-67.78º	62.79º-62.82º	3 x 1	460-490	6	0	0	6
TOTAL				0-490	57	18	34	107‡

*Two small islands 7 km apart. For the first Island (400 m south of Cook Island) samples were taken 25m apart. For the second (McLeod Island) samples were within 100 m^2. Acronyms as following: Elevation (Elev), algae-cyanobacteria (al-cy). ‡ One sample from MP and other from MS included soil-lichen (not shown in the table)

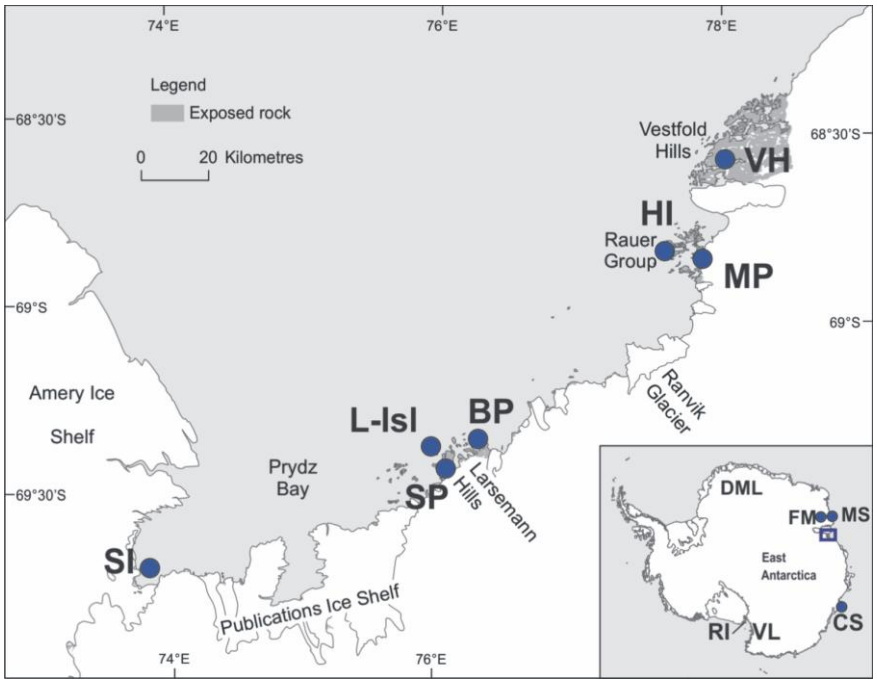


Figure 1. Maps showing the ten regions from East Antarctica (EA) where sampling was conducted (filled circles). Abbreviations: Framnes Mountains (FM), Mawson Station (MS), Casey Station (CS), Sansom Island (SI), Larsemann-Islands (L-Isl), Stornes Peninsula (SP), Broknes Peninsula (BP), Hop Island (HI), Mather Peninsula (MP), and Vestfold Hills (VH). Other sectors and regions across Antarctica mentioned in the text (not included in this study): Dronning Maud Land (DML), Victoria Land (VL), and Ross Island (RI). Adapted from maps provided courtesy of the Australian Antarctic Division.

Microfaunal Extraction

Microfauna were extracted from the soil samples using a modified sugar centrifugation method [62]. Extractions were performed on 100 g soil samples (wet weight) after which stones larger than 1 cm were removed. Soil was poured onto a coarse sieve (400 µm mesh size) and carefully rinsed with double distilled water. The suspension of fine material and water that flowed through the 400 µm mesh was kept in a tray 7 cm deep. This material was then poured onto a finer sieve (38 µm mesh size) and gently rinsed through, keeping the sieve at an angle of 30°, allowing the water and suspended sediment to filter through the mesh (and the post 38 µm filtrate was discarded). The fine soil retained on the 38 µm mesh sieve was gently washed into one or two 50 ml centrifuge tubes (depending on the quantity of fine soil, never exceeding 15 ml of soil per tube) and then topped up with water to 50 ml and mixed gently by inversion. Tubes were centrifuged at 500 RCF for 5 min, and the supernatant was decanted through a 38 µm mesh sieve (some animals retained in the sieve were recovered at this stage) whilst attempting to minimise the pouring out of any sediment onto the sieve. The tube was filled up with 1.3 M sucrose solution up to 50 ml and gently mixed by inversion to resuspend the pellet, and then centrifuged at 500 RCF for 1 min. The aqueous layer was then decanted into the 38 µm mesh sieve, again avoiding the transfer of any sediment from the pellet, and then back-washed into a clean 50 ml tube and storage at -20 °C until further analysis.

Microfaunal Isolation and classification

Tubes containing microfauna in frozen distilled water were thawed and poured into a petri-dish to be examined under a dissecting stereo microscope (Olympus SZ-PT, Japan) at magnification 10x to 40x. Before isolation of specimens, presence of rotifers, nematodes, tardigrades, mites and ciliates were recorded and sorted coarsely within a gridded petri-dish. Individual specimens were then counted and abundance for each of the taxa assessed. In cases where samples were difficult to sort due to excessive amount of suspended material, further dilution was required. Samples with a high abundance of microfauna were sub-sampled and the total abundance was extrapolated for 100 g of soil.

Taxa were divided into glass blocks using modified gel tips attached to micro-syringes. Representative morphotypes for each taxon, were retained for subsequent morphological analyses. Specimens were carefully transferred with an Irwin loop into a water droplet on a slide and imaged under microscope (Celestron- LCD Digital Microscope, USA) at 40x to 100x before placing in separate 2 ml Eppendorf tubes. The remaining microfauna not selected for imaging were stored at -20 °C.

For the abundance analyses, all Rotifera were pooled into a single category, as was the case for Tardigrada, Nematoda, Ciliophora and Acari. For the taxa composition analyses (based on presence/absence), Ciliophora (ciliates) and Acari (mites) had their own separate categories, Rotifera were subdivided into monogononts (non-bdelloid rotifers) and bdelloids. Bdelloid identification was based on presence or absence of wheel-organs. The three bdelloid groups included *Philodina* (wheel-organ bearing bdelloid), *Adineta* (lacking wheel-organs) and a group including unidentified bdelloids (mostly comprising contracted specimens). Tardigrada, were grouped according to their Order (Parachela, Apochela, Echiniscoidea); and Nematoda were categorised as *Plectus, Eudorylaimus, Scottnema, Halomonhystera* (genera) and cf. Panagrolaimidae (family). *Plectus* species were identified using de Man's ratios calculated from digital images (after [63]) and verified by comparison with published species descriptions (Appendix 2: Table S2).

Soil geochemistry

Soil geochemical analyses were performed for each of the 109 samples collected across EA. These analyses were conducted in Australia by APAL (Australian Perry Agricultural Laboratory) using standard chemical methods [64]. Subsamples of 100 g were analysed for electrical conductivity (EC), organic carbon (C), Olsen-available phosphorus (P), NO_3^- and NH_4^+. Analyses for soil moisture (moist) and pH were performed at the University of Adelaide using the methods described by Rayment & Lyons [64]. Soil moisture was calculated from an average of 40 g of wet soil, and percentage of moisture content (per gram of dry soil) was measured by weight loss of the subsample dried in an oven for 24 hours at 100°C. Soil pH (pH meter- Schott® Instruments) was determined from 20 g

subsamples after shaking a semi-liquid mixture (based on a soil/water ratio of 1:5) for 1 hour at room temperature. The suspension was stirred constantly during the measurement to minimize changes in electrode potential. Other categories considered in our analyses included: fine sediment (amount of fine sediments in sample ranging between 38 – 400 µm), and particle size (qualitative gradient from silt to coarse gravel).

Statistical Analyses

Defining abiotic categories

Environmental variables were elevation, aspect, slope, vegetation content in soil (moss, cyanobacteria, algae or lichen), and proximity to moss beds when present. Other categories included region, geology of the terrain, amount of fine sediment in the sample and the soil geochemical parameters analysed (EC, C, P, NO_3^-, NH_4^+, moisture and pH). In total, 16 categorical, abiotic variables were considered, with ten of these quantitative and six qualitative. The categories moss, algae-cyanobacteria (al-cy), and soil samples from moss beds were qualitative dichotomous (i.e., presence/absence); the categories region, geology and aspect were qualitative. Regions included: CS, VH, BP, SP, L-Isl, HI, MP, SI, FM and MS. Geology of terrain comprised three sub-categories as reported by tectonic studies [65,66]. It consisted on mainly archaean complexes (VH and CS); mixed archaean-proterozoic complexes (HI and MP); and mainly meso-neoproterozoic (BP, SP, SI, MS and FM). Aspect included three sub-categories (1, 2 and 0) representing north-facing, south-facing and east/west/flat-facing (respectively). North-west and north-east sites were merged under the sub-category north facing, and south-west and south-east merged under south facing.

Biotic and abiotic

Biotic and abiotic categories and the interaction between them were analysed using PRIMER v.6 [67]. Abiotic data for quantitative abiotic categories were logarithmically (base-10) transformed [68] to avoid right skewness (as detected using Draftman Plots before transformation) and a small constant was added (0.1) to avoid zero values (after [67]). Qualitative and log[0.1+*x*] transformed quantitative variables were normalised (for each entry of a single variable the mean is subtracted and divided by the standard deviation of that variable) and then subjected to a Principal Component Analysis (PCA) based on Euclidean distances (after [69,70]) in order to identify the most relevant categories and the cumulative percentage variation of PCAs. Points on the PCA ordination plot were colour coded by region to place the analysis in a geographical context. A preliminary colinearity test for normalised abiotic categories based on the resemblance matrix of the Draftsman Plot was first estimated in order to reduce the amount of variables (after [67]). Only one category (geology) was dropped from the analysis given its strong colinearity with one other variable (region) as observed in the resemblance matrix (0.9 correlation value). The second strongest correlation (0.7) was seen for P and NH_4^+ but was not high enough to be excluded from the analyses (after [67]). PCA was used to indirectly correlate parameters (vectors) and sampling sites. Resemblance matrices (for biotic data) were created for taxa composition and 4^{th} root transformed microfauna abundance based on Bray-Curtis similarity coefficients to correct for skewness in the data to achieve normality [17,68,71]. Matrices on taxa composition were also used to generate individual hierarchical clusters for rotifers, tardigrades, nematodes, and (combined) microfaunal taxa (rotifers, tardigrades, nematodes, ciliates and mites). To correlate the relative contribution of abiotic variables with microfauna abundance and taxa composition the Bioenv method (PRIMER v.6) was employed using the Spearman correlation coefficient (after [67]).The Pearson correlation method was also used to correlate biotic and abiotic variables using IBM-SPSS Statistics v19 (see Appendix 2: Table S3 for details).

Table 3. Sample size (a), Taxa absent (b), Abundance (c), Percentage of Abundance (d), and taxon composition percentage (e) of microfauna from 109 soil samples at ten regions.

	All sites	CS	VH	HI	MP	LH-BP	LH-SP	L-Isl	SI	MS	FM
(a) Sample size (number of samples collected)											
	109	14	22	16	7	14	9	11	3	7	6
(b) Taxa absent (number of samples with no visible microfauna)											
	4	0	0	3	0	0	0	0	0	1	0
(c) Abundance (average number of animals/g dry weight on occurrence)											
Rotifera	4756.3	264.3	576.8	244	802.8	312	1010.2	191.3	1279.3	70.5	5.1
Tardigrada	1363	113.6	101.3	42.7	35.1	8.2	711.8	57.1	183.5	109.5	0.1
Nematoda	326.4	58.5	75	16.7	20.2	3.7	7.9	7.6	120.7	13.3	2.8
Ciliophora	139.8	0.8	88.5	7.5	1	0	0.4	0	41.4	0	0.1
Acari	3.9	0.5	0.8	0.3	0	0.1	2	0.1	0	0	0
Total	**6589**	**438**	**842**	**311**	**859**	**324**	**1732**	**256**	**1625**	**193**	**8**
Average	**60**	**31**	**38**	**19**	**123**	**23**	**192**	**23**	**542**	**28**	**1**
(d) Percentage of Abundance											
Rotifera	**72.18**	60.37	68.47	78.43	93.44	96.29	58.32	74.68	78.73	36.48	63.34
Tardigrada	**20.68**	25.96	12.02	13.72	4.09	2.54	41.09	22.3	11.3	56.65	0.92
Nematoda	**4.95**	13.36	8.91	5.35	2.35	1.14	0.46	2.97	7.43	6.86	34.56
Ciliophora	**2.12**	0.19	10.51	2.41	0.12	0.01	0.02	0	2.55	0.01	1.05
Acari	**0.06**	0.12	0.09	0.09	0	0.02	0.12	0.06	0	0	0.13
(e) Taxon composition percentage (based on presence-absence)											
Rotifera	87.2	11.9	18.3	9.2	6.4	11	8.3	9.2	2.8	5.5	4.6
Monogononta	0	0	0.92	0	0	0	0	0	0	0	0.92
Unident-Bdell	78.9	11.9	14.7	8.3	6.4	10.1	7.3	9.2	2.8	3.7	4.6
Adineta	28.4	2.8	2.8	1.8	2.8	5.5	3.7	3.7	0.9	3.7	0.9
Philodina	18.3	2.8	5.5	0.0	0.0	2.8	0.9	2.8	0.9	2.8	0.0
Tardigrada	56.9	10.1	9.2	5.5	2.8	5.5	6.4	7.3	2.8	5.5	1.8
Parachela	56	10.1	9.2	5.5	2.8	5.5	5.5	7.3	2.8	5.5	1.8
Apochela	1.8	0	0	0	0	0	0	0.9	0	0.9	0
Echiniscoidea	8.3	1.8	0.9	0	0.9	0	0.9	2.8	0	0.9	0
Nematoda	71.6	10.1	15.6	7.3	5.5	10.1	5.5	8.3	1.8	3.7	3.7
Plectus	51.4	10.1	7.3	3.7	5.5	6.4	4.6	6.4	1.8	3.7	1.8
Eudorylaimus	24.8	0	8.3	1.8	4.6	3.7	1.8	3.7	0	0.9	0
Scottnema	22	0	8.3	1.8	1.8	4.6	1.8	1.8	0	0	1.8
Halomonhystera	4.6	0	2.8	0.9	0	0.9	0	0	0	0	0
Panagrolaimidae	1.8	0	0	0.9	0	0	0	0	0	0.9	0
Ciliophora	15.6	0.9	4.6	4.6	1.8	0.9	1.8	0	0.9	0.9	0.9
Acari[†]	22.9	5.5	3.7	0.9	3.7	1.8	5.5	2.8	0	0	0.9

[†] Including 27 samples (20 with mite specimens, and 7 with only mite exuviae).

Total abundance and average for the regions in **(c)** are given in bold. Percentage of abundance for all sites in **(d)** is shown in bold in 1st column. Taxon composition in **(e)** refers to presence of taxa in samples (no abundance data considered for this category). List of acronyms: Casey Station (CS), Vestfold Hills (VH), Hop Island (HI), Mather Peninsula (MP), Larsemann Hills-Broknes Peninsula (BP), Larsemann Hills-Stornes Peninsula (SP), Larsemann - Islands (L-Isl), Sansom Island (SI), Mawson Station (MS), Framnes Mountains (FM), and Unidentified Bdelloids (Unident-Bdell).

Results

Environmental assemblages

The PCA is presented in Figure 2, with vector length indicating the relevance of the abiotic variable in question, and the orientation of the vector showing the positive or negative influence in reference to the cluster of sites. The most significant abiotic variables (indicated by vector length) corresponded to C, soil samples from moss beds (Cs_bed), samples containing moss filaments (moss), NH_4^+, EC, P, pH and moisture; while the least significant variable was aspect. The distribution of samples among regions was better explained by PC1 (as observed for BP, FM and CS). CS samples were segregated to the right of PC1 (showing positive correlations to C, P and moss); while BP and FM tend to segregate along the PC1 axis to the left of the cluster (negative values). No clear trend was observed for the other regions along PC1 and PC2 axes (Fig. 2). Overall, PC1 explained 22.8% of the variation among environmental variables. The cumulative variation of PC1 and PC2 was 37.9%; while PC3 – PC5 had a cumulative variation of 28.4%. The vectors for logarithmic transformed variables (C, P and NH_4^+) were positively correlated and presented the highest contribution for PC1 (eigenvectors: 0.46, 0.41 and 0.41, respectively). While for PC2 the highest contribution was observed for Cs_bed, moss and by EC (eigenvectors: -0.51, 0.36 and 0.35, respectively). Cs_bed was positively correlated to moss samples but negatively correlated with pH. When examining other type of vegetation, we observed that algae-cyanobacteria (al-cy) was better explained by PC2 (eigenvectors: 0.27). A positive correlation was also observed between al-cy and NO_3^-, but a negative correlation with elevation (Fig. 2). To identify groups of correlated abiotic variables some of the associations between soil abiotic parameters indicated by the PCA are corroborated with results summarized in the Pearson correlation matrix (Appendix 2: Table S3) and geochemical parameters (Figs 3 – 6). In general, when considering soil geochemical parameters positive correlations were seen for: *(i)* EC and NO_3^-; *(ii)* P, NH_4^+, C and soil moisture.

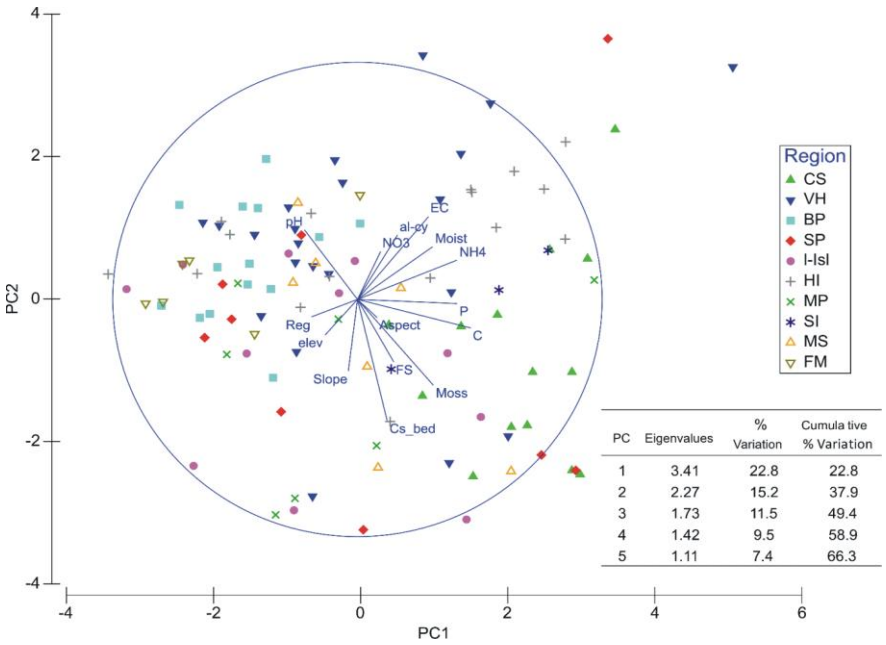


Figure 2. Principal component analysis (PCA) on log [x+0.1] transformed and normalized values of abiotic data from 109 sites. Symbol shapes represent region for each of the samples. Vectors labelled as region (Reg), elevation (elev), soil sample from moss bed (Cs_bed), fine sediment (FS), samples with moss filaments (moss), aspect, organic carbon percentage (C), phosphorus (P), $NH4^+$, moisture in soil (Moist), electrical conductivity (EC), samples containing alga-cyanobacteria (al-cy), $NO3^-$, and pH.

Taxon composition and terrestrial habitats

Taxon composition data (absence/presence) for 109 samples from the ten regions (Table 3) were used to identify closely related clusters based on Bray-Curtis similarity coefficient. Four hierarchical clusters were generated, one including all microfaunal taxa (rotifers, tardigrades, nematodes, ciliates and mites) as observed for each soil sample (Fig. 3A). The three other clusters represented taxon composition categories found for nematodes (Fig. 4A), rotifers (Fig. 5A), and tardigrades (Fig. 6A). Bdelloid rotifers were the most widespread taxon present in 87% of the samples, followed by nematodes in 71%, tardigrades in 57%, mites (including mite exuviae) in 23%, and ciliates in 15% (Table 3e). The presence of all microfauna taxa (combined) only occurred in three soil samples from CS, MP and SP (Fig. 3A). These three samples were from moss beds, with visible moss filaments and were characterised by high moisture (12 – 18%), wide ranges of C (0.8 – 2.9%), P (38 – 92 p.p.m.), and NH_4^+ (5 – 64 p.p.m.), and low NO_3^- (3.4 p.p.m.) and EC (0.06 – 0.15 dS/m; Fig. 3). The presence of the three most common taxa (rotifers,

tardigrades and nematodes) in the absence of ciliates and mites were found for 30 samples (Fig. 3A). Microfauna was absent in four samples (all with no visible vegetation); three of which were from Hop Island (Fig. 3), and two collected next to bird colonies. Rotifers (bdelloids) occurred as a single taxon in five samples, with a wide range of soil geochemical properties: EC (0.02 – 18.5 dS/m), NO_3^- (3.4 – 548 p.p.m.), NH_4^+ (4.8 – 345 p.p.m.), and P (4.5 – 469 mg/kg; Fig. 3). Nematodes were also found as the only taxon in five samples (without visible vegetation), but under more restricted concentrations of NO_3^- (3.4 p.p.m), C (0.01 – 0.22), EC (0.02 – 0.1 dS/m), and P (3.6 – 11.6 mg/kg; Fig. 3).

Nematode composition and habitats

All nematodes were identified using morphological measurements and de Man's ratios. For *Plectus*, morpho-types were compared with described populations for *Plectus murrayi* and *P. frigophilus* from continental Antarctica (Appendix 2: Table S2). Our study revealed the genus *Plectus* to be the most widespread, present in 51% of all 109 samples, followed by *Eudorylaimus* in 25%, *Scottnema* in 22%, *Halomonhystera* in 4.6% and cf. Panagrolaimidae in 1.8% of samples (Table 3e). *Plectus* occurred as the only nematode in 35 samples, followed by *Scottnema* (seven samples), *Halomonhystera* (three samples), and cf. Panagrolaimidae (one sample); while *Eudorylaimus* was always found in the presence of other nematode genera (Fig. 4A).The genus *Plectus*, was the only nematode genus present in all ten sampled regions (Fig.1, Table 3e). Although *Plectus* has been reported for EA [48,50,72] there are no published records for MS and FM (Table 1). *Plectus* was present in a wide range of environmental conditions (Fig. 4); with *P. murrayi* as the only nematode species observed from CS. *Plectus murrayi* was observed in samples with various ranges of C (0.01 – 9.9%), EC (0.01 – 48 dS/m), NH_4^+ (4.2 – 372 p.p.m.), NO_3^- (3.4 – 19 p.p.m.), P (2 – 171 mg/kg), pH (4.3 – 8) and moisture (0.25 – 77%). *Plectus frigophilus* was less tolerant of extreme conditions as *P. murrayi*, occurring in only five sites with no visible moss filaments, limited EC range (0.04 – 0.88 dS/m), and diverse ranges of C (0.05 – 9.9%), NO_3^- (3.4 – 12 p.p.m.), NH_4^+ (5.1 – 372 p.p.m.), pH (4.7 – 7.6), P (7.3 – 99 mg/kg) and moisture (6.5 – 77%). The minimum soil moisture requirements for *Plectus* species were higher than for *Scottnema* and *Eudorylaimus*.

Figure 3. ***(A)*** **Hierarchical cluster of taxa composition based on Bray-Curtis similarity coefficient (presence/absence of microfaunal taxa).** *(B)* Microfauna total abundance given in grams of dry weight of soil (Tot ab/gdw); and elevation at which samples were collected. *(C-E)* values for soil geochemical variables for 109 samples across EA. Geochemical variables (units and acronyms): phosphorus (P mg/kg), NH_3 (ppm), NO_3 (ppm), soil moisture percentage (Moist), electric conductivity (EC ds/m), and organic carbon percentage (C). The Order of samples for graphs *(B-E)* is the same as indicated in cluster *(A)*. Color-coded symbols identified by the Hierarchical cluster (separated by blue dotted line) represent microfaunal taxa categories (*MT-cat*): '0' no microfauna, '1' rot, '2' rot-mit, '3' rot-nem-mit, '4' rot-tar-mit, '5' rot-nem-tar-mit, '6' rot-nem-cil-mit, , '7' rot-nem-tar-cil-mit. '8' rot-nem-cil, '9' rot-nem-tar-cil, '10' rot-cil, '11' rot-tar-cil, '12' rot-nem '13' rot-tar, '14' tar-nem '15' rot-nem-tar, '16' mit, '17' nem-mit, '18' nem-cil, and '19' nem. Abbreviations used: rotifers (rot), tardigrades (tar), nematodes (nem), mites (mit), and ciliates (cil).

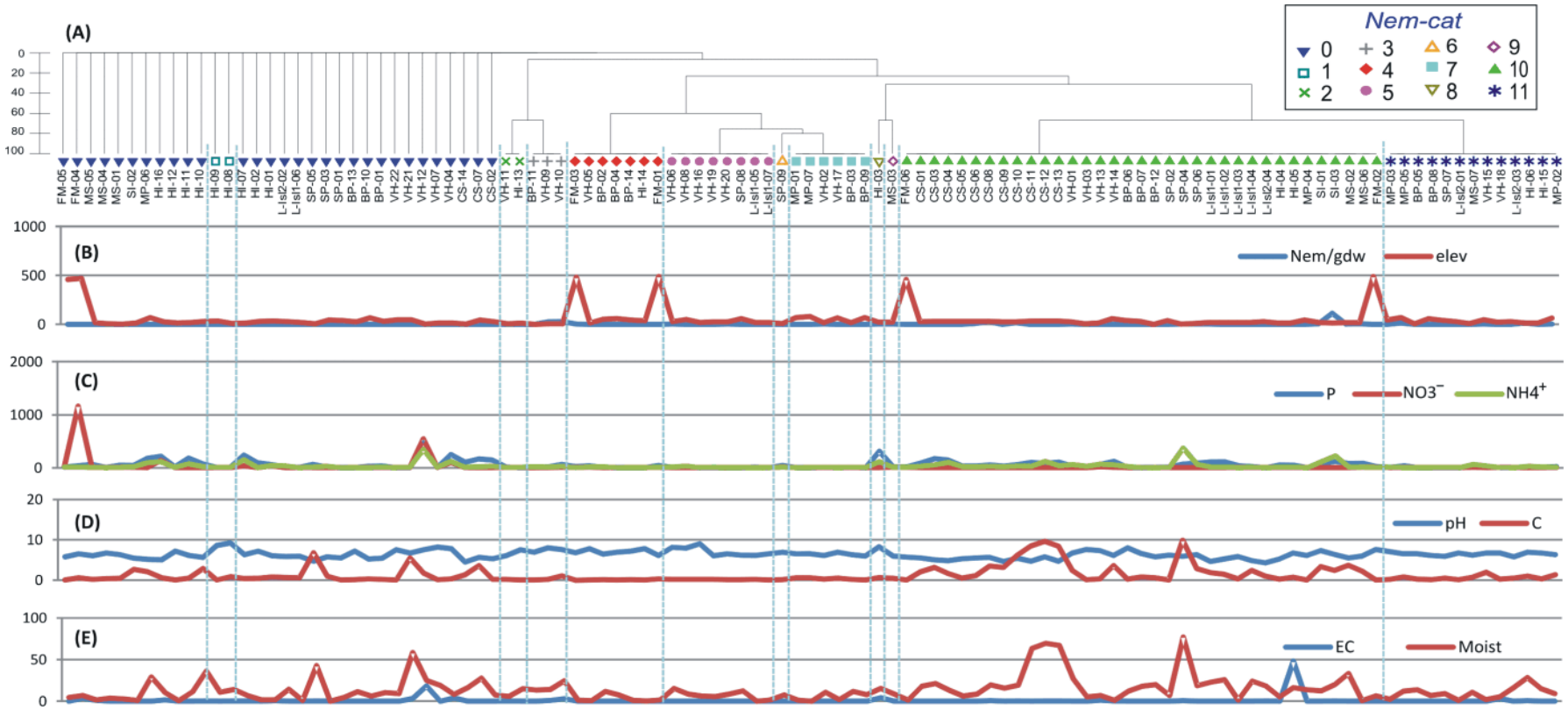


Figure 4. ***(A)*** **Hierarchical cluster of nematode composition based on Bray-Curtis similarity coefficient (presence/absence of morphologically identified taxa).** *(B)* Nematode total abundance given in grams of dry weight of soil (Nem/gdw); and elevation at which samples were collected. *(C-E)* values for soil geochemical variables for 109 samples across EA. Geochemical variables (units and acronyms): phosphorus (P mg/kg), NH_3 (ppm), NO_3 (ppm), soil moisture percentage (Moist), electric conductivity (EC ds/m), and organic Carbon percentage (C). The Order of samples for graphs *(B-E)* is the same as indicated in cluster *(A)*. Color-coded symbols identified by the Hierarchical cluster (separated by blue doted line) represent nematode categories (*Nem-cat*): '0' no nematodes, '1' undetermined, '2' Ha-Sc, '3' Ha, '4' Sc, '5' Sc-Eu, '6' Sc-Pt, '7' Pt-Eu-Sc, '8' Pa, '9' Pt-Pa, '10' Pt, and '11' Eu-Pt. Abbreviations used: *Plectus* (Pt), *Halomonhystera* (Ha), cf. Panagrolaimidae (Pa), *Scottnema* (Sc), and *Eudorylaimus* (Eu).

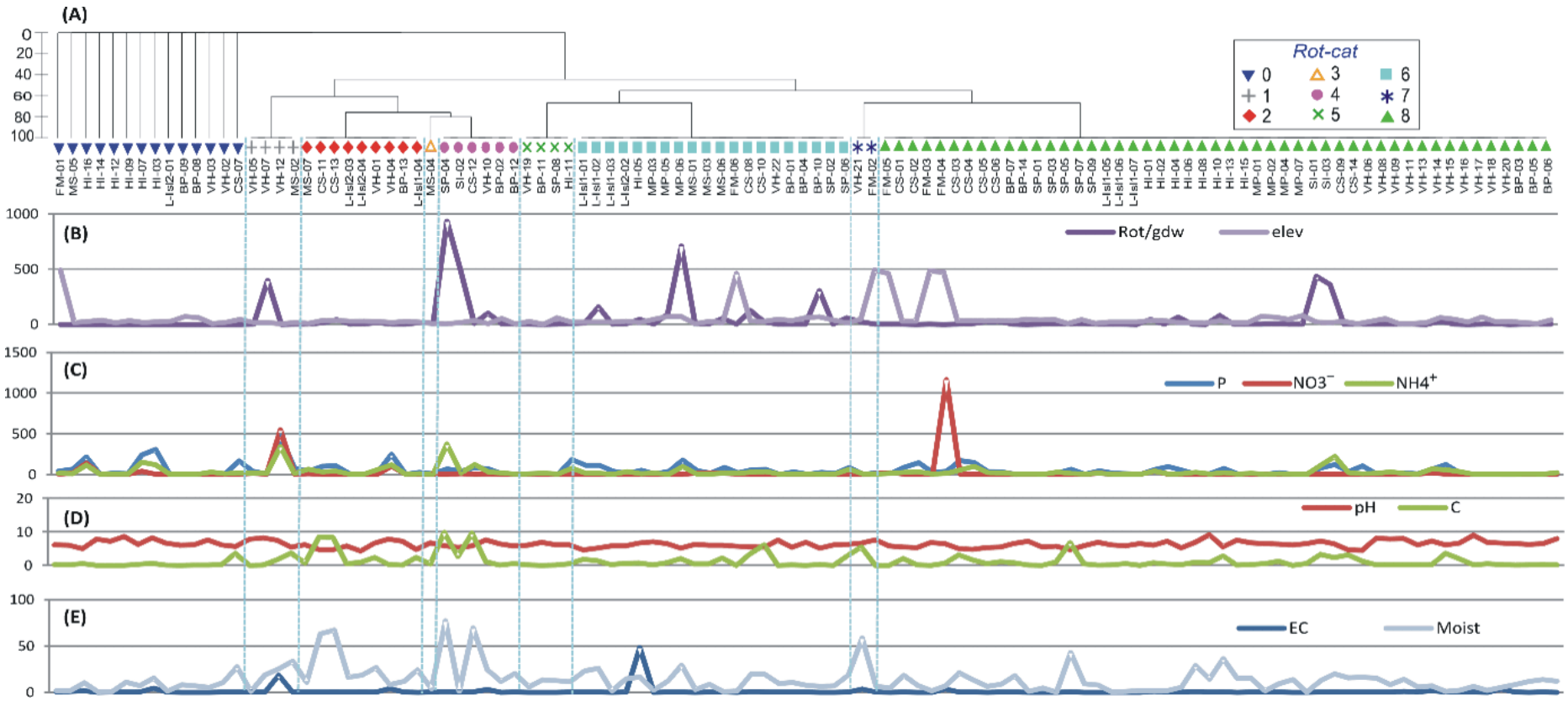


Figure 5. ***(A)*** **Hierarchical cluster of rotifer composition based on Bray-Curtis similarity coefficient (presence/absence of morphologically identified taxa).** *(B)* Rotifer total abundance given in grams of dry weight of soil (Rot/gdw); and elevation at which samples were collected. *(C-E)* values for soil geochemical variables for 109 samples across EA. Geochemical variables (units and acronyms): phosphorus (P mg/kg), NH_3 (ppm), NO_3 (ppm), soil moisture percentage (Moist), electric conductivity (EC ds/m), and organic carbon percentage (C). The Order of samples for graphs *(B-E)* is the same as indicated in cluster *(A)*. Color-coded symbols identified by the Hierarchical cluster (separated by blue dotted line) represent rotifer categories (*Rot-cat*): '0' no rotifers, '1' Ph, '2' Ph-ub, '3' Ph-Ad, '4' Ph-Ad-ub, '5' Ad, '6' Ad-ub, '7' ub-Monogonota, and '8' ub. Abbreviations used: *Adineta* (Ad), *Philodina* (Ph), and unidentified bdelloid (ub).

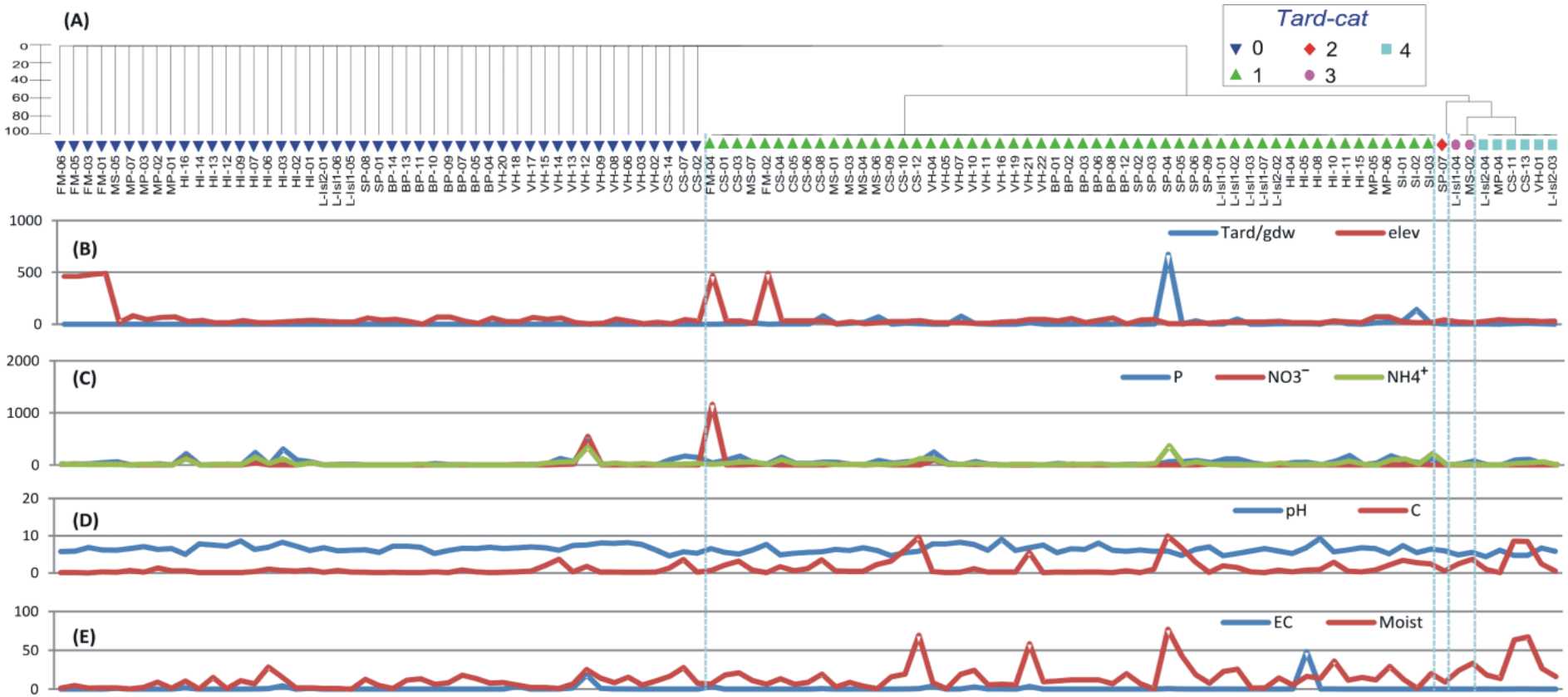


Figure 6. ***(A)*** **Hierarchical cluster of tardigrade composition based on Bray-Curtis similarity coefficient (presence/absence of morphologically identified taxa).** *(B)* Tardigrade total abundance given in grams of dry weight of soil (Tar/gdw); and elevation at which samples were collected. *(C-E)* values for soil geochemical variables for 109 samples across EA. Geochemical variables (units and acronyms): phosphorus (P mg/kg), NH_3 (ppm), NO_3 (ppm), soil moisture percentage (Moist), electric conductivity (EC ds/m), and organic carbon percentage (C). The Order of samples for graphs *(B-E)* is the same as indicated in cluster *(A)*. Color-coded symbols identified by the Hierarchical cluster (separated by blue dotted line) represent tardigrade categories (*Tard-cat*): '0' no tardigrades, '1' Parachela, '2' Echiniscoidea, '3' Parachela-Apochela-Echiniscoidea, and '4' Parachela-Echiniscoidea.

Scottnema specimens were collected from VH to FM (Fig. 1) in seven of the sampled regions (Table 3e). Records for this genus comprised the first records in these regions (Table 1). *Scottnema* was present in 30% of the nematode samples and always in environments of low EC (0.02 – 0.38 dS/m), NH_4^+ (4.5 – 18.6 p.p.m.), and C (0 – 0.55%), at low-moderate levels of moisture (0.1 – 15.4%), and various levels of P (3 – 44 mg/kg) and NO_3^- (18.4 – 3.4 p.p.m.). No visible cyanobacterial samples were associated with the presence of *Scottnema*. In samples where *Scottnema* was present, 58% of the time (14 samples) it occurred with *Eudorylaimus* (Fig. 4A) but never in the presence of the tardigrade *Echiniscus.* Soil geochemical variables seemed to be broader (in most cases) for *Eudorylaimus* than *Scottnema* (Fig. 4). *Eudorylaimus* was found from VH to MS (Fig.1, Table 3e) in soils of low-medium ranges of C (0.01 – 1.94%), and various levels of EC (0.01 – 3.5 dS/m), NH_4^+ (4.2 – 63.6 p.p.m.), NO_3^- (3.4 – 11 p.p.m.), moisture (0.11 – 28.6%), and P (1.4 – 40 mg/kg).

Halomonhystera was found in a total of five samples in VH, BP, and HI (Table 3e), and occurred as the only nematode genus in three of them (Fig. 4A). It was never observed co-occurring with *Plectus* or *Eudorylaimus* (Fig. 4A), but with bdelloids and ciliates in 80% of samples. *Halomonhystera* occurred mostly in coarse gravel samples with no visible moss filaments, low C (0.05-1.08%), moderate NO_3^- (3.4 – 7.5 p.p.m.), and various ranges of EC (0.04 – 3.02 dS/m), NH_4^+ (7 – 31 p.p.m.), moisture (5.8 – 24%), and P (4 – 67 mg/kg). Members of the family Panagrolaimidae have been recorded for EA [10], but as far as we are aware there are no previous records for the family in any of the ten sampled regions (Fig. 1). We found cf. Panagrolaimidae nematodes in two fine soil samples from HI and MS, and were the only nematode taxon from an ornithogenic soil (Fig. 4). Only one isolated specimen from a different genus (cf. *Hypodontolaimus*) was observed in a fine soil sample (HI-08) next to a saline lake (EC: 0.33 dS/m) without visible vegetation.

Rotifer composition and habitats

We identified the Classes Bdelloidea and Monogononta from our Antarctic soils. It was only possible to morphologically discern live-mobile bdelloid specimens (which constitute

less than one third of all specimens). Seven bdelloid species have been previously described in the literature for VH, Larsemann Hills, and CS [55,56,58] (Table 1). We were able to discern the genera *Adineta* and *Philodina* from some of the samples, but the remaining bdelloids were left as unidentified.. Bdelloids were present for all ten regions (Table 3) in soil samples varying in particle size from fine to coarse, with and without vegetation; and in the most extreme conditions in a variety of geochemical ranges: EC (0.01 – 48 dS/m), C (0 – 9.9%), P (1.4 – 469 mg/kg), NO_3^- (3.4 – 1163 p.p.m.), NH_4^+ (4.5 – 373 p.p.m.), moisture (0.11 – 77%) and pH (4.3 – 9.2; Fig. 5). The rotifer cluster (Fig. 5A) revealed nine categories, with the most common consisting of exclusively unidentified bdelloids (49 samples) in a single clade, followed by an 'unidentified bdelloids-*Adineta*' clade comprising 20 samples. *Philodina* and *Adineta* were found together in seven samples; while Monogononta (*Encentrum* cf., *Cephalodella* cf. and *Lepadella* cf.) was only observed in two samples from the sides of lakes with similar $NH4^+$ concentrations (7.5 – 7.8 p.p.m.) and close to neutral pH (6.7 – 7.6).

Tardigrade composition and habitats

Three Orders of tardigrades (Parachela, Apochela and Echiniscoidea) were identified in this study (Table 1). In samples with tardigrades, Parachela was the most dominant and present in all samples (except one) distributed across a broad type of habitats (Fig. 6). Parachela was present within the same extended NO_3^-, NH_4^+ and pH ranges as bdelloids, but in a narrower range of: EC (0.02– 48 dS/m), C (0.01 – 9.9%), P (1.9 – 249 mg/kg), and moisture (0.28 – 77). Parachela was recorded from 56% of the 109 samples followed by Echiniscoidea 8% and Apochela 1.8% (Table 3e). Apochela (represented by *Milnesium* sp.) occurred in two samples from L-Isl and MS together with the other two tardigrade Orders (Fig. 6), nematodes (*Plectus*) and rotifers. *Milnesium* was found in fine soils containing visible moss filaments, high moisture (24 – 33%) and P (27 – 79 mg/kg), moderate organic C (2.4 – 3.6%) and NH_4^+ (11 – 13 p.p.m.), and low pH (4.8 – 5.5). Echiniscoidea (represented by *Echiniscus* sp.) was present in nine samples (Fig. 6A) with different size soil particles, acidic pH (4.1– 6.6) and no visible al-cy. All samples including *Echiniscus* also contained bdelloid rotifers and *Plectus*.

Ciliate composition and habitats

Ciliates were not further classified and left as un-identified morpho-types. The exception was the morpho-species *Paradileptus* cf. *elephantinus* which was observed in a single soil sample collected at a bird moulting site in HI. Ciliates were observed in seven regions (Table 3), and occurred in a range of habitats from fine to coarse soil size, in dry to wet conditions (moisture: 1.55 – 58.4%), in presence and absence of vegetation; in soils with low to moderate ranges of EC (0.04 – 4.4 dS/m), and NO_3^- (3.4 – 41.5 p.p.m.); and soils with a wide range in C (0.05 – 6.8%), P (1.9 – 310 mg/kg), NH_4^+ (4.8 – 222 p.p.m.), and pH (4.7 – 8.24; Fig. 3).

Mite composition and habitats

The arthropod community in our EA soils was dominated by Prostigmata mites (cf. *Nanorchestes*, cf. *Tydeus*, and cf. *Stereotydeus*). They were found in seven of the ten geographic regions (Table 3) in a broad range of habitats (Fig. 3), from silty to coarse soils, in presence or absence of visible vegetation, and in soils presenting a wide range of EC (0.02 – 48 dS/m), pH (4.8 – 8.1), and C (0.01 – 6.14%) values; low to moderate values for P (3.9 – 169 mg/kg), NH_4^+ (4.5 – 64 p.p.m.), moisture (1.8 – 27.7%); and low NO_3^- concentrations (3.4 – 8.2 p.p.m.), which was corroborated by a negative correlation for NO_3^- and mite presence (Appendix 2: Table S3). One of the seven samples (LH-SP-04; Fig. 3) where mites were absent but mite exuviae present, was outside the maximum range observed for C, NH_4^+ and moisture values. Mites occurred in absence of any other taxa in one sample (CS-07; Fig. 3), which corresponded to the lowest NH_4^+ (18.9 p.p.m.), and the second highest P concentration (169.3 mg/kg) for all CS samples.

Microfaunal abundance and vegetation

The average invertebrate abundance for the microfaunal taxa in EA soils were 60 specimens per gdw, with the highest average of specimens per region found in SI (542 specimens/gdw); and the lowest for FM (Table 3c). The most abundant taxon was rotifers,

representing 72.2% of all invertebrates (average per sample: 44 specimens/gdw); followed by tardigrades 20.7% (12 specimens/gdw); nematodes 5% (3 specimens/gdw); ciliates 2% (1.3 specimens/gdw), and mites 0.06% (0.04 specimens/gdw; Table 3d). Abundance varied greatly among samples (Figs 3B – 6B). In 33% of the samples, it was less than 1 specimen/gdw; in 36% of samples, it ranged from 1-10 specimens/gdw, and in 13% of samples it was over 100 specimens/gdw (Appendix 2: Table S3).

From 109 samples, a total of 44 were identified with vegetation (moss, algae, cyanobacteria and/or lichen), which accounted for 82% of the total microfaunal abundance. There were 12 samples containing only al-cy as the only visible type of vegetation and accounted for 38% of microfauna abundance. Soil samples including moss (without visible al-cy) were 26 and represented 29% of the abundance (two of those sample also contained traces of lichens). Four samples included al-cy and moss (together) and contributed 14% of the abundance. Only two samples had lichen as the only form of vegetation and represented 2% of the abundance. Samples without visible vegetation (65 out of 109) included only 18% of the total microfauna abundance. Around 70% of the microfauna abundance was concentrated in six samples (Appendix 2: Table S1), with a single high moisture (77%) cyanobacteria sample from a lake edge (LH-SP-04; Fig. 3B – 6B) accounting for 24% of the total invertebrate abundance (49% of tardigrades and 20% of rotifers). In the case of ciliates, 61% of their total recorded density occurred in a single sample rich in cyanobacteria flakes and 58% of moisture content (VH-21; Fig.3B). For nematodes (*Plectus*), a soil sample with visible moss filaments from Sansom Island (SI-03; Fig. 4B) contained 35% of the total nematode density. Similarly, for mites, 31% of their entire density occurred in a single sample with moss filaments and moderate moisture content (9.2%) from Stornes Peninsula (LH-SP-07; Fig. 3).

Linkage between biotic and environmental parameters

Bioenv analyses were used to find the best combination for abiotic with biotic categories (taxa abundance and composition). For our study it was observed that the highest correlation among abiotic categories and taxa composition was a combination of P, NO_3^-,

soil moisture and elevation (ρ = 0.221; Table 4). NH_4^+ (ρ = 0.126) followed by P (ρ = 0.108) and EC (ρ = 0.094) represented the abiotic variables with strongest correlations when considering each individually (Table 4). For microfauna total abundance it was observed that EC (ρ = 0.204), C (ρ = 0.198) and NH_4^+ (ρ = 0.186) presented the highest correlation values when considered individually; while a combination of C and NO_3^- showed the highest two-variables correlation (ρ = 0.326; Table 4). The Bioenv analyses revealed that the best variables to explain nematode composition (all taxa combined) were al-cy (ρ = 0.15) and NO_3^- (ρ = 0.149); while abundance was better explained by NO_3^- (ρ = 0.206). A combination of P, NO_3^-, pH and al-cy had the strongest correlation values with nematode composition (ρ = 0.309); while the combined effect of P, NO_3^- and al-cy had the highest correlation with nematode abundance (ρ = 0.335; Table 5). Considering the most frequent nematode genera separately (*Plectus*, *Eudorylaimus* and *Scottnema*) we observed that the strongest correlations for *Plectus* were NO_3^- (ρ = 0.128) and al-cy (ρ =0.149); for *Eudorylaimus*, P (ρ = 0.272), pH (ρ =0.206) and C (ρ = 0.179); and for *Scottnema*, C (ρ =0.283), NO_3^- (ρ =0.213) and NH_4^+ (ρ = 0.19; Table 5).

Table 4. Result from Bioenv analysis showing the strongest correlations for abiotic variables (when considered individually or in connection to others) that best match the biotic matrices for microfauna total abundance and composition.

Taxa composition			Meiofauna total abundance		
Number of Variables	Correlation (ρ)	Selection of Variables	Number of Variables	Correlation (ρ)	Selection of Variables
1	**0.126**	4	1	**0.204**	1
1	0.108	3	1	0.198	2
1	0.094	1	1	0.186	5
4	**0.221**	3,4,6,9	1	0.16	3
3	**0.219**	3,4,6	1	0.129	4
2	**0.205**	4,6	4	**0.328**	1,2,4,5
3	0.204	4,6,9	4	0.327	2,4,5,13
4	0.2	1,3,4,6	2	**0.326**	2,4
3	0.195	3,4,9	3	**0.323**	2,4,5
4	0.195	1,4,6,9	4	0.317	2,4,5,7
4	0.194	3,4,6,7	4	0.316	2,3,4,5

Numbers in bold indicate best correlation values for the selected combinations of abiotic variables. Numbers under Selection of Variables correspond to: EC (1), C (2), P (3), NO_3^- (4), NH_4^+ (5), moisture (6), pH (7), elevation (9), and algae-cyanobacteria (13).

Table 5. Result from Bioenv analysis showing the strongest correlations for abiotic variables (when considered individually or in connection to others) that best match the biotic matrices for nematode composition (all taxa combined, *Plectus*, *Eudorylaimus* and *Scottnema*), and abundance.

Nematode composition											Nematode abundance		
Nematod taxa (all)			*Plectus*			*Eudorylaimus*			*Scottnema*				
No.V	Corr. (ρ)	Sel.V	No.V	Corr. (ρ)	Sel.V	No.V	Corr. (ρ)	Sel.V	No.V	Corr. (ρ)	Sel.V	No.V	Corr. (ρ)	Sel.V
1	0.15	13	1	0.128	4	1	0.272	3	1	0.283	2	1	0.206	4
1	0.149	4	1	0.107	13	1	0.206	7	1	0.213	3	1	0.182	1
1	0.142	3	1	0.083	7	1	0.179	2	1	0.19	5	1	0.178	13
1	0.119	7	4	0.233	4,7,9,13	1	0.144	5	1	0.164	13	1	0.116	3
1	0.107	1	4	0.229	4,6,7,13	4	0.36	2,3,7,9	4	0.353	2,3,5,13	3	0.335	3,4,13
4	0.309	3,4,7,13	4	0.222	4,7,10,13	4	0.357	2,3,4,7	4	0.351	2,5,7,13	4	0.325	3,4,11,13
4	0.299	4,7,9,13	3	0.22	4,7,13	4	0.355	3,4,7,9	3	0.351	2,5,13	4	0.321	1,3,4,13
4	0.294	3,4,9,13	4	0.214	3,4,7,13	4	0.347	2,3,4,9	4	0.349	1,2,5,13	4	0.318	2,3,4,13
4	0.292	2,4,7,13	4	0.21	4,6,7,9	3	0.34	3,4,7	3	0.348	2,3,13	4	0.311	3,4,5,13
3	0.289	4,7,13	4	0.21	4,6,9,13	3	0.339	3,7,9	4	0.345	2,3,7,13	4	0.311	3,4,12,13
3	0.277	3,4,13	4	0.21	2,4,7,13	4	0.333	3,4,7,10	4	0.343	2,3,4,13	3	0.308	2,4,13

Numbers in bold indicate best correlation values for the selected combinations of abiotic variables. Numbers under Selection of Variables correspond to: EC (1), C (2), P (3), NO_3^- (4), NH_4^+ (5), moisture (6), pH (7), elevation (9), fine sediments (10), region (11), moss in sample (12), and algae-cyanobacteria (13). Acronyms as following: Number of Variables (No.V), Correlation (Corr), Selection of Variables (Sel.V).

For rotifers, NO_3^- was the best variable to explain composition (ρ = 0.075); and when combined, C, NO_3^-, elevation and region (ρ = 0.15) were the strongest variables. Rotifer abundance was better explained by P (ρ = 0.154) and by the combined effect of C, P and NO_3^- (ρ = 0.205; Table 6). Individual Bioenv analyses based on presence/absence data were run for the bdelloid genera *Adineta* and *Philodina*. The highest correlation for *Adineta* corresponded to pH (ρ = 0.11) and NO_3^- (ρ = 0.213); while for *Philodina*, corresponded to moisture (ρ = 0.047) and slope (ρ = 0.037; Table 6). Bioenv results for tardigrade biotic parameters showed moisture to be the best single variable to explain tardigrade composition and abundance (ρ = 0.107 and ρ =0.141, respectively); and P, NO_3^-, moisture and elevation to have the strongest combined effect (ρ = 0.179 for composition, and ρ =0.141 for abundance; Table 7). We did not conduct separate analyses for tardigrade taxa, given that only few samples contained taxa other than Parachela (Echiniscoides in nine samples and Apochela in two samples). For ciliates, moisture was the strongest variable to explain presence and abundance (ρ = 0.092 and ρ =0.094, respectively). When considering a combination of variables it was seen that the highest correlation for ciliate presence involved NO_3^-, moisture and slope (ρ = 0.129); and for ciliate abundance involved moisture and slope (ρ = 0.135; Table 8). For mite presence, the highest

correlation value (when one or more variables were considered) corresponded to NO_3^- (ρ = 0.155), which was also the best value when correlated to mite abundance (ρ = 0.092); while the highest correlation value resulted for a combination of NO_3^- together with moisture, slope and elevation (ρ = 0.142; Table 9).

Table 6. Results from Bioenv analysis showing the strongest correlations for abiotic variables (when considered individually or in connection to others) that best match the biotic matrices for rotifer composition (all taxa combined, *Adineta* and *Philodina*), and abundance.

Rotifer composition									Rotifer abundance		
Rotifer taxa (all)			Adineta			Philodina					
No.V	Corr.(ρ)	Sel.V	No.V	Corr.(ρ)	Sel.V	No.V	Corr.(ρ)	Sel.V	No.V	Corr.(ρ)	Sel.V
1	**0.075**	4	1	**0.11**	7	1	**0.047**	6	1	**0.154**	3
1	0.062	3	1	0.075	4	1	0.037	8	1	0.134	1
1	0.039	5	4	**0.141**	4,6,7,11	1	0.035	14	1	0.121	5
4	**0.15**	2,4,9,11	3	**0.14**	3,4,7	4	**0.07**	6,8,9,14	1	0.107	2
3	**0.146**	2,4,9	4	0.138	3,4,7,11	4	0.069	8,9,12,14	4	**0.206**	1,2,3,4
4	0.146	2,4,9,15	3	0.138	4,6,7	3	**0.069**	8,9,14	4	0.206	2,3,4,7
4	0.146	2-4,9	2	**0.138**	4,7	3	0.069	6,9,14	3	**0.205**	2,3,4
4	0.142	1,2-4,9	4	0.137	3,4,6,7	4	0.068	3,6,9,14	4	0.205	2,3,4,5

Numbers in bold indicate best correlation values for the selected combinations of abiotic variables. Numbers under Selection of Variables correspond to: EC (1), C (2), P (3), NO_3^- (4), NH_4^+ (5), moisture (6), pH (7), slope (8), elevation (9), region (11), moss in sample (12), soil from moss bed (14), and aspect (15). Acronyms as following: Number of Variables (No.V), Correlation (Corr), Selection of Variables (Sel.V).

Table 7. Results from Bioenv analysis showing the strongest correlations for abiotic variables (when considered individually or in connection to others) that best match the biotic matrices for tardigrade composition and abundance.

Tardigrade composition			Tardigrade abundance		
Number of Variables	Correlation (ρ)	Selection of Variables	Number of Variables	Correlation (ρ)	Selection of Variables
1	**0.107**	6	1	**0.141**	6
1	0.104	3	1	0.119	3
1	0.068	9	1	0.101	9
4	**0.179**	3,4,6,9	1	0.068	4
3	**0.176**	3,6,9	4	**0.238**	3,4,6,9
2	**0.156**	3,6	3	**0.222**	3,6,9
3	0.156	4,6,9	3	0.212	4,6,9
4	0.154	3,6,9,15	3	0.2	3,4,6
4	0.153	1,3,6,9	4	0.199	1,3,6,9
4	0.149	3,6,7,9	4	0.198	2,4,6,9

Numbers in bold indicate best correlation values for the selected combinations of abiotic variables. Numbers under Selection of Variables correspond to: EC (1), P (3), NO_3^- (4), Moisture (6), elevation (9), and aspect (15).

Table 8. Results from Bioenv analysis showing the strongest correlations for abiotic variables (when considered individually or in connection to others) that best match the biotic matrices for ciliate presence/absence and abundance.

Ciliate presence/absence			Ciliate abundance		
Number of Variables	Correlation (ρ)	Selection of Variables	Number of Variables	Correlation (ρ)	Selection of Variables
1	**0.092**	6	1	**0.094**	6
1	0.062	8	1	0.07	8
3	**0.129**	4,6,8	2	**0.135**	6,8
2	**0.128**	6,8	3	**0.134**	4,6,8
4	**0.107**	4,6,8,11	3	0.113	6,8,11
3	0.107	6,8,11	4	**0.113**	4,6,8,11
4	0.106	3,4,6,8	3	0.111	3,6,8
3	0.106	3,6,8	4	0.111	3,4,6,8
4	0.105	4,6,8,15	4	0.11	4,6,8,15

Numbers in bold indicate best correlation values for the selected combinations of abiotic variables. Numbers under Selection of Variables correspond to: P (3), NO_3^- (4), Moisture (6), slope (8), region (11), and aspect (15).

Table 9. Results from Bioenv analysis showing the strongest correlations for abiotic variables (when considered individually or in connection to others) that best match the biotic matrices for mite presence/absence and abundance.

Mite presence/absence			Mite abundance		
Number of Variables	Correlation (ρ)	Selection of Variables	Number of Variables	Correlation (ρ)	Selection of Variables
1	**0.155**	4	1	**0.092**	4
1	0.058	10	1	0.06	10
1	0.05	7	1	0.057	9
2	**0.139**	4,7	1	0.043	6
4	**0.126**	4,8,10,13	4	**0.142**	4,6,8,9
4	0.124	4,5,7,10	4	0.14	4,5,6,9
4	0.123	4,7,8,13	4	0.136	4,5,6,10
4	0.122	4,7,10,13	4	0.135	4,6,7,10

Numbers in bold indicate best correlation values for the selected combinations of abiotic variables. Numbers under Selection of Variables correspond to: NO_3^- (4), NH_4^+ (5), Moisture (6), pH (7), slope (8), elevation (9), fine sediments (10), and algae-cyanobacteria (13).

Pearson correlation analysis (Appendix 2: Table S3) showed that microfauna abundance was positively correlated with C, NH_4^+, moisture, and fine sediments; and negatively correlated with pH and NO_3^-. Even when the three most common taxa (Rotifera, Tardigrada and Nematoda) were considered separately, positive correlations were observed for vegetation and C, while moisture was positively correlated with tardigrade, rotifer and ciliate abundance but not with nematodes (*Scottnema* showing a negative correlation).

NO_3^- was negatively correlated with mites, *Plectus* and *Eudorylaimus* presence, and with nematode abundance; while NH_4^+, P and C were all negatively correlated with *Eudorylaimus* and *Scottnema* presence. P and NH_4^+ were also found to have positive correlations with tardigrade and rotifer abundance (and ciliate abundance for NH_4^+); and EC showed correlations with *Scottnema* presence (negative) and ciliate presence and abundance (positive).

Discussion

Microfaunal distribution

There have been few soil microfaunal surveys for EA with most focusing on extremely restricted populations. Current knowledge on microfauna composition and abundance in EA is still incomplete, and in need of appropriate sampling. Considering previous research in other Antarctic regions, further sampling and molecular work is likely to reveal new species, resolve taxonomic problems and extend the known ranges of species. Studies for other Antarctic regions (Victoria Land) have revealed that nematodes were the most extensively distributed and abundant metazoan in soils [73]; but this is not the case for EA. In Dronning Maud Land, Sohlenius *et al*. [11] and Sohlenius & Boström [41] reported that the most commonly found taxon across samples were rotifers, followed by tardigrades and nematodes in similar proportions. Our results show that rotifers were also the most widespread group (Table 3e), followed by nematodes and then tardigrades (even though higher abundance was observed for tardigrades than nematodes), similar to previous studies in the VH [29].

Nematode occurrence and habitats

Nematode distribution in soil is affected by carbon content, moisture, and salinity [5,74]; even though the environmental requirements vary depending on the species. We observed that soils with higher moisture content, C, P and NH_4^+ were inhabited predominantly by

Plectus, while the opposite trend was observed for *Eudorylaimus* and *Scottnema*. *Scottnema* is reported to prefer dryer and saltier soils with lower organic matter than *Eudorylaimus* [5]. Based on Bioenv results (Table 5) we noticed that al-cy, NO_3^-, P and EC have an important contribution explaining nematode composition and abundance (no significant contribution was seen for moss samples). *Eudorylaimus* and *Scottnema* (Table 5) are driven by similar soil abiotic variables; with P, C, pH and NH_4^+ as strong drivers determining their presence. We observed *Scottnema* in soils with the lowest average EC (0.1 dS/m) and *Halomonhystera* in the highest (0.92 dS/m). Our findings support studies by Andrássy [43,49] that reveal a tendency of *Halomonhystera* towards more saline environments. The distribution of *Eudorylaimus* from our study appears to correspond to their predatory habits on other nematode species [61,75] whereby *Eudorylaimus* presence was always linked to potential prey (*Plectus* or *Scottnema*, but never in the presence of *Halomonhystera*) in a variety of soils with low-moderate C levels and for only 7% in samples with visible algae. Wall *et al*. [76] reported *Eudorylaimus* to be an algae-feeder and not an omnivore as previously recorded by others [15,16], but our results did not show a correlation among *Eudorylaimus* presence and al-cy in sample (Table 5; though we did not account for microscopic algae).

Scottnema was present in dry and low-abundance populated soils, but with nematodes as the most abundant taxon, indicating the low carrying capacity for the species in the habitats targeted. Low densities for *Scottnema* observed here do not seem to correspond to other studies across Antarctica (e.g. [13,16,17,61,77] which report it as an abundant and widespread species. The lowest nematode density was seen for cf. Panagrolaimidae which has been reported for habitats rich in nitrogen, mostly linked to ornithogenic soils in the vicinity of bird colonies [23,24,78]. Of the five genera recorded for this study, *Plectus* was observed for the broadest geochemical ranges (N, C, P, EC and pH) indicating higher tolerance levels to environmental stresses. *Plectus* is a bacterial feeder, which potentially increases the range of habitats where it can be found. Nevertheless, denser populations were seen in presence of al-cy that could offer food as well as sheltered microhabitats. It is important to highlight that nematode presence was never as wide when considering NO_3^- levels. We observed that samples with high NO_3^- (23-1163 p.p.m.) only harbor tardigrades,

ciliates and/or rotifers but no nematodes suggesting lower capabilities of the latter to adapt to NO_3^- rich environments.

Rotifer occurrence and habitats

The presence of bdelloid rotifers in 87% of soil samples (Table 3e) reflects not only their broad distribution but also the high tolerance level of the group towards extreme conditions. Wide ranges in abiotic and geochemical parameters (EC, C, P, NO_3^-, NH_4^+ and pH) were observed for samples including bdelloids, suggesting that the effect of a single variable does not drive bdelloid composition and is more the result of a combination of abiotic factors (Table 6; Figs 3, 5). Stronger contributions from single abiotic variables were observed by P, EC, NH_4^+ and C when considering rotifer abundance (Table 6). Our results also show a positive correlation between moisture and rotifer abundance (only seen for Pearson correlation analysis, Appendix 2: Table S3) as in other Antarctic regions [79]. Correlation of C with abundance was also reported by Sinclair & Sjursen [80] on Ross Island. We found 79% of total abundance occurred in samples with moss, algae or cyanobacteria. Soil pH seems to have an indirect role in determining abundance, given that three samples with higher bdelloid densities (LH-SP-04, MP-06 and SI-02) had low pH values (5.4 – 5.9) accounting for 45% of bdelloid abundance (Fig. 5). P and NH_4^+ also play an important role in bdelloid abundance; it was observed that a large proportion of bdelloids inhabit soils with moderate P content (69 – 123 mg/kg) representing 15% of total samples and accounting for almost half of rotifer abundance. Bdelloid numbers also seem to be indirectly affected by high NH_4^+ concentration in soils (98 – 373 p.p.m.) with contrasting results for the top 11 samples; four of those samples contributed 51% of the bdelloid abundance; but for three of those 11 samples no rotifers were observed.

Tardigrade occurrence and habitats

Tardigrades in the current study were mostly represented by the Order Parachela, a widely distributed Order reported elsewhere in Antarctica (e.g. [11,12,81,82]). However, contrary to previous studies, we found no species of the genus *Pseudechiniscus* (Order

Echiniscoidea), which has been reported as the most common tardigrade for the LH [83]. The Order Parachela was present in a variety of soil types, but mostly linked to soils with high levels of organic carbon and vegetation. In 98% of samples Parachela were found with bdelloid rotifers, suggesting similar habitat requirements, although rotifers were found across a greater range of soil properties (occurring with Parachela in 64% of cases). When looking at the Bioenv values for tardigrade abundance and composition we found soil moisture to be the strongest variable, followed by P, elevation and NO_3^- (Table 7). Positive correlations between tardigrade abundance and moisture were also observed by Kennedy [19] and Freckman & Virginia [73] for Antarctic soils. However, the highest tardigrade densities in our study were in samples with contrasting soil moisture concentrations (77% and 1.2%; Fig. 6). It is likely that tardigrade moisture-abundance correlations were driven by three of the four high abundance samples with high moisture content (19 – 77%), accounting for more than 60% of tardigrade abundance. The strong correlation for vegetation and abundance (97% of tardigrades in samples containing vegetation; Appendix 2: Table S3) was not reflected in the Bioenv values (Table 7). This could be explained as moisture driving vegetation growth and indirectly influencing tardigrade abundance.

The predatory and cosmopolitan species *Milnesium tardigradum* [29,84] was only found in two highly diverse samples co-occurring with two other tardigrade Orders (Echiniscoidea and Parachela), nematodes (*Plectus*) and bdelloid rotifers. Their presence is likely to be linked to presence of other microfaunal taxa, probably reflecting their feeding habits, but this is only based on two samples. *Echiniscus* sp. was observed in 8% of samples with various taxa, but never in samples where *Scottnema* occurred, suggesting that most suitable habitats comprise moderate to high soil moisture concentration (9 – 67%) that is likely to be outside the optimum requirements for *Scottnema*.

Ciliates and habitats

Due to their minute size (~70 – 100 µm in length) and oval shape, Ciliophora were not easily discernible. We restricted our study to live-mobile ciliates with visible cilia, and

abundance and presence of ciliates/protozoans in our samples is likely to have been underestimated (ciliates present in 15% of samples). Other studies, based on Victoria Land, also reveal low ciliate frequency [81,85] but high numbers of other protozoans (flagellates and amoeba). Ciliates were observed over a wide moisture range, but in our results most live-mobile ciliates (80% of samples) were present in high moisture soils (above 10%; Fig. 3) which might facilitate locomotion; also reported by Bamforth [86]. Bioenv values showed moisture and slope to be the strongest abiotic variables influencing presence and abundance of ciliates in soils (Table 8). EC was reported to play a role in ciliate populations; studies from dry pond sediments [87] showed ciliate occurrence at high EC conditions (13 – 27 dS/m). In contrast, ciliates in our study were only found within EC ranges 0.04 – 4.4 dS/m, and only two of the 109 samples analysed were above 13 dS/m and neither with visible ciliates.

Mite occurrence and habitats

Mites were the largest invertebrates but the least frequent of all taxa in our samples. Previous studies for EA have revealed a general paucity of this taxon from most sampling locations due to possible micro-habitat preferences [88,89]. Mites in Antarctica have been mostly linked to wet soils that support micro-algal growth (e.g. [9,80,89]); or in the vicinity of moss beds [22,30]. Based on the Bioenv individual correlation values we observed NO_3^- to be the strongest variable explaining mite presence and abundance (Table 9); no significant correlations were found for al-cy, moss, or proximity to moss beds with mite presence and abundance. Observations by Rounsevell [90] and Sinclair [24] who linked mite presence to food source (macroscopic algae), could not be supported with our findings, which show only 10% of samples to contain algae. In addition, most mites (56%) were present in soils with low to moderate moisture content (1.5 – 9.2%). Our data do not indicate a clear tendency for mites to favour wetter environments that sustain growth of algae; suggesting that other variables (besides moisture and algae) are influencing their presence. Convey [91] showed that temperature was the most obvious abiotic influence on micro-arthropod communities. The microclimate created by moss may provide a suitable habitat for mite survival, which are more susceptible to desiccation due to their size and

permeable cuticle [92,93]. The relative lack of samples with mites makes it difficult to tease out more complex associations, and abiotic variables other than vegetation, temperature and moisture are expected to be important. For example, we found that NO_3^- together with FS, slope and elevation seems to play a relevant factor in determining mite abundance (Table 9).

Correlating biotic and abiotic parameters

The Bioenv analyses linking abiotic variables with microfauna abundance and taxa composition (Tables 3 – 8) did not exceed correlation values of 0.206 when single variables were considered. Not surprisingly the effects were low since faunal ordination is not one-dimensional, and a single abiotic parameter does not provide a very successful match (e.g. [94]). Abiotic categories recorded during soil sampling and soil sieving did not play a major role compared to soil geochemical parameters. Single effects for the abiotic categories: region, aspect, slope, and particle size contributed poorly (or not at all) in determining interactions as seen in the PCA analysis (Fig. 3) and correlation values (Table 4 – 9). Salinity has been reported to influence diversity in Antarctic ecosystems. Magalhães *et al*. [9] showed a negative correlation between salt concentration and diversity; with salts increasing at higher elevations due to a longer exposure time of the terrain and more diluted in younger and active soils [7,9]. We found in our study a substantially higher region (FM) than the other nine (Table 2), with the lowest average of total microfauna (Table 3c) and low to medium EC values (0.01 – 3.66 d S/m) which were used as a proxy for salinity (*viz*. [9]). In our studies, salinity was correlated with microfauna abundance and composition; but correlations were not evident for elevation (when single effect of variable was considered) and microfauna (Table 4). Other variables such as organic matter, soil moisture and microbial diversity have also shown to play a role in determining microfauna distribution and diversity in Antarctica (e.g. [31,76]). In our study when organic C and soil moisture were correlated with microfauna abundance and composition, we noticed that microfauna composition was more strongly correlated to moisture, while microfauna abundance was more strongly correlated with C (Table 4). We did not account for microbial diversity.

Changes in soil geochemistry could be expected if seasonal variations are considered, in warmer periods we could expect higher accumulation of nutrients and C in lower sites as a result of greater meltwater (e.g. [13,20]). Changes in microfauna distribution could also be affected as a result of water availability [90]. We found a strong correlation of biotic factors (taxa abundance and composition) with NO_3^-, P, EC, and pH; and we may expect biotic-abiotic correlations to be altered as a result of seasonal changes.

Conclusions

We found that soil geochemical variables differed significantly among sites (Question 1) most likely as a result of variation in landscape formation/alteration, organic deposits from vegetation cover, ornithogenic inputs and shifting in nutrient accumulation due to melt-water runoff. Our study showed that abiotic variables are correlated with the composition of taxa (Question 2), with some taxa favouring *i)* close to neutral pH, drier and inorganic soils (*Scottnema*), *ii)* low NO_3, neutral pH, low-medium organic soils (*Eudorylaimus*), *iii)* saltier and less vegetated soils (*Halomonhystera*), *iv)* soils higher in phosphorous, NH_4^+, C and moisture (*Plectus*), *v)* more acidic soils without vegetation (*Echiniscus*), *vi)* acid-neutral soils high in moisture content *(Philodina)*, and *vii)* acidic soil (Adineta). Microfaunal abundance was significantly correlated with soil geochemistry (Question 3); we found that P, NO_3^-, EC and C are correlated with higher microfaunal densities of most common taxa (*Plectus murrayi*, *Adineta*, *Philodina* and Parachela); whereas in habitats with low pH, low moisture, low C, and high EC, the 'specialists' (also least abundant taxa) *Echiniscus, Scottnema, Eudorylaimus,* and *Halomonhystera* seem to do better. Our data indicate that region, slope and aspect did not play a major role in determining abundance. Our ability to address whether the occurrence of taxa is correlated with the presence of other taxa (Question 4) was confounded by determining if there is a biotic correlation among taxa, or if taxa co-occurring together are the result of similar micro-habitat requirements. It is most likely that any correlation (at least for non-predatory species) is the result of a connection between suitable soil geochemical conditions with soil productivity levels and microbial activity. For predatory species (*Milnesium,* and possibly

Eudorylaimus), we could expect their distribution to reflect availability of prey, as they were always present with other taxa.

To understand soil microfaunal abundance, taxa composition and distribution in Antarctica it is important to determine their correlation with soil geochemistry and other environmental parameters. Where a population exists is likely to be determined by a suite of soil geochemical factors, with NO_3^-, P and salinity as the main drivers; and to a lesser extent by pH, C and soil moisture. Microfaunal abundance and composition are more likely the result of soil abiotic properties and potentially historic landscape formation and alteration across multiple glacial cycles, rather than biotic interactions or the geographic region from which they were found.

Acknowledgments

We thank the Australian Antarctic Stations leaders and field personnel who contributed in logistics and support of our Antarctic field work. We are also extremely grateful to Rebecca Stonor, María Manjarrez, Pauline Glocke, Tanya Matic and Kathy Saint for laboratory assistance and support, and Steve Cooper, Chester Sands, Sandra McInnes, Byron Adams and Russell Shiel for their intellectual contributions. We also thank an anonymous reviewer and PLoS ONE academic editors Xiujun Wang and Erik Sotka for comments and suggestions that improved the manuscript.

References

1. Wall DH, Virginia RA (1999) Controls on soil biodiversity: insights from extreme environments. Applied Soil Ecology 13: 137-150.
2. Freckman DW, Virginia RA (1989) Plant-feeding nematodes in deep-rooting desert ecosystems. Ecology 70: 1665-1678.

3. Liang W, Mouratov S, Pinhasi-Adiv Y, Avigad P, Steinberger Y (2002) Seasonal variation in the nematode communities associated with two halophytes in a desert ecosystem. Pedobiologia 46: 63-74.
4. Pen-Mouratov S, Barness G, Steinberger Y (2008) Effect of desert plant ecophysiological adaptation on soil nematode communities. European Journal of Soil Biology 44: 298-308.
5. Barrett JE, Virginia RA, Wall DH, Parsons AN, Powers LE, et al. (2004) Variation in biogeochemistry and soil biodiversity across spatial scales in a polar desert ecosystem. Ecology 85: 3105-3118.
6. Barrett JE, Virginia RA, Hopkins DW, Aislabie J, Bargagli R, et al. (2006) Terrestrial ecosystem processes of Victoria Land, Antarctica. Soil Biology & Biochemistry 38: 3019-3034.
7. Pointing SB, Chan Y, Lacap DC, Lau MCY, Jurgens JA, et al. (2009) Highly specialized microbial diversity in hyper-arid polar desert. Proceedings of the National Academy of Sciences of the United States of America 106: 19964-12009.
8. Cary SC, McDonald IR, Barrett JE, Cowan DA (2010) On the rocks: the microbiology of Antarctic Dry Valley soils. Nat Rev Micro 8: 129-138.
9. Magalhães C, Stevens MI, Cary SC, Ball BA, Storey BC, et al. (2012) At Limits of Life: Multidisciplinary Insights Reveal Environmental Constraints on Biotic Diversity in Continental Antarctica. PLoS One 7: 1-10.
10. Wharton DA, Goodall G, Marshall CJ (2002) Freezing rate affects the survival of a short-term freezing stress in *Panagrolaimus davidi*, an antarctic nematode that survives intracellular freezing. CryoLetters 23: 5-10.
11. Sohlenius B, Boström S, Ingemar Jönsson K (2004) Occurrence of nematodes, tardigrades and rotifers on ice-free areas in East Antarctica. Pedobiologia 48: 395-408.
12. Sands CJ, Convey P, Linse K, McInnes SJ (2008) Assessing meiofaunal variation among individuals utilising morphological and molecular approaches: An example using the Tardigrada. BMC Ecology 8: 1-11.
13. Powers LE, Ho M, Freckman DW, Virginia RA (1998) Distribution, Community Structure, and Microhabitats of Soil Invertebrates along an Elevational Gradient in Taylor Valley, Antarctica. Arctic and Alpine Research 30: 133-141.

14. Virginia RA, Wall DH (1999) How Soils Structure Communities in the Antarctic Dry Valleys. Bioscience 49: 973-983.
15. Powers LE, Freckman DW, Virginia RA (1995) Spatial distribution of nematodes in polar desert soils of Antarctica. Polar Biology 15: 325-333.
16. Freckman DW, Virginia RA (1997) Low-diversity Antarctic soil nematode communities: Distribution and response to disturbance. Ecology 78: 363-369.
17. Poage MA, Barrett JE, Virginia RA, Wall DH (2008) The Influence of Soil Geochemistry on Nematode Distribution, Mcmurdo Dry Valleys, Antarctica. Arctic, Antarctic, and Alpine Research 40: 119-128.
18. Burkins MB, Virginia RA, Chamberlain CP, Wall DH (2000) Origin and distribution of soil organic matter in Taylor Valley, Antarctica. Ecology 81: 2377-2391.
19. Kennedy AD (1993) Water as a Limiting Factor in the Antarctic Terrestrial Environment: A Biogeographical Synthesis. Arctic and Alpine Research 25: 308-315.
20. Huiskes A, Convey P, Bergstrom D (2006) Trends in Antarctic Terrestrial and Limnetic Ecosystems: Antarctica as a Global Indicator. pp. 1-13.
21. Adams BJ, Wall DH, Gozel U, Dillman AR, Chaston JM, et al. (2007) The southernmost worm, *Scottnema lindsayae* (Nematoda): diversity, dispersal and ecological stability. Polar Biology 30: 809-815.
22. Hogg ID, Cary SC, Convey P, Newsham KK, O'Donnell AG, et al. (2006) Biotic interactions in Antarctic terrestrial ecosystems: Are they a factor? Soil Biology & Biochemistry 38: 3035-3040.
23. Sohlenius B (1988) Interactions between two species of *Panagrolaimus* in agar cultures Nematologica 34: 208-217.
24. Sinclair B (2001) On the distribution of terrestrial invertebrates at Cape Bird, Ross Island, Antarctica. Polar Biology 24: 394-400.
25. Block W (1984) Terrestrial microbiology, invertebrates and ecosystems. Laws RM (ed) Antarctic ecology Academic Press, London 1: 163-236.
26. Convey P (2001) Proceedings of the International Conference "Fingerprints" for Climate Change: Adapted Behaviour and shifting species ranges. In: Walther GR, Burga CA, Edwards PJ, editors. Terrestrial ecosystem responses to climate changes in the Antarctic. Switzerland: Plenum Publishers, New York.

27. Andrássy I (1998) Nematodes in the Sixth Continent. In: Peña Santiago R, editor. Journal of Nematode Morphlogy and Systematics. Jaén: Universidad de Jaén. pp. 107-186.
28. McGaughran A, Stevens MI, Hogg ID, Carapelli A (2011) Extreme glacial legacies: A synthesis of the Antarctic springtail phylogeographic record. Insects 2: 62-82.
29. Miller JD, Horne P, Heatwole H, Miller WR, Bridges L (1988) A survey of the terrestrial tardigrada of the Vestfold Hills, Antarctica. Hydrobiologia 165: 197-208.
30. Stevens MI, Hogg ID (2002) Expanded distributional records of Collembola and Acari in southern Victoria Land, Antarctica. Pedobiologia 46: 485-495.
31. Sohlenius B, Boström S (2008) Species diversity and random distribution of microfauna in extremely isolated habitable patches on Antarctic nunataks. Polar Biology 31:817-825.
32. Sohlenius B, Boström S (2009) Distribution and population structure of two bacterial-feeding nematode genera in ice-free areas in East Antarctica. Nematology 11: 189-201.
33. Gore DB, Rhodes EJ, Augustinus PC, Leishman MR, Colhoun EA, et al. (2001) Bunger Hills, East Antarctica: Ice free at the Last Glacial Maximum. Geology 29: 1103-1106.
34. Stevens MI, Hogg ID (2003) Long-term isolation and recent range expansion from glacial refugia revealed for the endemic springtail *Gomphiocephalus hodgsoni* from Victoria Land, Antarctica. Molecular Ecology 12: 2357-2369.
35. Stevens MI, Greenslade P, Hogg ID, Sunnucks P (2006) Southern Hemisphere springtails: Could Any Have Survived Glaciation of Antarctica? Molecular Biology and Evolution 23: 874-882.
36. Convey P, Gibson JAE, Hillenbrand CD, Hodgson DA, Pugh PJA, et al. (2008) Antarctic terrestrial life - challenging the history of the frozen continent? Biological Reviews 83: 103-117.
37. Convey P (2007) Antarctic Ecosystems. Encyclopedia of Biodiversity: 1-12.
38. Freckman DW, virginia RA (1990) Nematode ecology of McMurdo Dry Valley ecosystems. Antarctic Journal US 25: 229-230.

39. Wharton DA (2003) The environmental physiology of Antarctic terrestrial nematodes: a review. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 173: 621-628.
40. Sohlenius B, Boström S, Hirschfelder A (1995) Nematodes, rotifers and tardigrades from nunataks in Dronning Maud Land, East Antarctica. Polar Biology 15: 51-56.
41. Sohlenius B, Boström S (2005) The geographic distribution of metazoan microfauna on East Antarctic nunataks. Polar Biology 28: 439-448.
42. Hohberg K, Traunspurger W (2005) Predator–prey interaction in soil food web: functional response, size-dependent foraging efficiency, and the influence of soil texture. Biology and Fertility of Soils 41: 419-427.
43. Andrássy I, Gibson J (2007) Nematodes from saline and freshwater lakes of the Vestfold Hills, East Antarctica, including the description of *Hypodontolaimus antarcticus* sp. n. Polar Biology 30: 669-678.
44. Andrássy I (2008) *Eudorylaimus* species (Nematoda: Dorylaimida) of continental Antarctica. Journal of nematode morphology and systematics 11: 49-66.
45. Yeates GW (1979) Terrestrial nematodes from the Bunger Hills and Gaussberg, Antarctica. New Zealand Journal of Zoology 6: 641-643.
46. Kito K, Shishida Y, Ohyama Y (1991) *Plectus antarcticus* de Man, 1904 and *P. frigophilus* Kirjanova, 1958 (Nematoda: Plectidae), with emphasis on the male, from the Soya Coast, East Antarctica. Nematologica 37: 252-262.
47. Shishida Y, Ohyama Y (1986) A note on the terrestrial nematodes around Syowa station, Antarctica (extended abstract). Mere Nat Inst Res, Special Issue 259-260.
48. Kito K, Shishida Y, Ohyama Y (1996) New species of the genus *Eudorylaimus* Andrassy, 1959 (Nematoda: Qudsianematidae) from East Antarctica. Polar Biology 16: 163-169.
49. Andrássy I (2006) *Halomonhystera*, a new genus distinct from *Geomonhystera* Andrássy, 1981 (Nematoda: Monhysteridae). Meiofauna Marina 15: 11-24.
50. Kito K, Ohyama Y (2008) Rhabditid nematodes found from a rocky coast contaminated with treated waste water of Casey Station in East Antarctica, with a description of a new species of *Dolichorhabditis* Andrássy, 1983 (Nematoda: Rhabditidae). Zootaxa 1850: 43-52.

51. Dastych H (1984) The Tardigrada from Antarctic with descriptions of several new species. Acta Zoologica Cracoviensia 27: 377-436.
52. Convey P, McInnes SJ (2005) Exceptional tardigrade-dominated ecosystems in Ellsworth Land, Antarctica. Ecology 86: 519-527.
53. Miller WR, Heatwole H (1995) Tardigrades of the Australian Antarctic Territories - The Mawson coast, East Antarctica. Invertebrate Biology 114: 27-38.
54. Miller WR, Miller JD, Heatwole H (1996) Tardigrades of the Australian Antarctic Territories: the Windmill Islands, East Antarctica. Zoological Journal of the Linnean Society 116: 175-184.
55. Dartnall HJG (1995) Rotifers, and other Aquatic invertebrates, from the Larsemann Hills, Antarctica. Papers and Proceedings of the Royal Society of Tasmania 129: 17-23.
56. Dartnall HJG (2000) A limnological reconnaissance of the Vestfold Hills. ANARE Reports 141: 1-53.
57. Sohlenius B, Boström S, Hirschfelder A (1996) Distribution patterns of microfauna (nematodes, rotifers and tardigrades) on nunataks in Dronning Maud Land, East Antarctica. Polar Biology 16: 191-200.
58. Dartnall HJG (2005) Are Antarctic planktonic rotifers anthropogenic introductions. Quekett Journal of Microscopy 40: 137-143.
59. Pugh PJA, Convey P (2008) Surviving out in the cold: Antarctic endemic invertebrates and their refugia. Journal of Biogeography 35: 2176-2186.
60. Rounsevell DE, Horne P (1986) Terrestrial, parasitic and introduced invertebrates of the Vestfold Hills. In: Pickard J, editor. Antarctic Oasis Terrestrial environments and history of the Vestfold Hills. Sydney: Academic Press Australis. pp. 309-331.
61. Raymond M (2010) Cold-temperature adaptation in nematodes from the Victoria Land Coast, Antarctica. Dunedin: University of Otago. 1-312 p.
62. Freckman DW, Virginia RA (1993) Extraction of nematotes from Dry Valley Antarctic soils. Polar Biology 13: 483-487.
63. Fortuner R (1990) Ratios and indexes in nematode taxonomy. Nematologica 36: 205-216.

64. Rayment GE, Lyons DJ (2010) Soil chemical methods: Australasia. Collingwood, Vic.: CSIRO Publishing.
65. Harley S, Kelly N (2007) The impact of zircon–garnet REE distribution data on the interpretation of zircon U–Pb ages in complex high-grade terrains: an example from the Rauer Islands, East Antarctica. Chemical Geology 241: 62-87.
66. Corvino AF, Wilson CJ, Boger SD (2011) The structural and tectonic evolution of a Rodinian continental fragment in the Mawson Escarpment, Prince Charles Mountains, Antarctica. Precambrian Research 184: 70-92.
67. Clarke KR, Gorley RN (2006) PRIMER v6. User Manual/Tutorial. Plymouth.
68. Knox MA, Hogg ID, Pilditch CA, Lörz A-N, Nodder SD (2012) Abundance and diversity of epibenthic amphipods (Crustacea) from contrasting bathyal habitats. Deep Sea Research Part I: Oceanographic Research Papers 62: 1-9.
69. Graça MAS, Pinto P, Cortes R, Coimbra N, Oliveira S, et al. (2004) Factors Affecting Macroinvertebrate Richness and Diversity in Portuguese Streams: a Two-Scale Analysis. International Review of Hydrobiology 89: 151-164.
70. Kraufvelin P (2011) Scale-dependent distribution of soft-bottom infauna and possible structuring forces in low diversity systems. Marine ecology Progress series (Halstenbek) 426: 13-28.
71. Field JG, Clarke KR, Warwick RM (1982) A practical strategy for analysing multispecies distribution patterns. Marine ecology progress series 8: 37-52.
72. Andrássy I (2008) On the male of the Antarctic nematode species, *Plectus murrayi* Yeates, 1970. Journal of Nematode Morphology and Systematics 11: 87-89.
73. Freckman DW, Virginia RA (1998) Soil biodiversity and community structure in the McMurdo Dry Valleys, Antarctica, in ccosystem processes in a polar desert: The McMurdo Dry Valleys, Antarctica. Antarctic Research Series 72: 323-335.
74. Courtright EM, Wall DH, Virginia RA (2001) Determining habitat suitability for soil invertebrates in an extreme environment: the McMurdo Dry Valleys, Antarctica Antarctic Science 13: 9-17.
75. Yeates GW, Bongers T, De Goede RGM, Freckman DW, Georgieva SS (1993) Feeding habits in soil nematode families and genera—an outline for soil ecologists. The Journal of Nematology 25: 315-331.

76. Wall DH (2007) Global change tipping points: above-and below-ground biotic interactions in a low diversity ecosystem. Philosophical Transactions of the Royal Society B: Biological Sciences 362: 2291-2306.
77. Boström S, Holovachov O, Nadler S (2010) Description of *Scottnema lindsayae* Timm, 1971 (Rhabditida: Cephalobidae) from Taylor Valley, Antarctica and its phylogenetic relationship. Polar Biology: 1-12.
78. Porazinska D, Wall D, Virginia R (2002b) Invertebrates in ornithogenic soils at Ross Island, Antarctica. Polar Biology 25: 569-574.
79. Schwarz AMJ, Green JD, Green TGA, Seppelt RD (1993) Invertebrates associated with moss communities at Canada Glacier, southern Victoria Land, Antarctica. Polar Biology 13: 157-162.
80. Sinclair BJ, Sjursen H (2001) Terrestrial invertebrate abundance across a habitat transect in Keble Valley, Ross Island, Antarctica. Pedobiologia 45: 134-145.
81. Adams BJ, Bardgett RD, Ayres E, Wall DH, Aislabie J, et al. (2006) Diversity and distribution of Victoria Land biota. Soil Biology and Biochemistry 38: 3003-3018.
82. Czechowski P, Sands CJ, Adams B, D'Haese C, Gibson J, et al. (2012) Antarctic Tardigrada: a first step in understanding molecular operational taxonomic units (MOTUs) and biogeography of cryptic meiofauna. Invertebrate Systematics 26: 526-538.
83. Miller WR, Heatwole H, Pidgeon RWJ, Gardiner GR (1994) Tardigrades of the Australian Antarctic Territories - The Larsemann Hills, East Antarctica. Transactions of the American Microscopical Society 113: 142-160.
84. McInnes SJ (1994) Zoogeographic distribution of terrestrial/freshwater tardigrades from current literature. Journal of Natural History 28: 257-352.
85. Bamforth S, Wall D, Virginia R (2005) Distribution and diversity of soil protozoa in the McMurdo Dry Valleys of Antarctica. Polar Biology 28: 756-762.
86. Bamforth SS (1980) Terrestrial Protozoa. Journal of Eukaryotic Microbiology 27: 33-36.
87. Küppers GC, Claps MC, Lopretto EC (2009) Ciliates (Protozoa) from dried sediments of a temporary pond from Argentina. Revista Mexicana de Biodiversidad 80: 581-592.

88. Kennedy AD (1999) Microhabitats occupied by terrestrial arthropods in the Stillwell Hills, Kemp Land, East Antarctica. Antarctic Science 11: 27-37.
89. Heatwole H, Alter D, Charley J, Stephenson NC (1999) From bedrock to biota: weathering, physico-chemical properties, protozoans and micrometazoans of some soils of East Antarctica. ANARE Reports 140: 1-96.
90. Rounsevell D (1981) A Population of *Nanorchestes antarcticus* (Acari, Postigmata) at the Vestfold Hills, Antarctica: Australian Government Publishing Service.
91. Convey P (2000) How does cold constrain life cycles of terrestrial plants and animals? CryoLetters 21: 73-82.
92. Worland MR, Block W (1986) Survival and water loss in some Antarctic arthropods. Journal of Insect Physiology 32: 579-584.
93. Marshall DJ (1996) Comparative water relations of sub-Antarctic and continental Antarctic oribatid mites. Polar Biology 16: 287-292.
94. Clarke KR, Warwick RM (2001) Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition. Plymouth: PRIMER-E.

CHAPTER III

Morphological and molecular diversity at a regional scale: a step closer to understanding Antarctic nematode biogeography

Alejandro Velasco-Castrillón[1*] and Mark I. Stevens[1,2]

[1] *Australian Centre for Evolutionary Biology and Biodiversity, School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, South Australia 5005, Australia*

[2] *South Australian Museum, GPO Box 234, Adelaide, South Australia 5000, Australia*

[*]Corresponding author: Alejandro Velasco-Castrillón. E-mail: macalusio@yahoo.com

This chapter was published and formatted in a style appropriate for the journal *Soil Biology and Biochemistry.*

Statement of authorship

Title of Paper	Morphological and molecular diversity at a regional scale: a step closer to understanding Antarctic nematode biogeography
Publication Status	Published
Publication Details	This chapter was published as a research article Velasco-Castrillón, A. and Stevens, M. I. (2014). Morphological and molecular diversity at regional scale: a step closer to understanding Antarctic nematode biogeography. *Soil Biology and Biochemistry* 70:272-284

Author Contributions

By signing the Statement of authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Alejandro Velasco Castrillón		
Contribution to the Paper	Conceived and design the experiment, collected and sorted soil samples, conducted nematode extraction and identification, performed statistical analyses and interpreted data, produced figures and tables, wrote the manuscript.		
Signature		Date	11/11/2013

Name of Co-Author	Mark I Stevens		
Contribution to the Paper	Supervised the project, sought and won funding, assisted in data analyses, provided intellectual feedback, critically revised and approved manuscript.		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	09/10/2013

Preamble

Chapter III is based on nematode diversity mentioned in Chapter I and II. In this chapter we detailed the current state of knowledge of nematode diversity across Antarctica. Additionally to the nematode diversity list provided in Chapter I we have incorporated new species and distribution records from maritime and continental Antarctica, and performed phylogenetic and morphological analyses. In addition to the soil geochemistry analyses presented in Chapter I, we have complemented it with water biochemistry analyses from lakes were nematodes were collected. The content of this chapter has been published in the journal *Soil Biology & Biochemistry*.

Abstract

Antarctica is one of the harshest environments on earth and yet life has managed to persist on the continent for millions of years. While most of the continent is covered by snow and ice, in some coastal and mountain regions that do not have permanent cover terrestrial invertebrate fauna dominate. Nematodes are one of the most common taxa present in these environments, but despite their abundance very little work on diversity and distribution has been performed for the Phylum across the Antarctic continent. We examined nematodes from 123 limno-terrestrial samples from the vicinity of the Australian Antarctic Stations (62.8°E – 110.5°E) using the mitochondrial cytochrome *c* oxidase subunit I (COI) gene, and morphological analyses. We identified the nematodes *Plectus murrayi*, *Pl.*cf. *frigophilus*, *Scottnema* cf. *lindsayae*, *Halomonhystera* cf. *halophila*, *H.* cf. *continentalis* and *Eudorylaimus* spp. The distribution of these species appears to be determined by habitat type and salinity. We also made comparisons using the COI gene with nematodes from localised sampling from Dronning Maud Land, Francis Island (Antarctic Peninsula), and Tierra del Fuego (TF), and also with COI sequences from other worldwide locations. Contrasting levels of COI sequence divergence were identified among genera and species, ranging from low levels for *Pl. murrayi* (≤ 0.5%), medium levels for *S.* cf. *lindsayae* (≤ 2.1%) and *Halomonhystera* (≤ 4.3%), and high within *Pl.*cf. *frigophilus* (≤ 8.4%). Distribution ranges varied according to the species, with widespread ranges within Antarctica for *Pl. murrayi* and *Scottnema* cf. *lindsayae* (a range of over 2000 km); and distribution beyond Antarctica to TF for *Pl.*cf. *frigophilus*. Our results reveal the presence of cryptic species even when conservative approaches are applied in species delimitation.

Introduction

Nematodes are a major component of the Antarctic limno-terrestrial fauna, and have been described as one of the most dominant groups of metazoan found throughout the frozen continent (e.g. Sohlenius et al., 1996; Powers et al., 1998; Freckman and Virginia, 1998; Adams et al., 2006). Despite their worldwide dominance, little is known about the diversity of these microinvertebrates highlighting the need for further comprehensive research to assess biogeographical distribution of species and how these relate to the global fauna. Historically, most research has focussed on regions close to permanent stations, especially on the Antarctic Peninsula (AP) and Victoria Land (VL), and more recently in nunataks and coastal regions across the continent. The nematode fauna in Antarctica is now reported to include 67 species, 37 of which are found on the AP or its offshore islands (Maritime Antarctica), and 33 in Continental Antarctica (Table 1). Currently there is no apparent overlap among the nematode fauna from Maritime Antarctica (Sector 4) with the rest of the Antarctic continent (Sectors 1,2,3; Table 1). The exception are three unconfirmed records for the terrestrial species *Pl. murrayi* and the aquatic/semi-aquatic *Pl. frigophilus* from AP (Sector 4; Maslen and Convey, 2006), and the maritime species *Teratocephalus tilbrooki* from Sector 1 (Verlecar et al., 1996). Unfortunately with such records, without molecular or morphological data it is difficult to confirm species identifications and examine species connectivity and distributional ranges (see Table 1).

When considering the biogeographic distributions of all 67 Antarctic nematode species it becomes challenging to undertake continental-wide studies covering soil and water environments and relate these to biota from elsewhere in the world. Recent work on Antarctic limno-terrestrial nematodes has focussed on descriptions of new species based on distinguishing morphological characters (e.g. Andrássy, 1998; see Table 1). However, numerous studies have reported species as new records without providing any morphological information making it impossible to confirm identifications (see Table 1). The distinction between confirmed and unconfirmed records impacts on our capability to assess endemicity of the fauna, which is critical to examine Antarctic and southern hemisphere biogeography within a global context.

In recent years molecular studies have become more prevalent as an important tool to aid traditional taxonomy in assessing biodiversity, geographic distribution, species identification and descriptions (e.g. Courtright et al. 2000; Porazinska et al. 2009; Derycke et al. 2010, Stevens et al., 2011). Nuclear ribosomal DNA have been used in nematode species delimitation, but not always with a clear outcome given the low mutation rates presented by this group of genes (Smythe and Nadler, 2006; Nadler et al., 2006; Adams et al., 2007; Boström et al., 2010; Ristau et al., 2013). The implementation of a more rapidly evolving DNA region to discriminate among nematode species has come from the mitochondrial cytochrome *c* oxidase subunit I (COI) gene that provides high resolution to discern among closely related species (Hebert et al., 2003; Costa et al., 2007; Tavares and Baker, 2008; Ristau et al., 2013). This gene is one of the most widely-used markers in comparative phylogeography (Stevens and Hogg, 2003, Hogg and Hebert, 2004; Stevens et al., 2006; Ashton et al., 2008; Sands et al., 2008; Czechowski et al., 2012; Ristau et al., 2013) and although well-studied in invertebrates, most relevant to our study is that this gene has recently been optimised for nematodes (Prosser et al., 2013).

Collection of nematode specimens from throughout the Australian Antarctic Territory (62.8°E – 110.5°E) from soil and water samples were compared to more localised regions from soil samples in Dronning Maud Land (DML, 71ºS – 24ºE), Francis Island on the AP (69ºS – 65ºW), and Tierra del Fuego (TF, 54ºS – 69ºW). Using morphology together with the COI gene we aim to address the following three questions: (1) Does morphological diversity correspond to molecular diversity; (2) are nematodes from soil the same as those from lakes; and (3) given levels of diversity, are nematodes locally-endemic or widespread, and at what biogeographical scale?

Table 1 Geographical location of all nematode species recorded in continental and maritime Antarctica

		Sector 1 (45°W - 45°E)		Sector 2 (45°E - 135°E)						Sector 3 (135°E - 135°W)			Sector 4 (135°W- 45°W)	
														
	Area	DML	POC	MtV	MS-FM	SI	VH-LH	BH	CS	RI	VL	TM	M-AP	TF
Order Rhabditida														
Chiloplacoides antarcticus Heyns, 1994	C	**15 ^**												
***Cuticularia firmata* Andrássy, 1998**	M												**4 ^**	
***Dolichorhabditis tereticorpus* Kito & Ohyama, 2008**	C								**17 ^**					
***Panagrolaimus davidi* Timm, 1971**	C									**26,29 ^**	1, **29**			
Panagrolaimus magnivulvatus Timm, 1971	C	4,27,28,9 **^**												
Rhabditis krylovi Tsalolikhin, 1989	M												4 **^**	
***Scottnema lindsayae* Timm, 1971**	C-M	NR	25, 2		NR		NR			**4,11,26,29**	1,**4,11**,12,13,**29 ^**	2		
Order Plectida														
Ceratoplectus armatus (Butschli, 1873) Andrássy, 1984	M												4, 20	
Chiloplectus masleni Boström, 1997	C	4,**10 ^**												
***Plectus antarcticus* de Man, 1904**	M												4, 20 **^**	
***Plectus belgicae* de Man 1904**	M												4, 20 **^**	
***Plectus frigophilus* Kirjanova, 1958**	C-M		**25, 18**		NR		**8**	16,31 **^**	NR	**4, 29**	1,**4,29**		20?	NR
***Plectus insolens* Andrássy, 1998**	M												**4 ^**	
***Plectus meridianus* Andrássy, 1998**	M												**4 ^**	
***Plectus murrayi* Yeates, 1970**	C-M	**4,27,28,9**	**4**,25,**18**		NR	NR	23	7,16	7	**4,29,30 ^**	1,4,13		20?	
Plectus telekii Mulk & Coomans, 1978	C	21												33
***Plectus tolerans* Andrássy, 1998**	M												**4**, 20 **^**	
Teratocephalus pseudolirellus Maslen, 1979	M												20 **^**	
Teratocephalus rugosus Maslen, 1979	M												20 **^**	
***Teratocephalus tilbrooki* Maslen, 1979**	M	32											**4**, 20 **^**	
Order Dorylaimida														
***Amblydorylaimus isokaryon* Loof, 1975**	M												**4 ^**	
Calcaridorylaimus signatus (Loof, 1975) Andrássy, 1986	M												4 **^**	
Enchodelus signyensis Loof, 1975	M												4, 20 **^**	
***Eudorylaimus antarcticus* (Steiner, 1916) Yeates, 1970**	C									**4,6,29**	1,**4,6**,13,29 **^**			
Eudorylaimus coniceps Loof, 1975	M												4, 20 **^**	
***Eudorylaimus glacialis* Andrássy, 1998**	C				**6**		x			30	1,6 **^**			
***Eudorylaimus nudicaudatus* Heyns, 1993**	C	**4,6 ^**			x									
Eudorylaimus pseudocarteri Loof, 1975	M												4, 20 **^**	
***Eudorylaimus quintus* Andrássy, 2008**	C						**x,6 ^**				**6**			
Eudorylaimus sabulophilus Tijepkema, Ferris & Ferris, 1971	C	21												33
***Eudorylaimus sextus* Andrássy, 2008**	C						**x,6 ^**							
***Eudorylaimus shirasei* Kito, Shishida & Ohyama, 1996**	C		**6, 19**	4,**6,19 ^**	x						1			
Eudorylaimus spauli Loof, 1975	M												4, 20 **^**	
Eudorylaimus verrucosus Loof, 1975	M												4, 20 **^**	
Mesodorylaimus antarcticus Nedelchev & Peneva, 2000	M												**22 ^**	
Mesodorylaimus chipevi Nedelchev & Peneva, 2000	M												**22 ^**	
Mesodorylaimus imperator Loof, 1975	M												4, 20 **^**	

Table 1 (continued)

	Area	DML	POC	MtV	MS-FM	SI	VH-LH	BH	CS	RI	VL	TM	M-AP	TF
Mesodorylaimus masleni Nedelchev & Peneva, 2000	M												**22 ^**	
Mesodorylaimus signatus Loof, 1975	M												20 ^	
Rhyssocolpus paradoxus (Loof, 1975) Andrássy, 1986	M												4, 20 ^	
Order Monhysterida														
Eumonhystera vulgaris (de Man, 1880) Andrássy, 1981	M												4,20,3	
***Geomonhystera antarcticola* Andrássy, 1998**	C									29	1,4,29 ^			
Geomonhystera villosa (Butschli, 1873) Andrássy, 1981	M												4, 20	
Halomonhystera antarctica Cobb, 1914	C									5 ^				
***Halomonhystera continentalis* Andrássy, 2006**	C						**5**,8 ^							
Halomonhystera disjuncta Bastian, 1865	M												5	
Halomonhystera glaciei Blome & Riemann, 1999	M												5 ^	
***Halomonhystera halophila* Andrássy, 2006**	C						x,**5**,8 ^							
Halomonhystera uniformis Cobb, 1914	C									5 ^				
Order Tylenchida														
***Aglenchus agricola* (de Mann, 1884) Andrássy, 1954**	C	**24**												
Antarctenchus hooperi Spaull, 1972	M												4 ^	
***Apratylenchoides joenssoni* Ryss *et al.* 2005**	C	**24 ^**												
***Ditylenchus parcevivens* Andrássy, 1998**	M												**4 ^**	
Helicotylenchus diagonicus Dariling & Thorne, 1959	C	21												33
Helicotylenchus dihystera (Cobb, 1893) Sher, 1961	C	21												33
Helicotylenchus exallus Sher, 1966	C	21												33
***Paratylenchus nanus* Coob, 1925**	C	**24**												
***Pratylenchus andinus* Lordello, Zamith & Boock, 1961**	C	**24**												
Rotylenchus capensis Van den Berg, 1996	C	4 ^												
***Tylenchorhynchus maximus* Allen, 1955**	C	**24**												
Order Enoplida														
Paramphidelus antarcticus Tsalolikhin, 1981	M												4 ^	
Order Mononchida														
***Coomansus gerlachei* Jairajpuri & Khan, 1977**	M												**4,** 20 ^	
Order Aphelenchida														
Aphelenchoides haguei Maslen, 1979	M												4, 20 ^	
Aphelenchoides helicosoma Maslen, 1979	M												4, 20 ^	
Aphelenchoides vaughani Maslen, 1979	M												4, 20 ^	
Order Triplonchida														
Eutobrilus antarcticus Tsalolikhin, 1981	C							4,14 ^						
Order Chromadorida														
***Hypodontolaimus antarcticus* Andrássy & Gibson, 2007**	C						**8 ^**							

Specified Sectors (1 to 4) have been defined by Andrássy 1998. Species in bold provide a full description with figures. Species underlined are described but do not provide figures. Numbers indicate bibliographic referenes (those in bold indicate described species). Boxes in grey indicate records from the present study; new records are shown by 'NR'. Grey boxes with an 'x' suggest the presence of the species for our study based only on morphology (no COI data). References followed by '?' indicate unconfirmed species records. Symbol '^' represents the type locality for the species. List of acronyms: Continental Antarctica (C), Maritime Antarctica (M), Dronning Maud Land (DML), Prince Olav Coast (POC), Mount Vechernaya (MtV), area from Mawson Station to Framnes Mtns (MS-FM), Sansom Island (SI), area from Vestofold Hills to Larsemann Hills (VH-LH), Bunger Hills (BH), Casey Station (CS), Ross Island (RI), Victoria Land (VL), Transantarctic Mtns (TM), Maritime Antarctica and Antarctic Peninsula (M-AP), Tierra del Fuego (TF), and species recorded from elsewhere in the world (eW). Literature source: (1) Adams et al., 2006 (2) Adams et al., 2007; (3) Andrássy, 1981. (4) Andrássy, 1998; (5) Andrássy, 2006; (6) Andrássy, 2008a; (7) Andrássy, 2008b; (8) Andrássy & Gibson, 2007; (9) Boström, 1995; (10) Boström, 1996; (11) Boström et al., 2010; (12) Courtright et al., 2000; (13) Freckman & Virginia, 1997; (14) Gagarin, 2009; (15) Heyns, 1994; (16) Kirjanova, 1958; (17) Kito & Ohyama, 2008; (18) Kito et al., 1991; (19) Kito et al., 1996; (20) Maslen & Convey, 2006; (21) Bohra et al., 2010; (22) Nedelchev & Peneva, 2000; (23) Rounsevell & Horne, 1986; (24) Ryss et al., 2005; (25) Shishida & Ohyama, 1986; (26) Sinclair, 2001; (27) Sohlenius et al., 1995; (28) Sohlenius et al., 1996; (29) Timm, 1971; (30) Yeates, 1970; (31) Yeates, 1979; and (32) Verlecar et al., 1996.

Methods

Sampling areas

For practical reasons and in order to assign nematode diversity to Antarctica we have used the 45° sectors proposed by Andrássy (1998); as following: Sector 1 (45°W – 45° E), Sector 2 (45°E – 135° E), Sector 3 (135°E – 135° W), and Sector 4 (135°W – 45° W) (see Antarctic maps in Table 1).

Sampling in Antarctica occurred during three austral summers from 2007 to 2010; most of the sampling took place in Sector 2 (December 2009 – March 2010) from a geographical range of over 2000 km from Casey Station (CS) on Bailey Peninsula (66.28º S – 110.54º E) to Framnes Mountains (FM; 67.77º S – 62.82º E). Nematodes from soil samples in Sector 2 were obtained from 79 sites from CS, Vestfold Hills (VH), Hop Island (HI), Mather Peninsula (MP), Broknes Peninsula (BP), Stornes Peninsula (SP), Sansom Island (SI), Mawson Station (MS), and Framnes Mountains (Table 2). Samples were also collected from 44 tarn/lakes in Sector 2 (VH, HI, BP and SP), ranging in size from three metres diameter to large lakes over 450 m diameter. Restricted soil sampling occurred on Francis Island (summer of 2007-2008), Tanngarden and Brattnipane in DML (February 2009), and TF (summer of 2009) (see Table 2).

Table 2 Regions sampled from Antarctica and TF showing number of samples collected, and samples that produced positive COI sequences for nematodes.

Sector	Region	Coordinates		Sampling (area/ transect)	Elevation (m)	Samples with nematodes (total)	Samples with positive nematode sequences
		Latitude	Longitude				
2	Casey Station (CS)	66.28ºS	110.52º-110.54ºE	1.5 km2	4 - 44	11 soil	11 soil
2	Vestfold Hills (VH)	68.48º-68.60ºS	77.87º- 78.51ºE	340 km2	4 - 66	17 soil, 1 water	17 soil
2	Broknes Peninsula (BP)	69.38º- 69.4ºS	76.32º- 76.40ºE	7 km2	0 - 69	11 soil, 13 water	11 soil, 8 water
2	Stornes Peninsula (SP)	69.36º- 69.43ºS	75.99º- 76.14ºE	6 km2	4 - 59	15 soil, 14 water	15 soil, 13 water
2	Hop Island (HI)	68.82º- 68.83ºS	77.68º- 77.73ºE	4 km2	10 - 36	9 soil, 16 water	5 soil, 4 water
2	Mather Peninsula (MP)	68.85º- 68.86ºS	77.93º- 77.94ºE	1 km2	44 - 80	6 soil	4 soil
2	Sansom Island (SI)	69.71ºS	73.75º E	400 m2	15 - 20	2 soil	2 soil
2	Mawson station (MS)	67.60ºS	62.86º- 62.87ºE	0.48 km2	4 - 24	4 soil	5 soil
2	Framnes Mountains (FM)	67.77º- 67.78ºS	62.79º- 62.82ºE	3 km2	460 - 490	4 soil	3 soil
1	Dronning Maud Land (DML)	71.85º-71.87ºS	24.58º - 24.60ºE	47 km	1119 - 1331	3 soil	1 soil
4	Francis Island (AP)	69.58º- 69.65ºS	64.65º- 65.40ºW	31 km	113- 427	5 soil	2 soil
TF	Tierra del Fuego (TF)	54.41º-54.46ºS	69.23º-69.35ºW	17km	0 - 220	10 soil	3 soil

For more detailed information go to Appendix 3: Table S8

Sampling Methods

Soil samples were collected from 0 to 10 cm in depth given that previous studies have shown that the majority of nematodes inhabit this top 10 cm layer throughout the summer season (Powers et al., 1995). Soil samples (0.5 – 1.0 kg in weight) were excavated using a metal trowel which was carefully cleaned between sites to avoid cross contamination and subsequently placed in individual sterile bags, which were kept inside insulated containers while in the field and remained frozen from -20°C to -80 °C during transit and storage. Sampling took place in ice-free areas at a range of elevations (from 0 m to 2566 m asl) and various habitats to increase the probability that we would sample a wide diversity of nematodes. Soil samples range in organic content, vegetation, salinity, moisture, ornithogenic input, and soil geochemistry (Velasco-Castrillón, et al., 2014). Samples from tarn/lakes were collected in Sector 2 using a 30 cm diameter round frame with a 35 µm mesh bag and a detachable collection 50 ml tube at the end of the bag, with a 5 m line attached to the frame. The net was thrown into the water and pulled back quickly to avoid it from sinking. Sampling effort varied from 5 to 8 min depending on depth and size of the tarn/lake. Benthic communities were not targeted. Nematodes were stored in the 50 ml tubes filled with tarn/lake water and stored frozen from -20°C to -80 °C.

Nematode sorting and identification

Nematodes from soil were sorted using an adapted version of a sugar centrifugation method (Freckman and Virginia, 1993; Andrássy and Gibson, 2007). This method was carried out on 50-100g of soil (wet weight) after rocks over 1 cm had been removed, and follows the method detailed in Velasco-Castrillón et al. (2014). Nematodes were visualised under a stereo microscope (Olympus SZ-PT, Japan), and digital images were taken at magnifications ranging from 40X to 100X. Representatives of the morpho-types were slide-mounted using the wax-ring method by Hooper (1986; see Appendix 3: Preparation of nematode slides). Morphological identification was performed from digital images and slide mounted specimens. Measurements and de Man's ratios (see Appendix 3: De Man's ratios) were calculated only for those Antarctic nematodes classified to genus or species

(Appendix 3: Table S1 – S5) and compared with published species descriptions for Antarctic specimens (Table 3). We were able to identify *S.* cf. *lindsayae*, *Pl. murrayi*, *Pl.* cf. *frigophilus*, *H.* cf. *halophila*, *H.* cf. *continentalis* (Table 3), *Eudorylaimus* spp (Appendix 3: Table S4), the Family cf. Panagrolaimidae, and the family Criconematidae. No morphological comparisons were performed for Dorylaimida (collected in AP) or for cf. Panagrolaimidae (from Sector 2) given their low numbers. *Plectus* from TF were the only nematodes from outside Antarctica that we assessed morphological characters (Table 3).

Abiotic habitat parameters

Water and soil samples from Sector 2 were analysed for pH, electric conductivity (used as a proxy for salinity; *viz.* Magalhães et al., 2012), and moisture (for soil samples). Abiotic variables (pH, salinity, and moisture) were logarithmically transformed (base 10) to avoid skewness as observed in the Draftman Plots before transformation (using PRIMER v.6; Clarke and Gorley, 2006). Pearson's correlation coefficients were calculated for 121 samples using the IBM SPSS statistics package v20 (Armonk, NY: IBM Corp.). Correlations were performed for each combination of the log transformed abiotic variables and the presence of nematode taxa in soil and water samples (Appendix 3: Table S6).

DNA sequencing

DNA extraction, PCR, and COI sequencing were performed by the Canadian Centre for DNA Barcoding (CCDB) at the Biodiversity Institute of Ontario, University of Guelph, using standard laboratory protocols (Ivanova et al.,2006; Ivanova and Grainger, 2006). Total DNA was extracted from entire individuals and the mitochondrial COI gene amplified with a cocktail of specific primers (Prosser et al., 2013) (see Appendix 3: DNA, PCR and sequencing protocols).

Table 3 Species list, mitochondrial lineages, measurements, and de Man's ratios for specimens collected across our sampling regions. Measurements were compared to other populations.

Species	Region	Sec	Lin	s/w	N	Body length (µm)	Tail length (µm)	body width (µm)	de Man's ratios a	b	c	Ref
S. cf. *linds.*	**LH**	2	N18	s	7	410-680	32-50	28-38	15-21	-	12-15	p
S. cf. *linds.*	**HI-MP**	2	N18	s	9	450-740	33-50	28-45	16-20	-	13-15	p
S. cf. *linds.*	**VH**	2	N18	s	6	530-650	40-51	33-45	13-19	-	12-14	p
S. cf. *linds.*	**DM**	1	N19	s	2	650	45	35-40	16-19	-	14	p
S. cf. *linds.*	**FM**	2	N20	s	5	430-650	33-44	28-34	15-19	-	11-15	p
S. cf. *linds.*	**FM**	2	N21	s	1	440	40	30	15	-	11	p
S. linds.♀	La Croix Glacier	3	-	s	10	790 - 860	-	-	16.4	4.6	17.6	(7)
S. linds.♂	La Croix Glacier	3	-	s	10	770 - 880	-	-	19	4.5	15.7	(7)
S. linds.♀	Cape Hallet	3	-	s	7	512 ± 44	39 ± 2	29 ± 1.8	17.3 ± 0.5	3.9 ± 0.2	13.0 ± 0.7	(6)
S. linds.♂	Cape Hallet	3	-	s	5	568 ± 25	47 ± 3	30 ± 1	19.1 ± 0.8	4.2 ± 0.1	12.1 ± 0.3	(6)
S. linds.♀	Ross Island	3	-	s	8	640 - 720	-	-	16 - 20	3.8 - 4.6	14 - 16	(1)
S. linds.♂	Ross Island	3	-	s	6	550 - 730	-	-	19 - 22	4.1 - 4.3	13 - 15	(1)
S. linds.♀	Taylor Valley	3	-	s	28	570-730	37-51	-	14-19	3.9-5.0	13-16	(3)
S. linds.♂	Taylor Valley	3	-	s	24	540-720	43-54	-	16-21	3.7-4.8	11-15	(3)
Pl. murr ♀	**CS**	2	N10	s	5	800-920	85-110	28-38	22.1-28.9	3.8-4.8	7.4-9.4	p
Pl. murr ♀	**VH**	2	N10	s	5	810-910	100-110	28-36	24.6-28.9	4.1-4.7	8.1-9.0	p
Pl. murr ♀	**HI, MP**	2	N10	s	6	810-1080	80-110	33-44	22.3-28.4	4.3-4.8	7.8-10.8	p
Pl. murr ♀	**LH**	2	N10	s	7	800-1000	90-110	28-48	20-31.7	4.0-5.5	8.5-10.6	p
Pl. murr ♀	**MS-FM**	2	N10	s	5	810-880	95-105	37-44	20.0-21.9	3.9-4.2	8.1-9.1	p
Pl. murr ♀	Marble Point	3	-	s	25	600-820	-	-	15.2-24.8	4.7-6.0	6.5-9.1	(8)
Pl. murr ♀	Strand Moraines	3	-	s	16	683-882	-	-	18.6-31.5	4.6-5.5	6.6-8.3	(8)
Pl. murr ♀	Cape Hallet	3	-	s	16	817 ± 12	97 ± 2	31 ± 1	26.5 ± 0.3	4.4 ± 0.1	8.5 ± 0.2	(6)
Pl. murr ♀	Dry Valley	3	-	s	10	750 - 840	-	-	24 - 28	3.8 - 4.4	7.6 - 8.8	(1)
Pl. murr ♀	Soya coast	1	-	s	10	810-935	104-114	31-38	22.8-27.5	3.9-5.2	7.8-8.8	(5)
Pl. murr ♀	Marble Point	3	-	s	10	740 - 1100	-	-	20.1 - 27.2	3.8 - 4.7	8.3 - 10.5	(7)
Pl. murr ♀	Strand Moraines	3	-	s	10	1020-1190	-	-	21.5 - 27.6	4.3 – 4.7	9.1 – 10.3	(7)
Pl. murr ♀	Bunger Hills	2	-	s	34	650-1000	98-128	31-52	16.2-23.7	3.7-5.2	6.1-8.6	(9)
*Pl.*cf. *frig.*♀	**CS**	2	N12	s	1	1080	105	46	23.5	-	10.3	p
*Pl.*cf. *frig.*♀	**BP**	2	N12	w	7	1200-1800	110-160	45-70	24.3-34	4.6-5.2	10.3-11.7	p
*Pl.*cf. *frig.*♀	**SP**	2	N12	s/w	8	1380-1900	120-170	50-60	25-31.7	4.1-5.0	9.7-11.5	p
*Pl.*cf. *frig.*♀	**CS**	2	N11	s	1	1430	120	52	27.5	4.5	11.9	p
*Pl.*cf. *frig.*♀	**BP**	2	N11	w	5	1100-1700	100-150	40-55	24.4-35	4.2-4.9	10.8-12.1	p
*Pl.*cf. *frig.*♀	**SP**	2	N11	s/w	9	1300-2050	120-150	40-55	25.5-37.3	4.5-5.0	8.7-14.6	p
*Pl.*cf. *frig.*♀	**FM**	2	N11	s	2	930-1400	110-130	33-45	28.2-31.1	3.9	8.5-10.8	p
*Pl.*cf. *frig.*♀	**TF**	-	N22	s	1	970	-	38	25.5	-	-	p
*Pl.*cf. *frig.*♀	**TF**	-	N23	s	1	900	-	38	23.7	-	-	p
Pl frig. ♀	Obruchev Hills	2	-	s	3	1360-1887	-	-	23-28	4.8-5.1	10.6-12.5	(4)
Pl frig. ♀	Ross Island	3	-	s	10	1350-1720	-	-	24-33	4.9-5.4	9.9-11	(1)
Pl frig. ♀	Edmonson Point	3	-	s	5	1600-1820	-	-	23-24	4.7-5.0	11-Dec	(1)
Pl frig.♀	Soya coast	1	-	s	4	1455-1700	137-161	50-62	26.1-29.1	4.5-4.8	9.3-11.4	(5)
Pl frig. ♀	Marble Point	3	-	s	10	1400-1990	-	-	22.2-32.5	4.4-5.2	9.7-12.3	(7)
Pl frig. ♀	Strand Moraines	3	-	s	10	1540-2060	-	-	25.7-30.0	4.5-5.1	10.5-13.5	(7)
Pl frig. ♀	Bunger Hills	2	-	s	25	1190-1580	120-160	38-56	25.5-33	4.0-4.8	9.2-10.9	(9)
H. cf. *cont.*	**BP**	2	N16a	s	**1**	650	65	25	26	-	10	p
H. cf. *cont.*	**HI**	2	N16b	s	**2**	670-750	70-75	25-30	25-27	-	9.6-10	p
H. cf. *cont.*	**VH**	2	N17	s	**6**	470-710	55-70	20-35	19-26	-	8-12	p
H. cf. *cont.*	**HI**	2	-	s	**4**	510-790	50-68	22-28	23-28	-	10-12	p
H. cf. *halop*	**HI**	2	N15	w	**3**	1400-1600	90-120	45-50	31-32	-	13-16	p
H. cont. ♀	VH	2	-	w	3	520-580	-	-	20-24	-	9-11	(2)
H. cont. ♂	VH	2	-	w	4	420-560	-	-	20-23	-	10.4-11	(2)
H. halop.♀	VH	2	-	w	8	1000-1270	-	-	22-32	-	9-12	(2)
H. halop.♂	VH	2	-	w	8	820-1330	-	-	29-38	-	11-13	(2)

Regions 'in bold' correspond to our sampling areas. Rows in dark grey inside boxes correspond to species described from the type locality. Species acronyms: *Scottnema lindsayae* (*S. linds.*), *Plectus murrayi* (*Pl. murr.*), *Plectus frigophilus* (*Pl. frig.*), *Halomonhystera continentalis* (*H. cont.*), *Halomonhystera halophila* (*H. halophila*). Other acronyms: Broknes Peninsula (BP), Stornes Peninsula (SP), Hop Island (HI), Mather Peninsula (MP), Vestfold Hills (VH), *Dronning Maund Land* (DML), Framnes Mountains (FM), Mawson Station (MS), Casey Station (CS), mitochondrial lineage according to Fig.1 (Lin), specimens extracted from soil samples (s), specimens extracted from water samples (w), specimens measured (No), references from literature (ref), and present study (p). List of references: (1) Andrássy, 1998; (2) Andrássy, 2006; (3) Boström et al., 2010; (4) Kirjanova, 1958; (5) Kito et al., 1991; (6) Raymond, 2010; (7) Timm, 1971; (8) Yeates, 1970; and (9) Yeates, 1979. Gaps indicate no available data.

Sequence Analysis

A total of 369 COI sequences and 36 unique haplotypes were obtained from individuals sorted from soil and from tarn/lakes. COI sequences were visually inspected and sequence chromatograms were used to resolve ambiguous base calls. Sequences between 436 and 658 bp were used for the alignment in Geneious Bioinformatics package v3.8 (Biomatters, Ltd., Auckland, NZ). Sequences were verified as derived from the relevant taxa using the NCBI Blastn algorithm and compared to related taxa. Blastn searches using the 36 unique haplotypes identified a further 13 GenBank sequences (above 90% similarity). From these 13 GenBank sequences seven were included in our alignment (lineages N1, N2, N4-N7, N9; see Fig. 1). The remaining six GenBank sequences were within 1% divergence from any of the selected seven and were not included. COI sequences were translated into amino-acids choosing the appropriate frame and the invertebrate mitochondrial genetic code to check for stop-codons; after confirming absence of stop-codons, sequence alignments were performed with the default settings (cost matrix: 65% similarity; gap open penalty: 12; and gap extension penalty: 3). The general time reversible model with gamma rate variation (GTR + Γ) was selected as the best model under the Akaike Information Criterion (AIC) and the hierarchical likelihood ratio test (hLRTs) calculated within PAUP* v.4.0 beta10 (Swofford, 2002) using MRMODELTEST v.2.3 (Nylander, 2004) and MODELTEST v.3.7 (Posada and Crandall, 1998). Bayesian Inferences (BI) were calculated with MRBAYES v.3.2 (Huelsenbeck and Ronquist, 2001) using the GTR + Γ model. The tree-space was explored using four MCMC chains over 10,000,000 generation (to result in an average standard deviation of split frequencies of 0.005), a chain temperature of 0.2, sampling frequency of 100, and a burn-in of 25,000 generations. The program MEGA5 (Tamura et al., 2011) was implemented to generate Maximum likelihood (ML) trees with 1,000 bootstrap replicates and the GTR + Γ model. Similar topologies were observed comparing the Bayesian (Appendix 3: Fig. S1) and ML trees (Appendix 3: Fig. S2) generated using the 36 unique haplotypes and the seven representative GenBank sequences. For the pair-wise distance comparisons uncorrected p-distances among haplotypes were used (Appendix 3: Table S7). All sequences obtained in this study have been deposited in the Barcode of Life Data System (BOLD) database (www.barcodinglife.org) and GenBank (Appendix 3: Table S8).

Results

Nematode diversity

The nematode diversity from this study represents four of the most diverse Orders in Antarctica; Rhabditida, Plectida, Dorylaimida and Monhysterida (Fig. 1). The Order Tylenchida is represented by specimens from the family Criconematidae collected only from TF (Table 1). Other less common Orders not represented in our sampling are Aphelenchida, Mononchida, and Enoplida that have been found in Sector 4, and Chromadorida and Triplonchida, both rare from Sector 2 (Table 1).

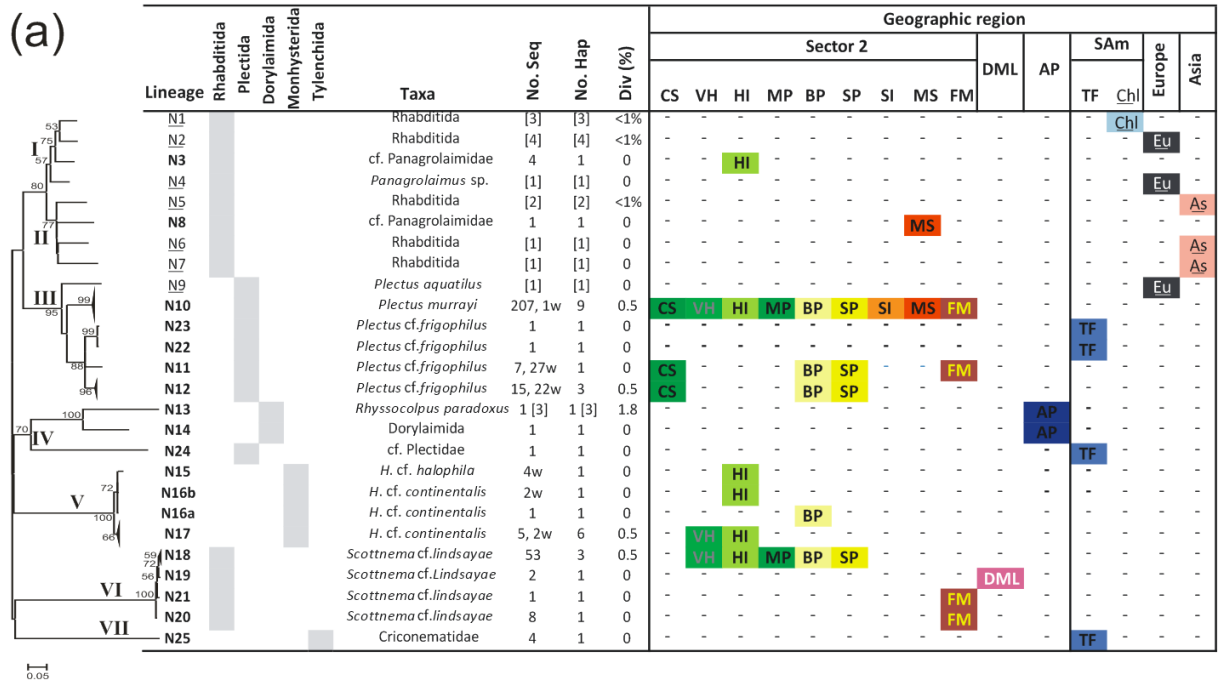


Figure 1 (previous page) Maximum Likelihood (GTR + Γ) tree of COI nematode sequences from Antarctica compared to other sequences from Tierra del Fuego (TF), Chile (Chl), Europe (Eu), and Asia (As). Confidence values at nodes were generated from 1,000 bootstrap pseudoreplicates (support values below 50% are not shown). COI lineages are represented from N1 to N25 (lineages below 1.0% p-distance were collapsed, with the exception of N13). Roman numerals correspond to Clades discussed in the text. Lineages and regions in bold indicate new sequences from our study; lineages and regions underlined indicate GenBank sequences (number of sequences and haplotypes shown in square brackets). The colour boxes in Fig. 1a represent the geographic region of sequences and the colour code refers to the maps in Fig. 1b. List of acronyms: number of sequences included in the specified clade (No. Seq), nematode sequences from water (w), number of haplotypes in each clade (No. Hap), P-distance percentage within-clade (Div %), Casey Station (CS), Vestfold Hills (VH), Hop Island (HI), Mather Peninsula (MP), Broknes Peninsula (BP), Stornes Peninsula (SP), Sansom Island (SI), Mawson Station (MS) and Framnes Mountains (FM), Dronning Maud Land (DML), Antarctic Peninsula (AP), South America (SAm), Prince Olav Coast (POC), Mount Vechernyaya (MtV), Bunger Hills (BH), Transantarctic Mtns (TM), Victoria Land (VL), and Ross Island (RI)

The final COI alignment contained 43 unique haplotypes with an AT-rich composition (A: 25%; T: 41%; G: 19%; and C: 15%) and pair-wise sequence divergences up to 47% (between N13 and Clade VI; Appendix 3: Table S7). The alignment of the 43 haplotypes revealed that sequences belonging to the Order Rhabditida (Clades I, II) and to the Order Tylenchida (Clade VII) had a 3bp deletion (position 349 – 351 bp). These sites were occupied by the amino acid Serine in *Plectus* (Order Plectida), *Eudorylaimus* (Order Dorylaimida) and *Halomonhystera* (Order Monhysterida). Rhabditida lineages belonging to the genus *Scottnema* (Clade VI) had a 3bp deletion (position 97 – 99 bp) corresponding to the amino acid Glycine in other Rhabditida (Clades I, II); and a deletion at sites 448 – 450 bp were observed for *Scottnema* (Clade VI) corresponding to Cysteine in all lineages (The exception was Valine in the genus *Plectus*). The ML analyses produced 26 distinctive lineages (all lineages < 1.0% maximum uncorrected p-distance were collapsed; Fig. 1). The seven GenBank sequences formed exclusively 'non-Antarctic' lineages nested within Clades I and II (N1, N2, N4 – N7, and N9; see Fig. 1). Sequences were also queried against the BOLD database and identified three matches (compared to sequences from lineage N13; Fig. 1) that were 1.4 – 1.8% divergent (p-distance) to *Rhyssocolpus paradoxus* collected in Maritime Antarctica.

Order Rhabditida

Morphological measurements and de Man's ratios for 32 *Scottnema* cf. *lindsayae* (Clade VI; Fig. 1) individuals were taken from Sector 2 and DML (Sector 1) and compared to records from VL (Sector 3; Table 3). Other Rhabditida, collected in two soil samples from HI and MS (lineages N3 and N8 respectively; Fig 1) were only possible to discriminate to the Family level (cf. Panagrolaimidae), and no morphological comparisons were undertaken for this group.

Rhabditida lineages were grouped by two highly divergent clades that were not monophyletic using nucleotide (Appendix 3: Fig. S1 – S2) or amino acid analyses (Appendix 3: Fig. S3). Clade I included three lineages (N1, N2 and N4) outside Antarctica and one 'new-lineage' (N3) from specimens collected in a soil sample from HI. Lineage N3 from Sector 2 included four identical sequences from cf. Panagrolaimidae and diverged by 9.8 – 11.6% (between-lineage-divergence 'bld') from N1 (from Chile), N2 (from Scotland) and N4 (from Netherlands) (Appendix 3: Table S7). Clade II diverged by 14.4 – 18.9% (bld) from clade I. Clade II was comprised by a single haplotype from Sector 2 (MS), a cf. Panagrolaimidae specimen and grouped with a further 3 lineages from China (N5 – N7; Fig. 1). Clades I and II were 37.4 – 42% divergent from the Antarctic Clade VI (N18 – N21) and 35 – 37% divergent from the TF clade (VII; Fig.1; Appendix 3: Table S7). Clade VI included closely related *Scottnema* cf. *lindsayae* specimens from our sampling regions (2.1% bld).

Most sampled *Scottnema* fall in lineage N18, all of which were collected within 130 km (Sector 2) in habitats with low moisture (0.11 – 15.4 %) and low salinity (0.01 – 0.38 dS/m; Appendix 3: Table S9). Lineage N18 consisted of 53 sequences and three haplotypes, and a maximum within-lineage-divergence (wld) of 0.5%. *Scottnema* cf. *lindsayae* lineage N20 from FM, consisted of eight identical sequences all from the same soil sample; while N21 also from FM (135 m from N20) was formed by a single sequence that diverged from N20 by 1.4%. A fourth *Scottnema* cf. *lindsayae* lineage (N19) was identified for DML (1500 km from N20) formed by two identical sequences. All *Scottnema* cf. *lindsayae* from DML revealed a single morpho-type.

Order Plectida

Plectus murrayi measurements and de Man's ratios were calculated for 28 adult females from soil samples from Sector 2 (Appendix 3: Table S2). Measurements were compared to published descriptions across Antarctica, including the holotype from Marble Point in VL (Yeates, 1970; Table 3). For *Pl.* cf. *frigophilus* we measured 33 specimens from soil and water samples from Sector 2 (and two nematodes from TF; Appendix 3: Table S3) and compared to published descriptions for other Antarctic regions, including the type specimens described by Kirjanova (1958) from Obruchev Hills in Sector 2 (Table 3).

The genus *Plectus* in our study were grouped in Clade III, though a single cf. Plectidae specimen from TF forms its own divergent lineage (N24, Clade IV). Most of the nematode sequences produced for this study belong to the species *Pl. murrayi* (Fig. 1). Our analyses showed that despite the large number of *Pl. murrayi* specimens collected across Sector 2 all of them fall within a single lineage (N10). This lineage included 208 sequences and nine haplotypes (0.5% wld) from nematodes collected in soil samples (and one specimen from a lake) from the nine regions in Sector 2 and covered a distance over 2000 km (Fig. 1). No nematodes from the Order Plectida were found in DML or AP samples.

Other specimens belonging to the genus *Plectus* were morphologically identified as *Pl.* cf. *frigophilus* but formed four separate lineages in Clade III up to 8.7% divergent from each other. Two of those lineages (N22 and N23) were formed by single individuals from TF with 1.4% sequence divergence. Lineage N11 included 34 sequences (27 from tarn/lakes) with only a single haplotype from specimens collected in Sector 2 over 2000 km apart. Lineage N12 comprised 37 sequences (22 from tarn/lakes) and three haplotypes (0.5% wld) from Sector 2 sites spanning 1470 km. Only one *Pl. murrayi* sequence was recorded for a low saline lake in BP (0.14 mS/cm). *Plectus aquatilus* from the Netherlands (N9) diverged by 16.4-16.7% from *Pl. murrayi*, and 16.2 – 17.6% from *Pl.* cf. *frigophilus* lineages (Fig. 1; Appendix 3: Table S7). Divergence between *Pl. murrayi* and *Pl.* cf. *frigophilus* lineages ranged between 13.5 – 15.5%. An unidentified cf. Plectidae specimen from TF (N24) diverged from other lineages by more than 40%, but shared a node with Dorylaimida from AP (N13 and N14; Fig. 1; Appendix 3: Table S7).

Table 4 Abiotic parameters ranges (salinity, pH, and moisture) for nematode taxa collected in soil and water samples from Sector 2

Nematode taxa	habitat	number of samples	EC (dS m^{-1})	pH	Moisture (%)
Plectus murrayi	soil	**55**	0.01 - 3.5*	4.3 - 9	0.25 - 69.4
Plectus murrayi	water	**1**	0.15	6.9	100
Plectus cf. *frigophilus*	soil	**4**	0.04 - 0.88	4.7 - 6.3	11.8 - 77
Plectus cf. *frigophilus*	water	**25**	0.11 - 1.7	4.9 - 7.9	100
Scottnema cf. *lindsayae*	soil	**25**	0.01 - 0.38	6 - 9	0.11 - 15.45
Eudorylaimus spp	soil	**27**	0.01 - 3.5	5.8 - 9	0.11 - 28.62
Eudorylaimus spp	water	**3**	0.23 - 9.9	6.5 - 8.6	100
Halomonhystera spp	soil	**5**	0.04 - 3.02	6.1 - 8	5.85 - 24.4
Halomonhystera spp	water	**17**	1.8 - 81.5	7.1 - 9.2	100
cf. Panagrolaimidae	soil	**2**	0.08 - 4.4	6 - 8.24	8.8 - 15.55

* A single *Pl. murrayi* was found in a soil sample with EC 48.1 dS m^{-1} (not included in Table)

Order Dorylaimida

Morphological measurements were carried out on 44 *Eudorylaimus* specimens collected from soil samples throughout Sector 2 (Appendix 3: Table S4). We found differences in size, ratios and supplement numbers for *Eudorylaimus* populations even within the same region. Body length varied from 1 mm to 2.4 mm, and supplements in mature males from 7 to 12 (Appendix 3: Table S4). Based on morphology, the nematodes from Francis Island (Sector 1) belonged to two distinctive Dorylaimida morpho-types not observed in Sector 2. Only two COI sequences were obtained for each of these morpho-types which diverged by 24.7% from each other (Fig. 1). These sequences split into two separate lineages (N13 and N14) and corresponded to samples collected in separate nunataks (22.5 km apart) from Francis Island. Lineage N13 was 1.4 – 1.8% divergent from *Rhyssocolpus paradoxus* specimens collected in Livingston Island in Maritime Antarctica (Sector 4), where this species has been previously reported (Table 1).

Order Monhysterida

Morphological measurements for 16 *Halomonhystera* specimens from soil and water samples from Sector 2 (VH, HI and BP) and de Man's ratios were compared to the type

specimens of *H. continentalis* and *H. halophila* described by Andrássy (2006) for saline lakes from VH (4 – 47 g salt/l; see Table 4). *Halomonhystera* cf. *continentalis* body length for adult nematodes varied between 470 um and 790 um, falling outside the maximum size range described from the type population (based on seven specimens). De Man's ratios ('a' and 'c') for our specimens seem to be slightly broader than the ones reported by Andrássy (2006) for the species (Table 3).

Clade V is formed by four closely related *Halomonhystera* lineages from Sector 2 across a range of 119 km with sequence divergence up to 4.3% (Fig 1; Appendix 3: Table S7). *Halomonhystera* cf. *halophila* (lineage N15) included four sequences and a single haplotype from a high-saline lake (46 mS/cm) from HI. *Halomonhystera* cf. *continentalis* was distributed across three lineages (N16a, N16b, N17). Lineage N16a consisted of one sequence from a soil sample in BP; lineage N16b was formed by two sequences and one haplotype from a HI lake; while N17 included seven sequences and six haplotypes (0.5% wld) from VH soil samples and HI lakes (Fig. 1). Even though the sequence divergence between cryptic lineages N16a and N16b was only 0.5%, they were placed in different lineages based on ML (Fig. 1; Appendix 3: Fig. S2), and Bayesian tree topologies (Appendix 3: Fig. S1).

Order Tylenchida

The Order Tylenchida was exclusively represented by a single lineage N25 from TF formed by four identical sequences from the Family Criconematidae (Clade VII; we did not attempt to identify the specimens further).

Linking species presence and abiotic parameters

Scottnema cf. *lindsayae* populations in the current study were found solely in soil samples with low salinity levels (< 0.38 dS/m) and low moisture content (<15.45%; Table 4; Appendix 3: Table S9). Soil samples from which *Pl. murrayi* (N10) were found varied greatly in pH, salinity, and moisture (Table 4; Appendix 3: Table S9). *Plectus* cf.

frigophilus collected from water samples came from 25 low saline tarn/lakes ranging in pH (4.9 – 7.8) and salinity concentration (0.11 – 1.7 dS/m; Table 4). *Eudorylaimus* were also collected from three tarn/lakes in VH, BP, and SP, ranging in salinity (0.23 – 9.9 mS/cm) and pH (6.5 – 8.6); while *Eudorylaimus* from 27 soils samples were found in low salinity and moisture concentration and similar pH to those in water (Table 4). When considering morphologically identified nematodes collected from water samples, we observed *Halomonhystera* to be the only nematode genus present in saline tarn/lakes (18 in total) ranging from 1.8 – 81.5 mS/cm. The only exception was a single *Eudorylaimus* specimen in a 9.9 mS/cm lake; while *Halomonhystera* from soils never exceeded salinity levels of 3.02 dS/m (Table 4).

The abiotic parameters measured for water and soil samples (pH, salinity, and moisture), together with presence-absence of nematode taxa (Appendix 3: Table S9) revealed several significant correlations (Appendix 3: Table S6). Positive correlations were observed for: *i*) *Plectus murrayi* and *Eudorylaimus* spp, *ii*) *Scottnema* cf. *lindsayae* and *Eudorylaimus* spp, *iii*) *Halomonhystera* spp with salinity and moisture, and *iv*) salinity, pH and moisture. Negative correlations were observed for: *i*) *Plectus murrayi* with *Pl.* cf. *frigophilus*, *Halomonhystera* spp and the three abiotic parameters, *ii*) *Pl.* cf. *frigophilus* with *Scottnema* cf. *lindsayae*, *Eudorylaimus* spp and *Halomonhystera* spp, *iii*) *Scottnema* cf. *lindsayae* with salinity and moisture, and *iii*) *Eudorylaimus* spp with *Halomonhystera* spp, salinity and moisture.

Discussion

Species boundaries

Our molecular work based on COI sequence divergence and combined with morphological data, clearly separates *Scottnema* lineages from *Plectus*, *Halomonhystera*, Dorylaimida, and cf. Panagrolaimidae (Fig. 1). Discerning among lineages or species was not always possible without the aid of the COI gene given the lack of diagnostic morphological

characters typical for nematodes (Derycke et al., 2010; Powers et al., 2011). Currently, there is little or no consistency in levels of COI divergence observed between species, and more data across genera and species are required to assess what thresholds may exist for nematodes. Elsasser *et al.* (2009) reported 5% genetic divergence separating interspecific parasitic nematodes of the genus *Dracunculus* (Order Spirurida). Derycke *et al.* (2010) reported a maximum divergence (K2P) of 0.59% for marine nematodes (including Order Rhabditida). Eamsobhana *et al.* (2010) reported uncorrected p-distances within parasitic nematode species of the Order Rhabditida to vary between 0.3% and 11.4%. Other studies of the COI gene on parasitic nematodes (including Rhabditida and Panagrolaimida) by Prosser *et al.* (2013) showed a divergence of 2% or greater separating operational taxonomical units (OTUs), and Ristau *et al.* (2013) found interspecific COI uncorrected p-distances in the aquatic genus *Tobrilus* (Order Enoplida) ranging from 9.6 to 15.4% and within *Tobrilus gracilis* identified three highly divergent 'cryptic' species ranging from 9.3 to 16.6%.

In our study the divergences in Clade III between *Pl. aquatilus* from Europe and our Antarctic *Pl.* cf. *frigophilus* and *Pl. murrayi* ranged from 13.5 to 17.6% (Appendix 3: Table S7). From the literature it is clear that establishing boundaries for nematode species delimitation varies widely among different groups. Greater understanding of these issues will come from studies that can link morphology and genes to delineate species boundaries.

Order Rhabditida

For the present study, *Scottnema* cf. *lindsayae* specimens were slightly shorter in body length (410 – 740 µm) than *Scottnema lindsayae* reported for Ross Island, Taylor Valley and Cape Hallet (Table 3), and significantly smaller than those described from the type location from La Croix Glacier (Table 3). However, de Man's ratios ('a' and 'c') for our specimens fall within the same ranges as the ones reported for other populations in Table 3 (Timm, 1971; Andrássy, 1998; Boström, 2010; Raymond, 2010). *Scottnema* cf. *lindsayae* specimens varied greatly in body size (body length, body width, and tail length) within the

same lineage, most likely as a result of individuals' age (Appendix 3: Table S1). However, it is also possible based on the widespread distribution across Antarctica and the large range in morphometrics, that *S.* cf. *lindsayae* is represented by more than one species.

Most of the work for the genus has been based on populations from Sector 3 in VL, Ross Island (Timm, 1971; Freckman and Virginia, 1998; Powers et al., 1998; Treonis et al., 1999; Virginia and Wall, 1999; Raymond, 2010; Boström et al., 2010), and Queen Maud Mountains (Adams et al., 2007). Also from Sector 2 at Syowa Station (Shishida and Ohyama, 1986) and Larsemann Hills (Velasco-Castrillón et al., 2014). *Scottnema* cf. *lindsayae* populations in the current study (Table 1) spanned 2000 km, from VH (Sector 2) to Brattnipane in DML (Sector 1; Fig. 1), increasing the known records across the continent and potentially making it one of the most widespread nematode species. Our closely related *S.* cf. *lindsayae* lineages (clade VI; Fig.1) diverged up to 2.1% (N19 and N21), slightly higher than the 2% species threshold suggested by Prosser *et al.* (2013) which included the Order Rhabditida. However, considering that lineages N20 and N21 were both collected from soil samples 135 m apart in FM and diverged by 1.4%, yet the remarkable distribution around Antarctica where *Scottnema* has been found it is likely that our data indicates a single species.

The other lineages within the Order Rhabditida in our study corresponded to specimens collected from Sector 2 belonging to the Family cf. Panagrolaimidae (N3 and N8); in Antarctica this Family is represented by the species *Panagrolaimus magnivulvatus* from DML and *Pa. davidi* from VL (Table 2). Inclusion of the family Panagrolaimidae within the Order Rhabditida was supported by phylogenies from Donn *et al.* (2011) and Raymond (2010). Our N3 and N8 lineages had a closer relationship to Rhabditida sequences from other non-Antarctic regions than to any of the Antarctic nematodes (Fig. 1), likely caused by inadequate taxon sampling. We could not identify our specimens further (only six specimens in two soil samples and the scarcity of morphological diagnostic characters). However, these would represent new records or new species once greater taxon sampling can be achieved. The tree topology reveals no resolution in determining the monophyly for the Order Rhabditida. The lack of reliability of the COI gene to discriminate between distantly related taxa has been well documented in previous studies (i.e. Stevens et al.,

2006; McGaughran et al., 2010; Czechowski et al., 2012) and using amino acid translation did not resolve this further (Appendix 3: Fig. S3); though polyphyly within Rhabditida has also been reported for the SSU rDNA (Meldal et al., 2007; van Megen et al., 2009; Donn et al., 2011).

Order Plectida

In our samples, measurements and de Man's ratios for adult *Pl. murrayi* in Sector 2 (Kito et al., 1991; Appendix 3: Table S2) fall within the accepted ranges for the species description from VL, Bunger Hills, Soya Coast, and DML (see Yeates, 1970; Timm, 1971; Andrássy, 1998; Raymond, 2010) with slightly longer specimens found for the regions LH, MP and HI. However, the type description for the species by Yeates (1970) and description by Timm (1971) for Strand Moraines (McMurdo Sound, VL), consisted of slightly shorter and larger animals respectively than the ones reported in other studies, suggesting that there may be some population level variation. No males were observed in our study, as for most Antarctic studies (e.g. Timm, 1971; Andrássy, 1998; Sohlenius and Boström, 2008; Raymond, 2010). Measurements and ratios also varied among populations in Sector 2, but it is clear from our results that the populations we examined are similar to those described from Soya coast (Kito et al., 1991) and Bunger Hills (Yeates, 1979), even though shorter tails were recorded for the present study.

When examining *Pl.* cf. *frigophilus*, body length for adult specimens were substantially larger than for *Pl. murrayi* (Table 3). We also observed differences in body lengths within *Pl.* cf. *frigophilus* even within adult specimens from the same samples (Appendix 3: Table S3), probably reflecting age differences and variation at the population level. *Plectus* cf. *frigophilus*, for this study were in the same size range as those recorded by Kirjanova (1958) and those described by Andrássy (1998), Kito *et al*. (1991) and Timm (1971; Table 3). Three small *Pl.* cf *frigophilus* from lineages N12 and N11 (Appendix 3: Table S3) fall below the body length range (< 1190 µm) observed by other studies. De Man's ratios for 'b' correspond to the expected range reported in the literature, while the minimum value for ratio 'c' fall just below the expected range, and maximum value for 'a' above that

described (Table 3). Morphological measurements for *Pl.* cf *frigophilus* from TF were restricted to body length (900 - 970 µm) and width (~38 µm).

Other *Plectus* species have been reported for continental Antarctica (besides *Pl. murrayi* and *Pl.* cf. *frigophilus*), but were later corrected to *Pl. murrayi* after careful examination. The species descriptions by Timm (1971) and Kito *et al.* (1991) for *Plectus antarcticus* do not correspond to the specimens described by de Man (1904); it is now accepted that their *'Pl. antarcticus'* was reported for continental Antarctica and does not occur in the maritime region (Andrássy, 1998; Maslen and Convey, 2006; Convey et al., 2008). Similarly, *Pl. acuminatus* from DML described by Boström (1995) and Sohlenius *et al.* (1995) was later confirmed as *Pl. murrayi* by Andrássy (1998).

In our study *Pl. murrayi* was the most abundant of all nematodes, it was present in all sampled regions in Sector 2, and was distributed over a distance of 2000 km. *Plectus murrayi* occurred in a wide range of soil habitats; probably reflecting a high dispersal capacity and high tolerance levels to diverse habitats (Velasco-Castrillón *et al.* 2014). Despite the abundance and widespread distribution in Sector 2, little sequence divergence was observed within the species (0.5% wld; Fig. 1). Only females were observed for this study (over 200); even though a study by Andrássy (2008b) has previously reported males (but were uncommon). *Plectus* cf. *frigophilus* was never as abundant or as common as *Pl. murrayi* in soil samples (found in single samples in FM, SP and CS), but it was the most common nematode collected from low salinity water samples (< 1.7 dS/m; Table S9); suggesting habitat preferences and physiological adaptations to withstand aquatic and low-saline environments.

We found that *Pl. murrayi* and *Pl. frigophilus* (from Sector 2) prefer different habitats, with the former occurring in terrestrial environs, while the latter in aquatic or semi-aquatic environments (Table 4). Cryptic morpho-species were observed for our *Pl.* cf. *frigophilus*, with two distinctive and highly divergent lineages from Antarctica (N11 and N12); and two closely related lineages from TF (N22, N23; Fig.1). The low sequence divergence observed for the two TF lineages (1.4%) suggests a single species. Divergence for the Antarctic lineages ranged between 8.0 and 8.4% even for specimens occurring sympatrically; while divergence between Antarctic and TF lineages ranged between 5.9

and 8.7% (Appendix 3: Table S7). Such divergence potentially suggests three separate species (two for Antarctica and one for TF; Fig. 1), similar to the cryptic species identified in *Tobrilus gracilis* (Ristau et al., 2013). What we currently know as *Pl. frigophilus* may actually be a species-complex (based on the COI data). Our records for TF extend the distribution range of this species-complex, and may support the unconfirmed *Pl. frigophilus* record from Alexander Island, west of AP (Sector 4; Maslen and Convey, 2006). Besides *Pl.* cf. *frigophilus* and *Pl. murrayi*, we found a distantly related cf. Plectidae lineage from TF (N24) which shared a common (but distant) node with Dorylaimida from Francis Island (40% sequence divergence). Polyphyly within the Family Plectidae has previously been observed in phylogenetic studies using the SSU rDNA (Holterman et al., 2006, van Megen et al., 2009).

Order Dorylaimida

Based on body length, morphological characters, ratios and distribution it is possible that our *Eudorylaimus* (which occurred in sites as far as 650 km from Mawson coast to VH) were represented by several species (*E. glacialis*, *E. quintus*, *E. sextus*, *E. nudicaudatus* and *E. shirasei*; Appendix 3: Table S4). Two specimens found at HI and MP were clearly larger than the others (2.32 – 2.4 mm), which matched the size and distribution reported by Andrássy (2008a) for *E. shirasei*. Smaller specimens from our samples in Sector 2 were similar in body length and male supplements to three *Eudorylaimus* species (*E. quintus*, *E. sextus*, and *E. glacialis*) described by Andrássy (2008a) and Kito *et al.* (1996); although some differences were observed for the de Man's ratios.

Eudorylaimus glacialis was recently reported for MS and FM (Andrássy, 2008a) extending the distribution from the type population in VL (Yeates, 1970). Our results indicate that morpho-types with similar measurements and de Man's ratios to the ones described for *E. glacialis* were present in VH, MP, BP and SP. Based also on morphology, *Eudorylaimus* cf. *sextus* appeared to be the most common of all *Eudorylaimus* found in VH. Previous records for *E. sextus* and *E. quintus* from VH describe *E. quintus* (1.6 – 2.04 mm) as a longer species than *E. sextus* (1.2 – 1.8 mm) (Andrássy, 2008a) but both species

have similar ‘b’ and ‘c’ ratios (Appendix 3: Table S4). Some of the specimens we found in MP fit ratios and number of supplements for *E. quintus*. Longer specimens (> 2.1 mm) were recorded for MP and HI and according to the descriptions for species of similar length they could fit within *E. nudicaudatus* (Syn. *E. nudicatilus*) or *E. shirasei* size range; even though higher ‘a’ ratios were observed in our specimens (47 – 55) atypical for the two species. Our ‘long specimens’ were also found to be longer than *E. nudicaudatus* and shorter than specimens described for *E. shirasei* from Mt Vechernaya (Kito et al., 1996). Morphological analyses from digital images are not sufficient to accurately fit any of our specimens within previously described species. We found that *Eudorylaimus* specimens were the most morphologically diverse in Sector 2 soils, although given the lack of COI sequences for the genus we were not able to corroborate molecular findings with morphology.

From Francis Island (Sector 4) only two Dorylaimida sequences (24.7% divergence) were represented by two morpho-types; each a separate lineage (N13 and N14) in Clade IV (Fig. 1). The high sequence divergence and the lack of amplification from the Order Dorylaimida across other Antarctic regions may reflect high sequence divergence at primer binding sites, despite the primers being designed using members of the Class Dorylaimea (Prosser et al., 2013).

Order Monhysterida

Records for *H. continentalis* and *H. halophila* are scarce, and the first records for the species go back to 2006 from VH (Andrássy, 2006). Specimens for the present study were collected at three sites from the same region as the type population (VH), and from HI and BP extending its overall distribution by over 100 km (Fig. 1). Some *Halomonhystera* specimens found in water samples from HI were clearly different from *H. continentalis* in body size and ratios (Table 3; Appendix 3: Table S5) and morphologically resembled the description for *H. halophila*. However, body length of our *H.* cf. *halophila* was slightly longer than the maximum size for *H. halophila* from the type population (Table 3). De

Man's ratio 'a' for *H.* cf. *halophila* specimens were within the range reported for *H. halophila*, while ratio 'c' was outside the maximum range.

The body size for *H.* cf. *continentalis* from our study exceeded records to those from the type population (Table 3), increasing maximum length from 580 µm to 790 µm, and maximum width from 28 µm to 35 µm. The size difference is likely the result of under-sampling at the type population (which was based on seven voucher specimens) rather than a new undescribed species given the sequence divergence (see below). Two morpho-types were clearly discernible, the *H.* cf. *continentalis* morpho-type (short nematodes represented by lineages N16a, N16b and N17) and the *H.*cf. *halophila* morpho-type (long nematodes, from lineage N15). Lineage N15 corresponded to specimens from a hyper-saline lake in HI, which diverged from N16 lineages by 2.3% and from N17 between 4.1 – 4.3 % (less than the divergence among N16 and N17). Interestingly, the morpho-type *H.* cf. *continentalis*, represented by lineages N16a and N16b, shared a common node with *H.*cf. *halophila* (N15); while lineage N17 was formed by a separate node. Sequence divergence among *H.* cf. *continentalis* lineages N16 (a-b) and N17 ranged from 1.8 to 2.5 % (Fig. 1; Table S7). Based on the divergence observed among our *H.*cf. *halophila* (N15) and the other *Halomonhystera* lineages, we suggest that a ~2% threshold may be indicative for separate species within the genus *Halomonhystera*.

Order Tylenchida

The Order Tylenchida represented by Clade VII shared a deep (sequence divergence 44 – 45%; Appendix 3: Table S7) and unsupported node with the *Scottnema* clade VI, as revealed by the ML, Bayesian and amino acid analyses (Fig. 1; Appendix 3: Fig. S1 – S3). It is not surprising we did not find the Order Tylenchida in Antarctica as it has only been found in Sectors 1 and 4 where we did not undertake detailed sampling.

Conclusions

Our work has revealed the nematode *Pl. murrayi* as the most abundant and highly conserved species (represented by a single lineage) of all nematodes in Sector 2 soils. *Plectus* cf. *frigophilus* reveals a conserved morpho-type, high COI divergence (up to 8.4%), and a widespread distribution with representatives in TF and Antarctica; raising the possibility of three separate species (two for Sector 2 and one for TF). The presence of *Eudorylaimus* spp. (Order Dorylaimida) in Sector 2 could only be established by morphological studies. *Eudorylaimus* in Sector 2 was confirmed as a commonly found genus, the most morphologically diverse and probably the richest in species diversity. We have also increased the geographic distribution records for *S.* cf. *lindsayae* populations, with our findings being the first confirmed records from Sector 2. So far, *S. lindsayae* has been the only *Scottnema* species described for Antarctica. Despite the morphological diversity, and widespread geographical distribution for *Scottnema lindsayae*, the possibility of more than one species could not be answered given the low sequence divergence found for our specimens in Sector 2 and Sector 3 (< 2.1%). We have also shown two distinct morpho-types for *Halomonhystera* in saline environments in Sector 2 and sequences divergence as high as 4.3%.

Habitat seems to play a role in determining species and distribution, with *Scottnema* cf. *lindsayae*, *Eudorylaimus* spp and *Plectus murrayi* mainly restricted to soil samples; while *Pl.* cf. *frigophilus* and *Halomonhystera* inhabit aquatic environments or saturated soils (moisture > 5.85% for *Halomonhystera*, and > 11.8% for *Plectus* cf. *frigophilus*). However, salinity concentration in water appears to be a factor segregating *Pl.* cf. *frigophilus* from *Halomonhystera* populations. We also reveal a widespread distribution within Antarctica for the commonly found *Plectus murrayi* and *Scottnema* cf. *lindsayae* (distributed over 2000 km), against short range endemics such as: *Eudorylaimus* spp, *Halomonhystera* and cf. Panagrolaimidae.

Micro-invertebrate taxonomy, with its recognised paucity and often ambiguity in morphological diagnostic characters, will significantly benefit from the inclusion of molecular data. Together with morphological analyses resolution of clear genus and

species boundaries are essential to further biogeographic studies in Antarctica. Here we have shown levels of morphological and molecular diversity for nematodes throughout the Antarctic continent that reveal both endemic and widespread species that challenge our perceptions of Antarctic nematodes.

Acknowledgements

This research was partially supported and funded by the Australian Antarctic Division (http://www.antarctica.gov.au/) Project (ASAC 2355 to MIS). We thank the University of Adelaide (http://www.sciences.adelaide.edu.au/) for a PhD scholarship to AVC and the South Australian Museum Mawson Trust for providing funding for the Sir Douglas Mawson Doctoral Scholarship (http://www.samuseum.sa.gov.au/). We deeply thank Mark Schultz and the Australian Antarctic Stations leaders and field personnel who contributed in logistics and support of field work in Antarctica. We are extremely grateful to the DNA sequencing agencies BOLD and the Canadian Centre for DNA Barcoding (CCDB). We also thank Rebecca Stonor, Maria Manjarrez, Pauline Glocke and Tanya Matic (The University of Adelaide) for their ongoing laboratory support. We are also grateful to John Gibson, Steve Cooper, Byron Adams and for their intellectual contributions. We thank John Gibson and Kerrie Davies for examining nematode specimens and confirming generic identifications. We also thank Cyrille D'Haese (MNHN), Paul Czechowski (The University of Adelaide), Chester Sands, Peter Convey, Paul Geissler (British Antarctic Survey) for providing soil samples from AP and DML. We are very greatful toto Allan Green, Leopoldo Sancho, Ana Pintado and María Arróniz-Crespo (Complutense University of Madrid) for providing soil samples and assistance in sorting nematodes from TF. We also want to thank the "Corporacion Nacional Forestal" (CONAF) from the Chilean government to grant permits to Dr. Leopoldo Sancho to collect soil samples in TF.

References

Adams, B.J., Bardgett, R.D., Ayres, E., Wall, D.H., Aislabie, J., Bamforth, S., Bargagli, R., Cary, C., Cavacini, P., Connell, L., Convey, P., Fell, J.W., Frati, F., Hogg, I.D., Newsham, K.K., O'Donnell, A., Russell, N., Seppelt, R.D., Stevens, M.I., 2006. Diversity and distribution of Victoria Land biota. Soil Biology and Biochemistry 38, 3003-3018.

Adams, B.J., Wall, D.H., Gozel, U., Dillman, A.R., Chaston, J.M., Hogg, I.D., 2007. The southernmost worm, *Scottnema lindsayae* (Nematoda): diversity, dispersal and ecological stability. Polar Biology 30, 809-815.

Andrássy, I., 1981. Revision of the order Monhysterida (Nematoda) inhabiting soil and inland waters. Opusc. Zool. Budapest 17, 13-47.

Andrássy, I., 1998. Nematodes in the Sixth Continent, In: Peña Santiago, R. (Ed.), Journal of Nematode Morphlogy and Systematics. Universidad de Jaén, Jaén, pp. 107-186.

Andrássy, I., 2006. *Halomonhystera*, a new genus distinct from *Geomonhystera* Andrássy, 1981 (Nematoda: Monhysteridae). Meiofauna Marina 15, 11-24.

Andrássy, I., 2008a. *Eudorylaimus* species (Nematoda: Dorylaimida) of continental Antarctica. Journal of nematode morphology and systematics 11, 49-66.

Andrássy, I., 2008b. On the male of the Antarctic nematode species, *Plectus murrayi* Yeates, 1970. Journal of nematode morphology and systematics 11, 87-89

Andrássy, I., Gibson, J., 2007. Nematodes from saline and freshwater lakes of the Vestfold Hills, East Antarctica, including the description of *Hypodontolaimus antarcticus* sp. n. Polar Biology 30, 669-678.

Ashton, G.V., Stevens, M.I., Hart, M.C., Green, D.H., Burrows, M.T., Cook, E.J., Willis, K.J., 2008. Mitochondrial DNA reveals multiple Northern Hemisphere introductions of *Caprella mutica* (Crustacea, Amphipoda). Molecular Ecology 17, 1293-1303.

Bohra, P., Sanyal, A., Hussain, A., Mitra, B., 2010. Five new records of nematodes from East Antarctica. Journal of Threatened Taxa 2, 974-977.

Boström, S., 1995. Populations of *Plectus acuminatus* Bastian, 1865 and *Panagrolaimus magnivulvatus* n. sp.(Nematoda) from nunatakks in Dronning Maud Land, East Antarctica. Fundamental and Applied Nematology 18, 25-34.

Boström, S., 1996. *Chiloplectus masleni* sp. nov. and variability in populations of *Plectus acuminatus* Bastian 1865 (Nematoda: Plectidae) from the nunatak Basen, Vestfjella, Dronning Maud Land, East Antarctica. Polar Biology 17, 74-80.

Boström, S., Holovachov, O., Nadler, S., 2010. Description of *Scottnema lindsayae* Timm, 1971 (Rhabditida: Cephalobidae) from Taylor Valley, Antarctica and its phylogenetic relationship. Polar Biology, 1-12.

Clarke, K.R., Gorley, R.N., 2006. PRIMER v6. User Manual/Tutorial, Plymouth.

Convey, P., Gibson, J.A.E., Hillenbrand, C.D., Hodgson, D.A., Pugh, P.J.A., Smellie, J.L., Stevens, M.I., 2008. Antarctic terrestrial life - challenging the history of the frozen continent? Biological Reviews 83, 103-117.

Costa, F.O., deWaard, J.R., Boutillier, J., Ratnasingham, S., Dooh, R.T., Hajibabaei, M., Hebert, P.D.N., 2007. Biological identifications through DNA barcodes: the case of the Crustacea. Canadian Journal of Fisheries and Aquatic Sciences 64, 272-295.

Courtright, E.M., Wall, D.H., Virginia, R.A., Frisse, L.M., Vida, J.T., Thomas, W.K., 2000. Nuclear and mitochondrial DNA sequence diversity in the Antarctic nematode Scottnema lindsayae. Journal of Nematology 32, 143-153.

Czechowski, P., Sands, C.J., Adams, B., D'Haese, C., Gibson, J., McInnes, S.J., Stevens, M.I., 2012. Antarctic Tardigrada: a first step in understanding molecular operational taxonomic units (MOTUs) and biogeography of cryptic meiofauna. Invertebrate Systematics 26, 526-538.

De Man, J.G., 1904. Nématodes libres. Expédition Antarctique Belge. *Resultats du voyage du S.Y. Belgica en 1897-1898-1899.* Zoologie 3-55.

Derycke, S., Vanaverbeke, J., Rigaux, A., Backeljau, T., Moens, T., 2010. Exploring the Use of Cytochrome Oxidase c Subunit 1 (COI) for DNA Barcoding of Free-Living Marine Nematodes. PLoS One 5, e13716.

Donn, S., Neilson, R., Griffiths, B., Daniell, T., 2011. Greater coverage of the phylum Nematoda in SSU rDNA studies. Biology and Fertility of Soils 47, 333-339.

Eamsobhana, P., Lim, P.E., Solano, G., Zhang, H., Gan, X., Yong, H.S., 2010. Molecular differentiation of Angiostrongylus taxa (Nematoda: Angiostrongylidae) by cytochrome c oxidase subunit I (COI) gene sequences. Acta Tropica 116, 152-156.

Elsasser, S.C., Floyd, R., Hebert, P.D.N., Schulte-Hostedde, A.I., 2009. Species identification of North American guinea worms (Nematoda: *Dracunculus*) with DNA barcoding. Molecular Ecology Resources 9, 707-712.

Freckman, D.W., Virginia, R.A., 1993. Extraction of nematotes from Dry Valley Antarctic soils. Polar Biology 13, 483-487.

Freckman, D.W., Virginia, R.A., 1997. Low-diversity Antarctic soil nematode communities: Distribution and response to disturbance. Ecology 78, 363-369.

Freckman, D.W., Virginia, R.A., 1998. Soil Biodiversity and Community Structure in the McMurdo Dry Valleys, Antarctica, in Ecosystem Processes in a Polar Desert: The McMurdo Dry Valleys, Antarctica. Antarctic Research Series 72, 323-335.

Gagarin, V., 2009. A revision of the genus *Eutobrilus* Tsalolikhin, 1981 (Nematoda, Triplonchida). Inland Water Biology 2, 205-212.

Hebert, P.D.N., Ratnasingham, S., deWaard, J.R., 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London Series B-Biological Sciences 270, S96-S99.

Heyns, J., 1994. *Chiloplacoides antarcticus* n. gen., n. sp. from western Dronning Maud Land, Antarctica (Nematoda: Cephalobidae). Fundamental and Applied Nematology 17, 333-338.

Hogg, I.D., Hebert, P.D.N., 2004. Biological identification of springtails (Hexapoda : Collembola) from the Canadian Arctic, using mitochondrial DNA barcodes. Canadian Journal of Zoology-Revue Canadienne De Zoologie 82, 749-754.

Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., Bakker, J., Helder, J., 2006. Phylum-Wide Analysis of SSU rDNA Reveals Deep Phylogenetic Relationships among Nematodes and Accelerated Evolution toward Crown Clades. Molecular Biology and Evolution 23, 1792-1800.

Hooper, D.J., 1986. Handling, fixing, staining and mounting nematodes, Southey, J.F. (Editor), Laboratory Methods for Work with Plant and Soil Nematodes. Her Majesty's Stationary Office, London, pp. 34-38.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754-755.

Ivanova, N., Grainger, C., 2006. Pre-made frozen PCR and sequencing plates. CCDB Advances, Methods Release No 4.

Ivanova, N.V., Dewaard, J.R., Hebert, P.D., 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes 6, 998-1002.

Kirjanova, E.S., 1958. Antarctic specimens of freshwater nematodes of the genus *Plectus bastian* (Nematoda, Plectidae). Information Bulletin of the Soviet Antarctic Expedition 3, 101-103.

Kito, K., Ohyama, Y., 2008. Rhabditid nematodes found from a rocky coast contaminated with treated waste water of Casey Station in East Antarctica, with a description of a new species of *Dolichorhabditis* Andrássy, 1983 (Nematoda: Rhabditidae). Zootaxa 1850, 43-52.

Kito, K., Shishida, Y., Ohyama, Y., 1991. *Plectus antarcticus* de Man, 1904 and *P. frigophilus* Kirjanova, 1958 (Nematoda: Plectidae), with emphasis on the male, from the Soya Coast, East Antarctica. Nematologica 37, 252-262.

Kito, K., Shishida, Y., Ohyama, Y., 1996. New species of the genus *Eudorylaimus* Andrassy, 1959 (Nematoda: Qudsianematidae) from East Antarctica. Polar Biology 16, 163-169.

Magalhães, C., Stevens, M.I., Cary, S.C., Ball, B.A., Storey, B.C., Wall, D.H., Türk, R., Ruprecht, U., 2012. At Limits of Life: Multidisciplinary Insights Reveal Environmental Constraints on Biotic Diversity in Continental Antarctica. PLoS One 7, 1-10.

Maslen, N.R., Convey, P., 2006. Nematode diversity and distribution in the southern maritime Antarctic - clues to history? Soil Biology & Biochemistry 38, 3141-3151.

McGaughran, A., Convey, P., Redding, G.P., Stevens, M.I., 2010. Temporal and spatial metabolic rate variation in the Antarctic springtail *Gomphiocephalus hodgsoni*. Journal of Insect Physiology 56, 57-64.

Meldal, B.H.M., Debenham, N.J., De Ley, P., De Ley, I.T., Vanfleteren, J.R., Vierstraete, A.R., Bert, W., Borgonie, G., Moens, T., Tyler, P.A., Austen, M.C., Blaxter, M.L., Rogers, A.D., Lambshead, P.J.D., 2007. An improved molecular phylogeny of the Nematoda with special emphasis on marine taxa. Molecular Phylogenetics and Evolution 42, 622-636.

Nadler, S.A., De Ley, P., Mundo-Ocampo, M., Smythe, A.B., Patricia Stock, S., Bumbarger, D., Adams, B.J., De Ley, I.T., Holovachov, O., Baldwin, J.G., 2006. Phylogeny of Cephalobina (Nematoda): molecular evidence for recurrent evolution of probolae and incongruence with traditional classifications. Molecular Phylogenetics and Evolution 40, 696-711.

Nedelchev, S., Peneva, V., 2000. Description of three new species of the genus *Mesodorylaimus* Andrássy, 1959 (Nematoda: Dorylaimidae) from Livingston Island, Antarctica, with notes on *M. imperator* Loof, 1975. Russian Journal of Nematology 8, 161-172.

Nylander, J., 2004. MrModeltest 2.3.(program distributed by the author) Evolutionary Biology Centre. Uppsala University, Sweden.

Porazinska, D.L., Giblin-Davis, R.M., Faller, L., Farmerie, W., Kanzaki, N., Morris, K., Powers, T.O., Tucker, A.E., Sung, W., Thomas, W.K., 2009. Evaluating high-throughput sequencing as a method for metagenomic analysis of nematode diversity. Molecular Ecology Resources 9, 1439-1450.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817-818.

Powers, L.E., Freckman, D.W., Virginia, R.A., 1995. Spatial distribution of nematodes in polar desert soils of Antarctica. Polar Biology 15, 325-333.

Powers, L.E., Ho, M., Freckman, D.W., Virginia, R.A., 1998. Distribution, Community Structure, and Microhabitats of Soil Invertebrates along an Elevational Gradient in Taylor Valley, Antarctica. Arctic and Alpine Research 30, 133-141.

Powers, T., Harris, T., Higgins, R., Mullin, P., Sutton, L., Powers, K., 2011. MOTUs, Morphology, and Biodiversity Estimation: A Case Study Using Nematodes of the Suborder Criconematina and a Conserved 18S DNA Barcode. Journal of Nematology 43, 35.

Prosser, S., Velarde-Aguilar, M., León-Règagnon, V., Hebert, P., 2013. Advancing nematode barcoding: A primer cocktail for the cytochrome c oxidase subunit I gene from vertebrate parasitic nematodes. Molecular Ecology Resources.

Raymond, M., 2010. Cold-Temperature Adaptation in Nematodes from the Victoria Land Coast, Antarctica. University of Otago, Dunedin, pp. 1-312.

Ristau, K., Steinfartz, S., Traunspurger, W., 2013. First evidence of cryptic species diversity and significant population structure in a widespread freshwater nematode morphospecies (Tobrilus gracilis).

Rounsevell, D.E., Horne, P., 1986. Terrestrial, parasitic and introduced invertebrates of the Vestfold Hills, In: Pickard, J. (Ed.), Antarctic Oasis. Terrestrial environments and history of the Vestfold Hills. Academic Press Australis, Sydney, pp. 309-331.

Ryss, A., Bostrom, S., Sohlenius, B., 2005. Tylenchid nematodes found on the nunatak Basen, East Antarctica, Annales Zoologici. Museum and Institute of Zoology, Polish Academy of Sciences, pp. 315-324.

Sands, C.J., Convey, P., Linse, K., McInnes, S.J., 2008. Assessing meiofaunal variation among individuals utilising morphological and molecular approaches: An example using the Tardigrada. BMC Ecology 8, 1-11.

Shishida, Y., Ohyama, Y., 1986. A note on the terrestrial nematodes around Syowa station, Antarctica (extended abstract). Mere Nat Inst Res, Special Issue, 259-260.

Sinclair, B., 2001. On the distribution of terrestrial invertebrates at Cape Bird, Ross Island, Antarctica. Polar Biology 24, 394-400.

Smythe, A.B., Nadler, S.A., 2006. Molecular phylogeny of *Acrobeloides* and *Cephalobus* (Nematoda: Cephalobidae) reveals paraphyletic taxa and recurrent evolution of simple labial morphology. Nematology 8, 819-836.

Sohlenius, B., Boström, S., 2008. Species diversity and random distribution of microfauna in extremely isolated habitable patches on Antarctic nunataks. Polar Biology 31, 817-825.

Sohlenius, B., Boström, S., Hirschfelder, A., 1995. Nematodes, rotifers and tardigrades from nunataks in Dronning Maud Land, East Antarctica. Polar Biology 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 Biology 16, 191-200.

Stevens, M.I., Hogg, I.D., 2003. Long-term isolation and recent range expansion from glacial refugia revealed for the endemic springtail *Gomphiocephalus hodgsoni* from Victoria Land, Antarctica. Molecular Ecology 12, 2357-2369.

Stevens, M.I., Greenslade, P., Hogg, I.D., Sunnucks, P., 2006. Southern Hemisphere springtails: Could Any Have Survived Glaciation of Antarctica? Molecular Biology and Evolution 23, 874-882.

Stevens, M.I., Hogg, I.D., 2006. Molecular ecology of Antarctic terrestrial and limnetic invertebrates and microbes. Trends in Antarctic Terrestrial and Limnetic Ecosystems.

Stevens, M.I., Porco, D., D'Haese, C.A., Deharveng, L., 2011. Comment on "taxonomy and the DNA barcoding enterprise" by Ebach (2011). Zootaxa 2838, 85-88.

Swofford, D., 2002. PAUP* version 4.0. Phylogenetic Analysis Using Parsimony (and Other Methods).

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28, 2731-2739.

Tavares, E.S., Baker, A.J., 2008. Single mitochondrial gene barcodes reliably identify sister-species in diverse clades of birds. BMC Evolutionary Biology 8, 81.

Timm, R.W., 1971. Antarctic Soil and Freshwater Nematodes from the McMurdo Sound Region. Proceedings of the Helminthological Society of Washington 38, 42-52.

Treonis, A.M., Wall, D.H., Virginia, R.A., 1999. Invertebrate Biodiversity in Antarctic Dry Valley Soils and Sediments. Ecosystems 2, 482-492.

van Megen, H., van den Elsen, S., Holterman, M., Karssen, G., Mooyman, P., Bongers, T., Holovachov, O., Bakker, J., Helder, J., 2009. A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. Nematology 11, 927-950.

Velasco-Castrillón, A., Schultz, M.B., Colombo, F., Gibson, J.A.E., Davies, K.A., Austin, A.D., Stevens, M.I., accepted manuscript. Distribution and diversity of microfauna from East Antarctica: assessing the link between biotic and abiotic factors. PLoS ONE.

Verlecar, X., Dhargalkar, V., Matondkar, S., 1996. Ecobiological studies of the freshwater lakes at Schirmacher Oasis, Antarctica.

Virginia, R.A., Wall, D.H., 1999. How Soils Structure Communities in the Antarctic Dry Valleys. (Cover story). Bioscience 49, 973-983.

Yeates, G.W., 1970. Two Terrestrial Nematodes from the McMurdo Sound Region, Antarctica, with a Note on *Anaplectus arenicola* Killick, 1964. Journal of Helminthology 44, 27-34.

Yeates, G.W., 1979. Terrestrial nematodes from the Bunger Hills and Gaussberg, Antarctica. New Zealand Journal of Zoology 6, 641-643.

CHAPTER IV

Mitochondrial DNA reveals hidden diversity for tardigrades and rotifers from across the Antarctic realm

Alejandro Velasco-Castrillón[1*], Chester J. Sands[2], Sandra McInnes[2], Mark B. Schultz[3], María Arróniz-Crespo[4], Cyrille D'Haese[5], John A. E. Gibson[6], Byron Adams[7], Tim Page[8], Andrew D. Austin[1], Steven J. B. Cooper[1,9] and Mark I. Stevens[1,9]

[1] *Australian Centre for Evolutionary Biology and Biodiversity, School of Earth and Environmental Sciences, The University of Adelaide, South Australia, SA 5005, Australia*

[2] *British Antarctic Survey, Madingley Road, Cambridge, Cambridgeshire CB3 0ET, United Kingdom*

[3] *Department of Genetics, Bio21 Institute, The University of Melbourne, Parkville, Victoria, 3010, Australia*

[4] *Facultad de Farmacia, Departamento de Biologı´a Vegetal II, Universidad Complutense de Madrid, 28040, Madrid, Spain*

[5] *UMR 7205 CNRS, Origine, Structure et Evolution de la Biodiversite, Departement Systematique et Evolution,Museum national d'Histoire naturelle, CP50 – Entomologie, 75231 Paris cedex 05, France*

[6] *Institute of Marine and Antarctic Studies, University of Tasmania, Private Bag 129, Hobart, Tasmania 7001, Australia*

[7] *The College of Life Sciences, Brigham Young University, Provo Utah 84602, USA*

[8] *Australian Rivers Institute, Nathan campus, Griffith University, 170 Kessels Road QLD 4111, Australia.*

[9] *South Australian Museum, GPO Box 234, Adelaide, South Australia 5000, Australia*

[*] Corresponding author: Alejandro Velasco-Castrillón. E-mail: macalusio@yahoo.com

This chapter has been formatted in a style appropriate for the journal *Biodiversity*

Statement of authorship

Title of Chapter	Mitochondrial DNA reveals hidden diversity for tardigrades and rotifers from across the Antarctic realm
Publication Status	Accepted and submitted (see below)
Publication Status	Rotifer section of this chapter has been accepted to the journal *Biodiversity*. Tardigrade section has been submitted to the journal *Invertebrate Systematics* (both as research articles) Velasco-Castrillón A., Page T. J., Gibson J. A. E., and Stevens M. I. Surprisingly high levels of biodiversity and endemism amongst Antarctic rotifers uncovered with mitochondrial DNA. *Biodiversity* Velasco-Castrillón A., Sands C.J., McInnes S. J., Schultz M. B., Arróniz-Crespo M., D'Haese C. A., Gibson J. A. E., Adams, B. J., Page T. J., Austin A. D.,Cooper S. J. B., and Stevens M. I. Mitochondrial DNA reveals hidden diversity for tardigrades from across the Antarctic realm. *Invertebrate Systematics*

Author Contributions

By signing the Statement of authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Alejandro Velasco Castrillón		
Contribution to the Paper	Conceived and design the experiment, collected and sorted soil samples, conducted rotifer and tardigrade extraction and identification, performed statistical analyses and interpreted data, produced figures and tables, wrote the manuscript.		
Signature		Date	11/11/2013

Name of Co-Author	Chester J Sands		
Contribution to the Paper	Provided literature, supplied soil samples and some tardigrade sequences, assisted in phylogenetical analysis, critically revised manuscript.		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	14/10/13

Name of Co-Author	Sandra J McInnes		
Contribution to the Paper	Provided a thorough and critical revision of the manuscript, assisted in the production of tardigrade species list, provided sequences and identifications.		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	11/10/2013

Name of Co-Author	Mark B Schultz		
Contribution to the Paper	Collected soil samples, revised manuscript.		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	03/10/2013

Name of Co-Author	María Arróniz-Crespo		
Contribution to the Paper	Provided soil samples, assisted in in micro-invertebrates sorting, critically revised manuscript.		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	10/10/2013

Name of Co-Author	Cyrille D'Haese		
Contribution to the Paper	Provided soil samples		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	08/10/2013

Name of Co-Author	John A E Gibson		
Contribution to the Paper	Provided literature, taxonomic expertise, critically revised manuscript.		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	09/10/2013

Name of Co-Author	Byron Adams		
Contribution to the Paper	Provided some tardigrade sequences, critically revised manuscript.		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	19/10/2013

Name of Co-Author	Timothy J Page		
Contribution to the Paper	Provided some rotifer sequences, critically revised manuscript.		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	02/10/2013

Name of Co-Author	Steve J B Cooper		
Contribution to the Paper	Assisted in phylogentic analyses, critically revised manuscript.		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	1/11/2013

Name of Co-Author	Andrew D Austin		
Contribution to the Paper	Revised manuscript.		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	29/10/13

Name of Co-Author	Mark I Stevens		
Contribution to the Paper	Supervised the project, sought and won funding, assisted in data analyses, provided constant intellectual feedback, critically revised manuscript.		
Consent from Co-Author	I give consent for Alejandro Velasco Castrillón (candidate) to include this manuscript for examination towards the degree of Doctor of Philosophy.		
Signature		Date	09/10/2013

Preamble

Chapter IV follows a similar structure as Chapter III, but deals with the current state of knowledge on bdelloid rotifers and tardigrade diversity across Antarctica. In addition to the diversity tables presented in Chapter I we have now incorporated rotifer and tardigrade new haplotypes (identified to different taxonomic levels) and distribution records across several sectors from continental and maritime Antarctica. We have also performed phylogenetic analyses using our sequences and sequences available in the public domain. The content of this chapter has been divided in two manuscripts (rotifer and tardigrade). The rotifer manuscript has been accepted for publication in the Journal *Biodiversity*. The tardigrade manuscript has been submitted to the journal *Invertebrate Systematics*.

Abstract

Antarctica is one of the most inhospitable habitats on the planet, with challenging environmental conditions due to its freezing temperatures, prolonged winters and lack of liquid water. Whereas most of the continent (99.7%) is permanently covered by ice and snow, some coastal areas and mountain ridges have remained ice-free and maintain life. Some of the more dominant microfaunal organisms in soil and limno-terrestrial habitats are tardigrades and bdelloid rotifers, but despite their presence little is known of their diversity and distribution across the frozen continent. Here we identify and analyse mitochondrial *COI* sequences from microfauna across Antarctica and compare them with sequences from Tierra del Fuego (TF) and other worldwide locations. From 420 Antarctic tardigrade sequences we identified 85 unique haplotypes for *Echiniscus*, *Milnesium*, *Macrobiotus*, *Diphascon*, *Acutuncus antarcticus*, and unidentified Parachela. For the Antarctic bdelloid rotifers we generated 514 sequences and identified 119 unique haplotypes for *Philodina*, *Adineta* and unidentified bdelloids. The highest sequence divergence within any genus was observed for the tardigrade *Echiniscus* (31%), and for the rotifer *Adineta* (18%). The closest sequence similarity observed from our Antarctic micro-invertebrates and those North of 60°S was 5.3% for bdelloid sequences from Antarctica and TF. In general, higher widespread ranges were observed for bdelloids, while tardigrades were mostly short-range endemics. Our extensive coverage across Antarctica reveals separate haplotypes that may represent potential species exceeding what is currently known from morphology even when conservative methods are employed for species delimitation.

Introduction

Antarctic bdelloid rotifers and tardigrades are a major component of the soil, and one of the most dominant groups of metazoan found in the harsh polar environment (Sohlenius et al. 1996; Sohlenius and Boström 2008; Convey et al. 2008). Despite their dominance in the frozen continent, little is known about their diversity and origin. It has been suggested that Antarctic terrestrial fauna could have survived glaciation in ice-free areas and may be a remnant of the Gondwanan super-continent (Stevens et al. 2006). However, such studies

that explore extinction and recolonisation versus persistence in refugia are lacking for the majority of the Antarctic taxa (e.g. Stevens and Hogg 2006; Convey et al. 2009). When considering the patchy distribution reported for Antarctic limno-terrestrial rotifers and tardigrades (e.g. Sohlenius and Boström 2005; Adams et al. 2006) research to date has failed to provide clear species identifications or boundaries resulting in poor resolution of geographical knowledge for the distribution of taxa.

It has been discussed in the literature that the taxonomic resolution for most microfaunal groups constitutes an ongoing dilemma leading in many cases to misclassification and underestimation of the diversity (Adams et al. 2006; Fontaneto et al. 2009; see also Stevens et al. 2011). Comprehensive studies redescribing and describing new species from the continent are lacking; currently only a few studies have discussed tardigrades and bdelloid rotifers from Antarctica (e.g. Dastych 1984; Miller et al. 1994; Miller and Heatwole 1995; Dartnall 2000; De Smet and Gibson 2008; Pilato and Binda 2010). Additionally, well documented and descriptive morphological work across a species' distributional range is rare. Given the low number of specialists in the field the addition of molecular tools can assist in discerning among cryptic species in addition to determining accurate distribution of species. For example, the bdelloid rotifers' and tardigrades' conserved morphology and minute size has clearly limited establishing accurate understanding of levels of diversity and actual species distributions (e.g. Sands et al. 2008a; Fontaneto et al. 2009; Czechowski et al. 2012).

Records of tardigrade and bdelloid species based on morphological taxonomy have been subjected to constant change. Recent studies incorporating molecular data have allowed scrutiny of species hypotheses based on phenotypic or geographical similarities and have revealed complexes of cryptic species (Salomone et al. 2007; Kaya et al. 2009). The most notorious case is represented by the cosmopolitan bdelloids *Macrotrachela quadricornifera*, *Rotaria rotatoria*, and *Adineta vaga*, which have been shown to include numerous lineages (21, 34, and 36, respectively) (Fontaneto et al. 2009, Fontaneto et al. 2011). A similar case was observed for the tardigrade *Macrobiotus macrocalix* (from Europe and Asia) which is now known to represent a complex of lineages (Bertolani et al. 2011). For the case of the tardigrade *Milnesium tardigradum*, known to be a cosmopolitan

species with a pan-Antarctic distribution, it is now recognised to comprise at least two species, including *Milnesium antarcticum* (Tumanov 2006; Smykla et al. 2012).

Micro-invertebrate diversity across Antarctica is not homogenously distributed, due to the majority of research being conducted near research stations (Andrássy and Gibson 2007). Such biases create clear problems for understanding the patterns of species distributions and the biogeography of Antarctica. For example Graham sector and sub-Antarctic islands contain the greatest apparent tardigrade diversity but are also the most studied geographic regions (Convey and McInnes 2005). Currently, there have been reported 63 tardigrade species, forty-one of them for continental Antarctica, and 19 species shared between continental and maritime Antarctica (Table 1). The bdelloid species list comprises 36 species, 31 of which occur outside Antarctica (Table 2). An important aspect to understand distribution ranges in Antarctica is to recognise the presence of those species beyond Antarctica. Long-range dispersal as a result of ocean currents, air currents, and transport by more mobile animals has often been proposed as a mechanism explaining the presence of microfauna in Antarctica (Marshall and Pugh 1996). However, McInnes and Pugh (1998) questioned the survival capability of microfauna exposed to long-range dispersal even during the anhydrobiotic phase. It is therefore possible that the presence of 'alien species' in Antarctica could be the result of misidentification (McInnes 1995a).

Molecular studies have developed in recent years as a significant way to complement morphological work in assessing biodiversity, species identification, and descriptions (Sands et al. 2008a; Stevens et al. 2011). Ribosomal RNA genes (e.g. 18S) have been used in the species delimitation process of tardigrades and rotifers, but lack resolution to resolve closely related species (e.g. Sands et al. 2008b; Robeson et al. 2009; Guidetti et al. 2009). Here we used a 710 bp fragment of the mitochondrial gene, cytochrome *c* oxidase subunit I (*COI*), that has demonstrated good resolution to discern among closely related species (Hebert et al. 2003; Costa et al. 2007; Fontaneto et al. 2008). This gene is also one of the most regularly used markers in phylogeography and it has been implemented as the 'barcoding gene' for metazoans (Simon et al. 1994; Sunnucks 2000; Stevens and Hogg 2003; Ashton et al. 2008). More rencently, it has also been used for species delimitation of tardigrades and rotifers (eg. Guidetti 2009; Czechowski et al. 2012; Fontaneto et al. 2009, 2011).

In order to ascribe diversity to Antarctic geographical areas, and only for practical reasons, the continent was divided into 60 degree longitude sectors (see Pugh 1993); with continental Antarctica including Maud, Enderby, Wilkes, Scott, Byrd and Ronne; and maritime Antarctica including the Antarctic Peninsula (Palmer and Graham sectors), South Orkney Islands and South Shetland Islands (see Fig. 1). We have also included the sub-Antarctic islands of South Georgia (SG), Kerguelen Island (KI) and Marion Island (MI). We sequenced tardigrades and bdelloid rotifers from soil samples collected in Maud, Enderby, Wilkes, Scott, and Graham sectors and TF, and compared these with *COI* sequences from across the world (Fig. 2; Table 3). Specimens were also collected from water samples in the Enderby sector, from Stornes Peninsula to Vestfold Hills (76ºE – 78ºE). Using the *COI* gene we aim to: (1) investigate the diversity and distribution of tardigrades and bdelloid rotifers from across the Antarctica, and (2) assess whether species lineages (identified based on mitochondrial DNA) are endemic to the Antarctic continent or have widespread distributions outside Antarctica.

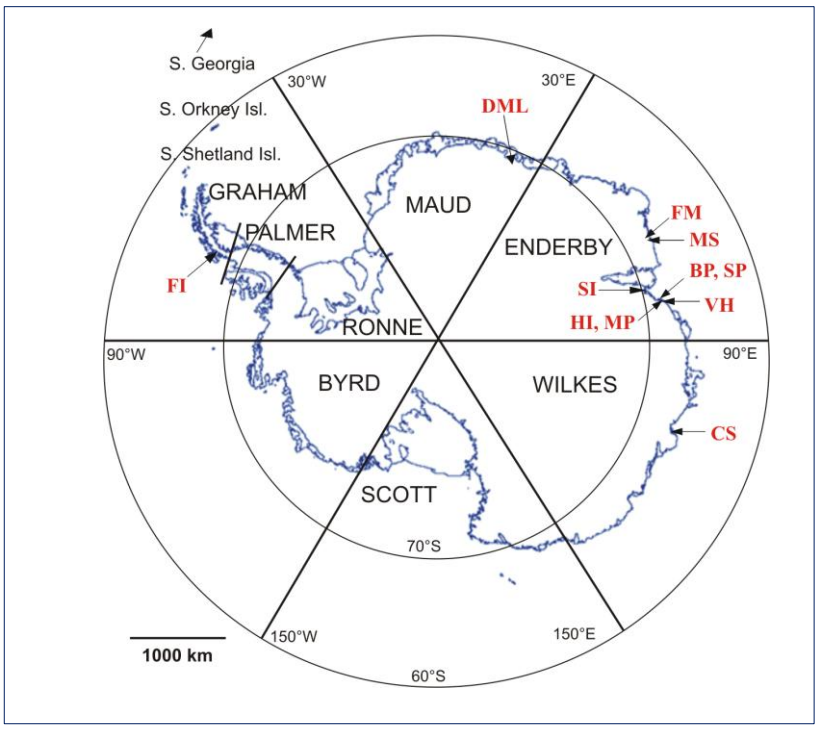


Figure 1. Map of Antarctica showing eight sectors (see Pugh, 1993), South Shetland Islands, South Orkney Islands, South Georgia, and the eleven regions where sampling took place (Dronning Maud Land 'DML', Framnes Mountains 'FM', Mawson Station 'MS', Sansom Island 'SI', Broknes Peninsula 'BP', Stornes Peninsula 'SP', Hop Island 'HI', Mather Peninsula 'MP', Vestfold Hills 'VH', Casey Station 'CS' and Francis Island 'FI').

Table 1 Geographical distribution of tardigrade species recorded in Antarctica, including type localities and species records outside Antarctica

	Continental Antarctica												Maritime Antarctica			sub Ant	Not - Antarctica		
	Maud	Enderby					Wilkes		Scott			Ronne	AP						
Tardigrade species	DML	EnL	MS-FM	PCM	VH-LH	Ga	BH	CS-wm	RI	VL	QM	EIL	PS	GS	SS-SO	SG	TF	SA	oAnt
Order Echiniscoidea																			
Echiniscus spp (lineages for this study)					2L			1L				1L			1L	3L			
Echiniscus corrugicaudatus McInnes, 2010												28							
Echiniscus jenningsi Dastych, 1984			**15**	e1										3, 10	3, **9,29**				
Echiniscus kerguelensis Richters, 1904		[27,18]													[27]				14
Echiniscus macronyx Richters, 1907																3			
Echiniscus pseudowendti Dastych, 1984	24	**9**											e1						
Echiniscus punctus (McInnes, 1995)															3,29				
Mopsechiniscus imberbis (Richters, 1908)																3, 5		[5]	[5]
Oreella mollis J. Murray, 1910															3	3			14,13,18
Pseudechiniscus cf. *suillus* (Ehrenberg, 1853)		**9**			**16**			10,17							3, **9,29**				9,16
Pseudoechiniscus novaezeelandiae Richters, 1903					21,14,16													14	14,**18**
Testechiniscus meridionalis Murray, 1906															3, **9,29**				
Order Parachela																			
Acutuncus antarcticus (Binda & Pilato, 2000)	23,24,25	27, **18**	15	12	21,12,**16**	**4**		10,17	1	1,22	2L			3	27,3,**9**	3			9,14
Amphibolus volubilus Durante Pasa & Maucci, 1975															[27]				
Calohypsibius cf. *ornatus* (Richters, 1900)																3			e2
Dactylobiotus cf. *ambiguus* (Murray, 1907)														12	3, **9,29**	3			
Diphascon spp (lineages for this study)	1L?		1L?		2L?						1L		1L			3L	2L?		
Diphascon ongulensis Morikawa, 1962		27																	
Diphascon (Adropion) greveni Dastych, 1984														3	3, **9,29**				
Diphascon (Adropion) maucci Dastych & McInnes, 1996															3				
Diphascon (Adropion) tricuspidatum Binda & Pilato, 2000										1, 2									
Diphascon (Diphascon) alpinum J. Murray, 1906															[**27**]				14
Diphascon (Diphascon) dastychi Pilato & Binda, 1999										1,**19**									
Diphascon (Diphascon) higginsi Binda, 1971															[**27**]				
Diphascon (Diphascon) langhovdense Sudzuki, 1964	23,24	**9,18,6**			16			10,17							3, **9**			14	9, 14
Diphascon (Diphascon) mirabilis Dastych, 1984															3, **9**	3			
Diphascon (Diphascon) pingue ('Vaierty A') Marcus, 1936									17						3, 10	3,**9**,19	3,**9**,10,19		14,19,13
Diphascon (Diphascon) pingue ('Vaierty B') Marcus, 1936		19													10	9	10		
Diphascon (Diphascon) polare Pilato & Binda, 1999										1,**19**									
Diphascon (Diphascon) scoticus Murray, 1905									[1]						[e3]				
Diphascon (Diphascon) victoriae Pilato & Binda, 1999										1,**19**									
Diphascon (Diphascon?) puniceum Jennings, 1971	e1				14?										3, **9**,14,**29**				14,13
Diphascon sanae Dastych, Ryan & Watkins, 1990	**11**		**15**	e1								3	3						
Hebesuncus ryani Dastych & Harris, 1994	23,25											3	3?						
Hebesuncus schusteri Dastych, 1984	24	27,**9**												3	3, **9**				e2
Hebesuncus mollispinus Pilato, McInnes & Lisi, 2012													20	20		20			
Hexapodibius boothi Dastych & McInnes, 1994															27,3,**8**				
Hypsibius allisoni Horning, Schuster & Grigarick, 1978					14														14

Table 1 (continued)

Tardigrade species	DML	EnL	MS-FM	PCM	VH-LH	Ga	BH	CS-wm	RI	VL	QM	ElL	PS	GS	SS-SO	SG	TF	SA	oAnt
Hypsibius cf. *convergens* (Urbanowicz, 1925)									[1]							[3]		14	14,13
Hypsibius cf. *dujardini* (Doyère, 1840)														3	3, **9,29**	3,**9**		14	9,14
Hypsibius cf. *mertoni simoizumii* (Sudzuki, 1964)							e1		1										
Hypsibius pallidus Thulin, 1911																3, **9**			e2
Isohypsibius asper J. Murray, 1906													e1		**27**,3,**9,29**	3			9?,14
Isohypsibius improvisus Dastych, 1984		**9**													**9**				
Isohypsibius laevis (McInnes, 1995)															3,**29**				
Isohypsibius papillifer J. Murray, 1905															3,**27,29**	3			14
Isohypsibius prosostomus Thulin, 1928																3, **9**			e2
Isohypsibius saracenus Pilato, 1973		[27]																	
Macrobiotus spp (lineages for this study)			1L							3L	3L								
Macrobiotus blocki Dastych, 1984	23, 24	**9**	**15**	12															
Macrobiotus cf. *hufelandi* (Schultze, 1833)	23						e1							3, 10		3, **9**			9,13
Macrobiotus cf. *liviae* (Ramazzotti, 1962)																3, **9**			
Macrobiotus cf. *polaris* (Murray, 1910)									1,12	1									
Macrobiotus harmsworthi coronatus(Utsugi, 1991)		27,**7**																	
Macrobiotus harmsworthi (Barros, 1942)	e1														[27]				18
Macrobiotus krynauwi Dastych & Harris, 1995	23,24,25,**7**				14		4							3,10,12	3,**9,29**	3, **9**		14	9,14
Macrobiotus meridionalis Richters, 1909										22									
Macrobiotus montanus J. Murray, 1910		[27]																	18,e2
Macrobiotus mottai Binda & Pilato, 1994										1									
Macrobiotus polaris Dougherty & Harris, 1963										1									13
Macrobiotus weinerorum Dastych, 1984		**9**		12	**16**														
Minibiotus stuckenbergi Dastych, Ryan & Watkins, 1990	3, **11**		**15**	e1															
Minibiotus vinciguerrae Binda & Pilato, 1992										1									
Ramajendas frigidus Pilato & Binda, 1990								17		1									
Ramajendas renaudi Ramazzotti, 1972														3, **9**	3				
Ramazzottius cf. *oberhäuseri* (Doyère, 1840)	e1								1	1			e1	3		3		14	9,14,13
Order Apochela																			
Milnesium antarcticum Tumanov, 2006										22					**26**				
Milnesium cf. *tardigradum (*Doyère, 1840)	23,24	**9, 18**	15	e1	16						NR	3	3	3	3, **9**	3			3,9,14,13,18

Numbers indicate the literature source (those in bold indicate detailed described species). Boxes in grey indicate records from the present study. Numbers in grey boxes followed by 'L' indicate the number of Lineages found for this study (see Fig. 2). New record is shown by 'NR'. Numbers followed by '?' indicate uncertainty for the record. Boxes with black borders indicate type locality. Records in '[ ]' are probably misidentification. Under the heading 'Endemic' the acronyms 'C-M' indicate presence for Continental and Maritime Antarctica, and 's-A' indicate presence for sub-Antarctica. Other acronyms: Dronning Maud Land (DML), Enderby Land (EnL), Mawson Station - Framnes Mtns (MS-FM), Prince Charles Mtns (PCM), area from Vestfold Hills to Larsemann Hills (VH-LH), Gaussberg (Ga), Bunger Hills (BH), Casey Station and Windmill islands (CS-wm), Ross Island (RI), Victoria Land (VL), Queen Maud Mtns (QM), Ellsworth Land (ElL), Antarctic Peninsula (AP), Palmer sector (PS), Graham sector (GS), South Shetland and South Orkney Islands (SS-SO), sub-Antarctica (sub Ant), South Georgia (SG), Tierra del Fuego (TF), South America (SA), outside Antarctica (oAnt). Literature source: (1) Adams et al. 2006, (2) Binda and Pilato 2000, (3) Convey and McInnes 2005, (4) Dastych 1991, (5) Dastych 1999, (6) Dastych 2003, (7) Dastych and Harris 1995, (8) Dastych and McInnes 1994, (9) Dastych 1984, (10) Dastych 1989, (11) Dastych et al. 1990, (12) Gibson et al. 2007, (13) Guil and Giribet 2012, (14) Miller et al. 1988, (15) Miller and Heatwole 1995, (16) Miller et al. 1994, (17) Miller et al. 1996, (18) Murray 1910, (19) Pilato and Binda 1999, (20) Pilato et al. 2012, (21) Rounsvell and Horne 1986, (22) Smikla et al. 2012, (23) Sohlenius and Boström 2005, (24) Sohlenius et al. 1995, (25) Sohlenius *et al.* 2004, (26) Tumanov 2006, (27) Utsugi and Ohyama 1993, (28) Utsugi and Ohyama 1991, (29) McInnes 1995b, (30) McInnes 2010, (e1) Australian Antarctic Data Centre (https://data.aad.gov.au/aadc), (e2) Fauna Europea (http://www.faunaeur.org), (e3) Smithsonian National Museum of Natural History (http://invertebrates.si.edu).

Table 2. Geographical distribution of bdelloid species recorded in Antarctica, including type localities and species records outside Antarctica

Bdelloid rotifers	Ant (not specified)	Continental Antarctica								AP	mA	sub Ant	outside Ant
		Maud sector	Enderby sector		Wilkes sector			Scott sector					
		DML	MS-FM	VH-LH	Haswell	BH	CS	RI	VL		Signy Isl	South Georgia	
Adineta spp (lineages for this study)			3L	6L			3L						
Adineta barbata Janson, 1893	16	18, 19,e1		3				14,9,6		e1	6,e1	5,e1	11,16
Adineta gracilis Janson, 1893	16	18, 19,e1	1	1L	1	e1	1L	14,1,**6**		e1	10,1,6,e1		10,11,16
Adineta grandis Murray, 1910	16	e1		3,2,e1	15,1	12,e1	4	**14**,**6**,9	13,17,e1	e1	10,1,6,e1		16
Adineta longicornis Murray, 1906	16							14		e1			16
Adineta steineri Bartoš, 1951	16	18,19,e1											11,16
Adineta vaga vaga (Davis, 1873)*	16	18, 19,e1						14,6	23	e1			10, 11, 16
Habrotrocha angularis Murray, 1910	16							**14**,**6**,9		e1			20
Habrotrocha angusticollis angusticollis Murray, 1905	16												16
Habrotrocha constricta Dujardin, 1841	16	18, 19,e1		3,2,e1		e1	4	8,**6**,9	17, 23	e1	6,e1	5	11,16
Habrotrocha crenata crenata Murray, 1905	16												16
Habrotrocha elusa elusa Milne, 1916*	16	18, 19,e1				e1							16
Habrotrocha gulosa Milne, 1916						20,e1							16
Habrotrocha pulchra (Murray, 1905)	16												16,e2
Habrotrocha tridens Milne, 1886	16	18, 19,e1								e1			16,e2
Macrotrachela ambigua Donner, 1965		18,e1											16,e2
Macrotrachela concinna Bryce, 1912	16										6,e1		16,e2
Macrotrachela constricta Milne, 1886								14		e1			
Macrotrachela habita Bryce, 1894	16	18, 19,e1						14,6,9		e1			11,16,e2
Macrotrachela insolita De Koning, 1947	16	18, 19,e1				20,e1		8,6	17	e1			16
Macrotrachela kallosoma Schulte, 1954	16												16,e2
Macrotrachela libera Donner, 1949		18, 19,e1											16,e2
Macrotrachela musculosa Milne, 1886	16												16,e2
Macrotrachela cf. *ligulata* Haigh, 1965		18,e1											16
Macrotrachela nixa Donner, 1962	16	18,e1				20,e1							16,e2
Macrotrachela quadricornifera quadricornifera Milne, 1886*	16			3,e1									11,16
Macrotrachela timida Milne, 1916		18, 19,e1											16,e2
Mniobia burgeri Bartoš, 1951	16												16
Mniobia russeola (Zelinka, 1891)	16			3,e1									11,16
Mniobia symbiotica (Zelinka, 1886)		18, 19,e1											16,e2
Otostephanos torquatus (Bryce, 1913)		18, e1											
Philodina sp (lineages for this study)			1L	1L			1L						
Philodina alata Murrayi, 1910	16			13		12,13,7,e1		**14**,13,6,9	22,23,e1	e1			
Philodina antarctica Murrayi, 1910	16							**14**,6,9	23,e1	e1			
Philodina cf. *gregaria* (lineages for this study)			1L	1L					1L				
Philodina gregaria Murrayi, 1910	16	e1		3,2,e1	15,1	12,1,e1	4	8,**14**,1,**6**	13,22,23,e1	6,e1	1,6,e1		
Philodina jeanneli de Beauchamp, 1940	16												e1
Philodina plena (Bryce, 1894)	16												16,e2
Rotaria rotatoria Pallas, 1766									17			5,e1	10,11,16

Table 2 (previous page). Numbers indicate the literature source (those in bold indicate species descriptions). Boxes in grey indicate records from the present study. Numbers in grey boxes followed by 'L' indicate the number of Lineages found for this study (refer to Fig. 2). Boxes with black borders indicate type locality. * indicates species recorded as subspecies by Segers (2007). List of acronyms: Antarctica (Ant), Dronning Maud Land (DML), Mawson Station - Framnes Mtns (MS-FM), area from Vestfold Hills to Larsemann Hills (VH-LH), Bunger Hills (BH), Casey Station (CS), Ross Island (RI), Victoria Land (VL), Antarctic Peninsula (AP), maritime Antarctica (mA), sub Antarctica (sub Ant). Literature source: (1) Dartnall 1983, (2) Dartnall 1995, (3) Dartnall 2000, (4) Dartnall 2005a, (5) Dartnall 2005b, (6) Dartnall & Hollowday 1985, (7) De Smet & Gibson 2008, (8) Donner 1972, (9) Dougherty & Harris 1963, (10) Fontaneto et al. 2008, (11) Fontaneto et al. 2007, (12) Gibson 2000 (unpublished), (13) Hansson et al. 2012, (14) Murray 1910, (15) Opalinski 1972, (16) Segers 2007, (17) Smykla et al. 2010, (18) Sohlenius & Boström 2005, (19) Sohlenius et al. 1995, (20) Sudzuki 1979, (21) Suren 1990, (22) Vincent & James 1996, (23) Webster-Brown et al. 2010, Australian Antarctic Data Centre (https://data.aad.gov.au) (e1), and Fauna Europea (http://www.faunaeur.org/) (e2).

Methods

Sampling areas

Sampling across Antarctica occurred during the summers of 2007 to 2010, covering Maud, Enderby and Wilkes sectors, and Francis Island (FI) off the east coast of Graham Land, Antarctic Peninsula (Fig. 1). Soil sampling in Wilkes and Enderby sectors was conducted from December 2009 to March 2010 covering a distance of ~2000 km from the Australian base Casey Station (CS) on Bailey Peninsula (66.28º S – 110.54º E) to Framnes Mountains (FM) (67.77º S – 62.82º E). Tardigrades and Rotifers were extracted from a total of 96 soil sample sites from CS, Vestfold Hills (VH), Hop Island (HI), Mather Peninsula (MP), Sansom Island (SI), the Australian base Mawson Station (MS), FM, and the Broknes (BP) and Stornes Peninsulas (SP), both in the Larsemann Hills (Table 3). Separate field parties collected four soil samples at FI during the summer of 2007-2008; and eigth soil samples from Tanngarden and Brattnipane in Dronning Maud Land (DML) in February 2009 (see Table 3). Net-water samples from lakes and tarns were included as part of the sample collection; we collected from 41 water bodies in the Enderby sector; ranging in size from small tarns 3 m in diameter to large lakes over 450 m in diameter. To increase the geographic area of the samples, we include tardigrades and rotifers from eight soil samples collected from recently deglaciated areas of Tierra del Fuego (TF) (54.4º S – 69º W). Our data was complemented with unpublished sequences of tardigrades from previous collections throughout Antarctica and maritime Antarctic islands (sequences provided by

co-authors). Tardigrades were collected (and sequenced) from Marion Island, King George Island, South Georgia (SG), Signy Island (SgI), Ellsworth Mountains (EM), Queen Maud Mountains (QM), Hidden Valley (HV), and Ross Island (RI) in Victoria Land. In the case of rotifers, specimens from cyanobacterial mats from Ross Island lakes were also included in the analysis.

Table 3. Regions sampled from Antarctica (Wilkes, Enderby, Maud, and Palmer sectors) and Tierra del Fuego showing samples collected and samples that produced positive tardigrade COI sequences.

Sector	Region	Coordinates		Sampling	Elevation	Samples extracted	Samples with positive PCR	
		Latitude	Longitude	area/transect	(m)		Tardigrade	Rotifer
Wilkes	Casey Station (CS)	66.28ºS	110.52º-110.54ºE	1.5 km^2	4 - 44	13s	11s	13s
Enderby	Vestfold Hills (VH)	68.48º-68.60ºS	77.87º- 78.51ºE	340 km^2	4 - 66	20s, 7w	5s, 3w	17s, 2w
Enderby	Broknes Peninsula (BP)	69.38º- 69.4ºS	76.32º- 76.40ºE	7 km^2	0 - 69	13s, 17w	5s, 10w	12s, 8w
Enderby	Stornes Peninsula (SP)	69.36º- 69.43ºS	75.99º- 76.14ºE	6 km^2	4 - 59	19s, 15w	10s, 14w	19s, 5w
Enderby	Hop Island (HI)	68.82º- 68.83ºS	77.68º- 77.73ºE	4 km^2	10 - 36	9s, 3w	5s	6s
Enderby	Mather Peninsula (MP)	68.85º- 68.86ºS	77.93º- 77.94ºE	1 km^2	44 - 80	7s	1s	7s
Enderby	Sansom Island (SI)	69.71ºS	73.75º E	400 m^2	15 - 20	3s	3s	3s
Enderby	Mawson station (MS)	67.60ºS	62.86º- 62.87ºE	0.48 km^2	4 - 24	6s	5s	6s
Enderby	Framnes Mountains (FM)	67.77º- 67.78ºS	62.79º- 62.82ºE	3 km^2	460 - 490	5s	0	5s
Maud	Dronning Maud Land (DML)	71.97º-72.10ºS	23.83º - 23.47ºE	100 km^2	1317-1389	8s	7s	3s
Palmer	Francis Island (FI)	69.60º- 69.66ºS	64.37º- 64.86ºW	27 km	114- 405	4s	1s	2s
South America	Tierra del Fuego (TF)	54.41º - 54.46ºS	69.23º- 69.35ºW	18 km	10 - 220	8s	6s	7s

Numbers under 'Samples extracted' and 'Samples with positive PCR' followed by 's' indicate soil samples; numbers followed by 'w' indicate water samples

Sampling Methodology

The top 0 – 10 cm of soil was excavated, as previous studies have shown that the majority of invertebrates inhabit this layer (Powers et al. 1995). Each soil sample (0.5 – 1.0 kg) was excavated using a metal trowel (cleaned after each use in order to avoid cross contamination between sites), and transferred to sterile Whirl-pak® bags (1.24 l) that were stored at -20 °C to -80°C. Samples were collected at diverse elevations (from 0 m to 1389 m asl; Table 3), ranging in vegetation content, soil particle size and soil geochemical properties (after Velasco-Castrillón et al. 2014). The various types of habitats and geographic regions were considered to capture the widest biodiversity in the terrestrial environment. Different size water bodies from the Enderby sector were sampled employing a 30 cm diameter frame with a mesh bag (35 µm size) and a removable 50 ml

tube (at the bottom of the bag) attached to a 5 m line. The net was thrown to the water and recovered quickly to preclude it from sinking. Rotifers and Tardigrades recovered from the net were stored in 50 ml tubes filled with water from the sampled lake. Water and soil samples were subsequently analysed for electric conductivity (EC) (used as a proxy for salinity; *viz.* Magalhães et al. 2012), pH, and moisture content (in soils)

Rotifer and tardigrade sorting and identification

Rotifers and tardigrades from soil were extracted using an adapted version of a sugar centrifugation protocol (Freckman and Virginia 1993). Extractions were carried out on 50-100g soil samples (wet weight), following methodology described in Velasco-Castrillón et al. (2014). Animals captured in a 38 µm mesh size were placed into a petri dish and examined under a stereo Microscope (Olympus SZ-PT, Japan). Selected rotifer and tardigrade specimens were divided into glass blocks using modified gel-tips. Representatives of each apparent morpho-type were transferred with an Irvin loop into a water droplet on a slide positioned under a digital microscope (Celestron- LCD Digital Microscope, USA). Digital images were taken at magnifications from 40X to 100X, and then the animal was transferred to a unique well in a 96-well microplate. Unused tardigrades and rotifers were put back into tubes and stored at -20 °C. Morphological identification (with the help of experts) was carried out at different levels depending on the taxa considered. For tardigrades, the specimens identified correspond to: *Echiniscus* spp., *E. jenningsi*, (Order Echiniscoides), *Milnesium* spp. (Order Apochela), *Diphascon* spp., *D. puneceum*, *Macrobiotus* spp. and *Acutuncus antarcticus* (the latter three genera belonging to the Order Parachela). For rotifers, given the difficulty in distinguishing between cryptic rotifer morpho-types and specimens that have contracted into a tight ball, the identification was only possible for live-mobile *Philodina* sp., *P.* cf. *gregaria*, *Adineta* spp. and *A. gracilis* (both genera from the class Bdelloidea), the remaining bdelloids were left as unidentified. *Adineta* specimens lack the wheel organ typical of other bdelloids and have a 2/2 dental formula (see Dartnall 2000). The wheel organ-bearer *Philodina* sp. comprised specimens smaller than *P.* cf. *gregaria* with a large pair of ciliary discs.

DNA sequencing

Representatives of the morpho-types were selected for sequencing and placed in a 96-well microplate in 12 µl of 100% ethanol. Mitochondrial DNA extraction and sequencing were undertaken at the Biodiversity Institute of Ontario, University of Guelph, using the laboratory protocols from Ivanova et al. (2006), and Ivanova and Grainger (2006). Total DNA was extracted from the whole specimen and amplified with different sets of primers according to phylum and success rate. The Folmer primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG -3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'; Folmer et al.1994) amplified up to 658 bp fragment of the mitochondrial *COI* gene. For some specimens, these primers (LCO1490 + HCO2198) were tailed with standard flanking sequences M13F (5'-TGTAAAACGACGGCCAGT-3') and M13R (5'-CAGGAAACAGCTATGAC-3'; Messing 1993) to allow subsequent sequencing. When amplifications failed with the Folmer (non-tailed) primers, a cocktail of primers combining in equal proportions Folmer with LepF1 (5'-ATTCAACCAATCATAAAGATATTGG -3') and LepR1 (5'-TAAACTTCTGGATGTCCAAAAAATCA -3'; Folmer et al. 1994; Hajibabaei et al. 2006) were used.

PCR products of *COI* were amplified on the thermocycler (Mastercycler® ep gradient, Eppendorf®) with the following conditions: 94 °C for 1 min for initial denaturation, 5 cycles of 94 °C for 30 sec, annealing at 45-50 °C for 40 sec, and extension at 72 °C for 1 min, followed by 30-35 cycles at 94 °C for 30 sec, 40 sec at 51-54°C, and 1 min at 72 °C, with a final extension of 72 °C for 10 min. The 12.5 µl PCR reaction mix for one reaction included 2 µl of DNA template, 0.06 µl of Taq DNA Polymerase (Platinum®), 0.125 µl of each 10 µM primer (Invitrogen™), 2 µl of ultrapure water, 0.0625 µl of 10 mM dNTPs (New England Biolabs®), 6.25 µl of 10% trehalose, 1.25 µl of 10X PCR buffer for Platimum *Taq* (Invitrogen™), and 0.625 µl of 50 mM $MgCl_2$ (Invitrogen™). The PCR products were visualised by electrophoresis on a 2% agarose E-gel® stained with Ethidium bromide. Amplification products from the PCR were cleaned-up following the Sephadex® protocol and sequenced in a 3730xl DNA Analyzer (Applied Biosystems) (Ivanova and Grainger 2006).

Sequence analyses

COI sequences were visually checked and sequence chromatograms were examined (when required) to resolve unclear base calls; short sequences were removed and longer ones (ranging from: 418 – 660 bp for tardigrades, and 473 – 658 bp for rotifers) used for the alignment performed with Geneious Bioinformatics package v3.8 (Biomatters, Ltd., Auckland, NZ). All sequences were translated into amino-acids to check for stop-codons (using the invertebrate mitochondrial genetic code); alignments were performed with the default settings (cost matrix: 65% similarity; gap open penalty: 12; and gap extension penalty: 3). Sequences were verified to correspond to the relevant phylum and compared with individual haplotypes using the Barcode of Life Data System (BOLD) search engine, and the Blastn algorithm implemented in GenBank. Nucleotide sequence divergence was calculated using uncorrected p-distances as implemented in MEGA5 (Tamura et al. 2011). The general time reversible model with invariant sites and gamma distribution (GTR+I+Γ) was chosen as the best model by Modeltest v.3.7 (Posada and Crandall 1998), and MrModeltest v.2.3 (Nylander 2004) under the Akaike Information Criterion (AIC). Analyses to produce likelihood scores for the models of evolution were performed with PAUP* v.4.0beta10 (Swofford 2002). The selected model (GTR+I+Γ) was used to generate Maximum Likelihood (ML) trees with 1000 bootstrap replicates in the program MEGA5 (Tamura et al. 2011); and to produce trees using Bayesian Inferences (BI) with MrBayes v.3.2 (Huelsenbeck and Ronquist 2001). For the Bayesian analyses the trees were generated using two replicates, four chains per replicate (three heated and one cold chain) over 5,000,000 generation for tardigrades and 15,000,000 generations for rotifers (to result in an average standard deviation of split frequencies below 0.01), a chain temperature of 0.2, sampling frequency of 100, and a burn-in of 25% (12,500 generations for tardigrades and 37,500 for rotifers). For the pair-wise distance comparisons we used uncorrected p-distances among haplotypes (Appendix 4: Table S1, Table S2). *COI* sequences generated in this study have been uploaded to GenBank (Appendix 4: Table S3, Table S4) and archived in BOLD in the ANTAR (Antarctic Invertebrates) project (www.barcodinglife.org) together with images and collateral information for each voucher specimen.

Results

Tardigrada

Tardigrades molecular diversity

We obtained a total of 438 sequences (418 – 658 bp), of which 323 sequences came from Enderby and Wilkes sectors (from 45 soil and 27 net-water samples), 17 sequences from Tanngarden in DML (from seven soil samples), one sequence from FI, 18 sequences from six soil samples collected in TF (Table 3), and an extra 79 sequences from other Antarctic locations (Marion Island, King George Island, SG, SgI, EM, QM, HV and RI). Sequences were grouped into lineages according to pair-wise sequence divergence using p-distance. We identified 63 lineages (within-lineage haplotype divergence [wld] did not exceed more than 1.6%), of which 39 were formed exclusively by 'unique haplotypes' (generated from this study), four lineages (Ta28, Ta29, Ta42, Ta44) included 'unique haplotypes' and GenBank haplotypes, and 20 lineages (158 sequences) were comprised exclusively by GenBank haplotypes (Appendix 4: Table S1 for p-distances, and Table S3 for accession numbers). We generated ML and Bayesian trees based on a total of 130 haplotypes. The tardigrade consensus alignment (from 63 lineages) was found to be highly AT-rich (A: 27.9%; T: 36.1%; G: 16.1%; C: 19.9%), with tardigrades belonging to the class Eutardigrada (51 lineages with 44 (Ta1 – Ta44) in Parachela and 7 (Ta45 – Ta51) in Apochela) and the class Heterotardigrada (12 lineages in Echiniscoidea (Ta52 – Ta63) Fig. 2.

Order Parachela

Fourteen lineages of the Order Parachela (nine from TF) were not identified to genus or species rank (indicated as 'Parachela' in Fig. 2) as no close GenBank sequences were found. However, they were distinct enough to form their own lineages with 'between-lineage divergence' (bld) greater than 3.4% (Ta22 – Ta23). Ta15 was represented by 34 sequences (three haplotypes) from Wilkes and Enderby sectors (CS, HI, MP and SP) covering a distance of almost 1500 km. Ta11 was represented by six sequences forming three haplotypes (0.7% wld) from two regions in VH and MS separated by over 650 km.

The three remaining unidentified Antarctic Parachela lineages were formed by single sequences from Francis Island (Ta12), and from DML (Ta14, Ta16). Three of the lineages (Ta11, Ta12 and Ta14) were observed that share a basal node with *Diphascon* clades (Fig. 2; Appendix 4: Fig. S2). None of the TF soil tardigrades were identified by morphological taxonomy, but the 15 specimens sequenced formed 12 haplotypes with 8 exclusive TF lineages (Fig. 2; Appendix 4: Fig. S1, Fig. S2).

Diphascon COI lineages showed a monophyletic group of lineages, covering 43 sequences in nine lineages (Ta1 – Ta7, Ta10, T13; Fig. 2). Lineage Ta1 comprised a single sequence from a sub-Antarctic island South Georgia; its closest lineage corresponded to a GenBank sequence from King George Island (Ta2 - maximum of 12.2% bld; Appendix 4: Table S1), spatially 1500 km distant. Lineage Ta3 from BP and SP comprised 19 sequences and three haplotypes (0.5% wld) from soil/lakes. One haplotypes, formed lineage Ta4, which was sister to four other Signy Island GenBank sequences (1% wcd) (Ta5). These were associated (6.4 – 7.6% bld) with a grouping of two South Georgian sequences (Ta6 - 1.8% wld) and seven South Georgian sequences, three haplotypes, (Ta7 - 1.4% wld). A Queen Maud Mtns sequence (Ta10) diverged (16%) from an unidentified Parachela fom VH and MS (Ta11) (Appendix 4: Table S1). Six *Diphascon puneceum* sequences (T13), from Signy Island, formed five haplotypes (1.6% wld). We also observed three Parachela lineages (Ta8, Ta9, Ta14) that complemented the Diphascon clade (Ta1 – Ta14) (Fig. 2).

The most commonly found tardigrade throughout continental Antarctica was *Acutuncus antarcticus*, which formed two highly divergent lineages Ta28 and Ta29 (18.3 – 20.4 bld). Lineage Ta28 (263 sequences and 27 haplotypes (1.6% wld), including three GenBank sequences) covered the widest geographical area, comprising the sectors Maud, Enderby, Wilkes and Scott and lineage Ta29 (13 sequences and four haplotypes, including one GenBank sequence) was more restricted from QML and DML sites about 2600 km apart (Fig. 2). We found our *Acutuncus antarcticus* lineages (Ta28 and Ta29) shared a deep node with a group comprising a GenBank King George Isl *Hypsibius* (T25), two GenBank European *Borealibius* sequences (T26), and four unidentified TF Parachela (Ta22, Ta23,

Ta24, and Ta27) (Fig. 2); though branch support for the clade Ta22 – Ta29 was low (Fig. 2; Appendix 4: Fig. S1, Fig. S2).

Both the ML and Bayesian consensus trees revealed a well-supported *Macrobiotus* clade (Fig. 2; Appendix 4: Fig. S1, Fig. S2) including 14 lineages of morphologically identified *Macrobiotus* spp. from continental and maritime Antarctica and one unidentified Parachela lineage from TF (Ta30 – Ta44; Fig. 2). The maximum sequence divergence of 26.8% bld was between *Macrobiotus* clades Ta30 and Ta38 (Appendix 4: Table S1). Within the *Macrobiotus* clade, five lineages (Ta30, Ta31, Ta34, Ta41, Ta43 from Graham, Palmer and Maud sectors) were formed exclusively by GenBank sequences. All DML (Maud sector) *Macrobiotus* sequences, from specimens collected in Tanngarden and Brattnipane (50 km apart) grouped closely together in three lineages Ta42, Ta43 and Ta44 (4.6% bld), with T42 and T44 combining newly collected specimens for this study and GenBank sequences. The Brattnipane lineage (Ta43) consisted of ten sequences, three haplotypes (1.8% wld), while the Tanngarden lineages comprised Ta42 (11 sequences, four haplotypes (0.7% wld)) and T44 (31 sequences, seven haplotypes (0.7% wld)). The QM (three lineages - four sequences) and HV (three lineages - 11 sequences, six haplotypes) *Macrobiotus* sub-clade (Ta35 – Ta40), ~700 km apart, consisted of six closely well supported lineages (8% bld; Fig.1; Appendix 4: Fig. S2). A single *Macrobiotus* sequence was obtained from an egg collected in FM (Ta33), with the closest GenBank sequence provided by AI Ta34 (20.6% bld; Fig. 2).

The remaining Parachela lineages Ta16, Ta17, Ta18, and Ta20, included a single DML sequence (Ta16) with the nearest GenBank match of two European *Ramazzottius oberhaeuseri* lineages (Ta17 and Ta18), which diverged by 16 – 20% (bld). Lineage Ta20 was formed by a single *Murrayon* GenBank sequence from Europe (possibly the result of a misidentification given that *Murrayon* should be placed within the Macrobiotid line). Ta20 shared a root node (17.6 – 21.3% bld) with unidentified Parachela lineages from TF Ta19 and Ta21; and showed low support (< 50%; Fig. 2) but high posterior probability (1.0; Appendix 4: Fig. S2).

Order Apochela

The genus *Milnesium* was the only representative of the Order Apochela and formed a monophyletic clade (Ta45 – Ta51) well supported by the Bayesian and ML analyses (Fig. 2; Appendix 4: Fig. S1, Fig. S2). Seven *Milnesium* lineages, including 13 sequences and nine haplotypes were identified in the analyses (8.2 – 25.4% bld). Three of those lineages were formed exclusively by GenBank sequences (Ta48 – Ta50) and represented the nearest matches for the five *Milnesium* haplotypes found in this study. Two of those lineages (Ta48 and Ta49) were labelled as *Milnesium tardigradum* in GenBank. Lineage Ta49, from Germany, represented work redescribing *Milnesium tardigradum sensu stricta* and the sister lineage Ta48 (formed by two sequences and two haplotypes with < 1% sequence divergence from Germany and Japan) was found to diverge by 19.7% (Appendix 4: Table S1). The third GenBank sample formed lineage Ta50, a single sequence from Charcot Island (Palmer sector), which was the closest match to Ta51 (8.2% bld), a single haplotype (three sequences) from EM 1000 km away. Three new lineages (Ta45 – Ta47) complemented the *Milnesium* clade; formed by Ta45 comprising three sequences and two haplotypes (0.5% wld) from Marion Island and, the sister lineages Ta46 and Ta47 (diverging by 21%) formed by single continental Antarctic haplotypes. Ta46 comprised two sequences from specimens collected in Stornes Peninsula (EA) and Ta47 a single sequence from QM, ~2400 km away.

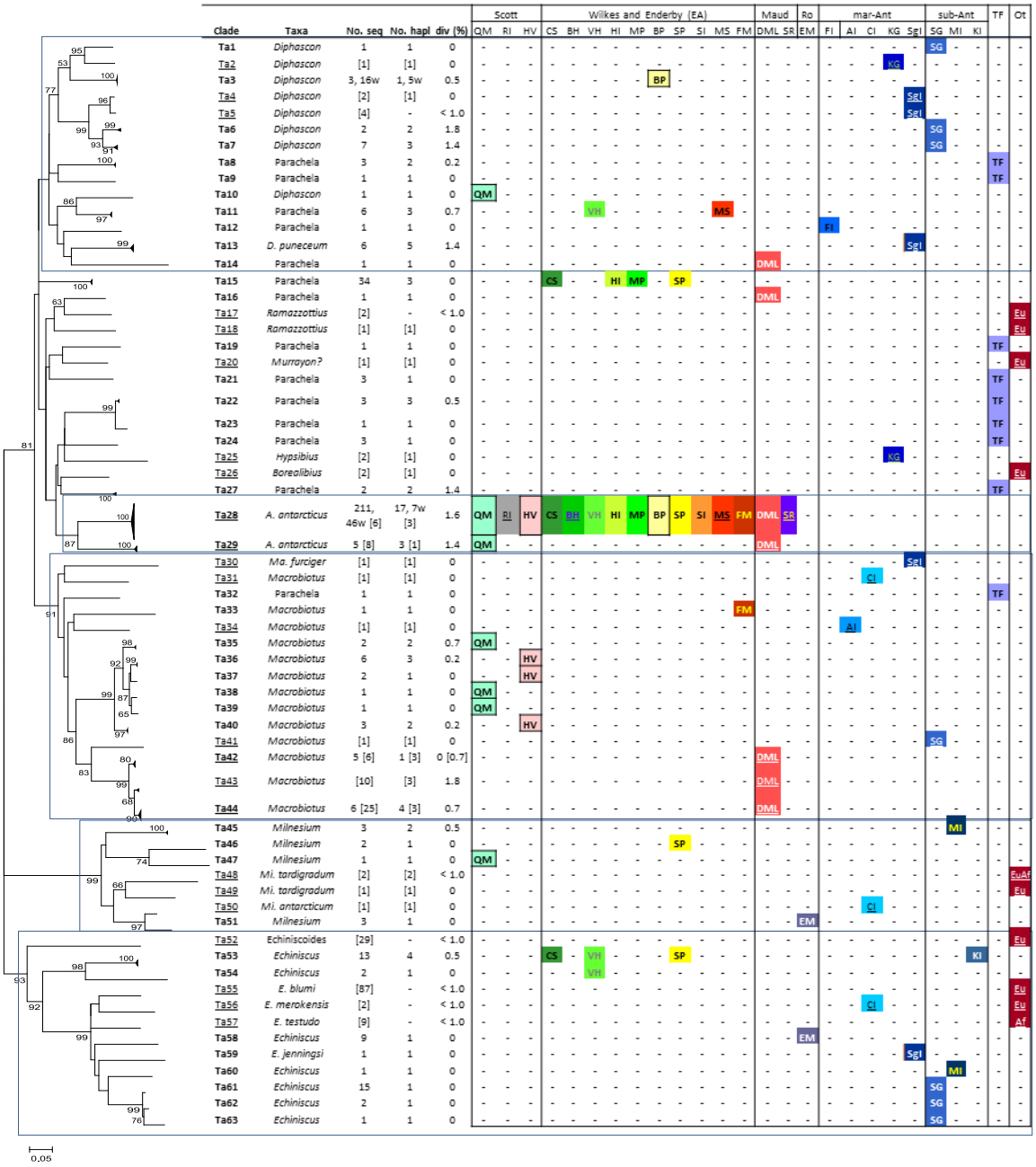
Order Echiniscoidea

The Order Echiniscoidea was represented by an unidentified *Echiniscoides* (GenBank - European sequences) (Ta52) and eleven *Echiniscus* lineages Ta53 – Ta63 (2.5 – 31% bld). The 29 sequences forming the *Echiniscoides* lineage Ta52 diverged by less than 1% in sequence similarity. Three of the *Echiniscus* lineages formed by GenBank sequence from Europe, Africa and maritime Antarctica (Ta55 – Ta57), diverging 16 – 18% (bld; Appendix 4: Table S1). Ta55 was formed by 87 *Echiniscus blumi* European sequences (<1% wld), Ta56 by a European *E. merokensis* and an unnamed maritime Antarctica *Echiniscus* (<1% wld) (the locality for this lineage should be questioned given that there

are no previous records for *E. merokensis* from Antarctica), and Ta57 with nine African *E. testudo* sequences (<1% wld). These three lineages formed the closest matches for our Antarctic *Echiniscus* sequences.

Two highly divergent *Echiniscus* lineages Ta53 and Ta54 (21% bld) were revealed, with sequences that showed a 3 bp deletion (position 292 – 294 bp) in the general tardigrade alignment. We found this position highly variable in the *Echiniscus* lineages examined, with four possible amino-acids (Valine, Alanine, Isoleucine, and Serine). Lineage Ta53 was formed by 13 sequences of four haplotypes (0.5% wld) from CS, VH, SP and sub-Antarctic Kerguelen Island, covering a distant of 2200 km; a soil sample from VH produced a single haplotype from two sequences Ta54. SG *Echiniscus* sp. formed a sub-clade with three closely related lineages (Ta61 – Ta63). Two (Ta61 and Ta62) diverged by 2.5% (bld), which diverged from the third lineage (Ta63) by 6% (bld; Appendix 4: Table S1). These three SG haplotype lineages (Fig. 2) were closely aligned with Ta60, a single Marion Island sequence (14.6 – 17.2% bld) 6600 km away and diverged from EM *Echiniscus corrugicaudatus* (Ta58) (21% bld) and SgI *Echiniscus jenningsi* (Ta59) (Fig. 2).

Figure 2 (following page)**.** ML tree of COI tardigrade sequences from Antarctica compared to other sequences from Tierra del Fuego (TF), and other (Ot) sites, e.g. Europe (Eu), and Africa (Af). Confidence values at nodes were generated from 1,000 bootstrap pseudoreplicates (support values below 50 are not shown). COI lineages are represented from Ta1 to Ta63. Lineages and regions in bold include new sequences from this study. Lineages and regions underlined include sequences gathered from GenBank (number of sequences and haplotypes shown in square brackets). Refer to 'Appendix 4: Table S3' for the accessions numbers. Monophyletic clades for *Diphascon*, *Acutuncus antarcticus*, *Macrobiotus*, *Milnesium* and Echiniscoidea are shown enclosed in separate boxes. The colour boxes represent the geographic region of sequences, which colour code refers to the maps at the bottom of the figure. List of acronyms: number of sequences included in the specified clade (No. seq); sequences from specimens collected from water samples (w); number of haplotypes in each clade (No. hapl); P-distance percentage within-clade (div %); Queen Maud Mtns (QM); Ross Island (RI); Hidden Valley (HV); Casey Station (CS); Bunger Hills (BH); Vestfold Hills (VH); Hop Island (HI); Mather Peninsula (MP); Broknes Peninsula (BP); Stornes Peninsula (SP); Sansom Island (SI); Mawson Station (MS) and Framnes Mountains (FM); Dronning Maud Land (DML); Shackleton Range (SR); Ronne sector (Ro); Ellsworth Mtns (EM); maritime Antarctica (mar-Ant); Francis Island (FI); Alexander Island (AI); Charcot Island (CI), King George Island (KG); Signy Island (SgI); sub-Antarctica (sub-Ant); South Georgia (SG); Marion Island (MI); and Kerguelen Island (KI).


Clade
Taxa
No. seq
No. hapl
div (%)
Scott
QM RI HV
Wilkes and Enderby (EA)
CS BH VH HI MP BP SP SI MS FM
Maud
DML SR
Ro
EM
mar-Ant
FI AI CI KG SgI
sub-Ant
SG MI KI
TF
Ot
Ta1 Diphascon 1 1 0
Ta2 Diphascon [1] [1] 0
Ta3 Diphascon 3, 16w 1, 5w 0.5
Ta4 Diphascon [2] [1] 0
Ta5 Diphascon [4] - < 1.0
Ta6 Diphascon 2 2 1.8
Ta7 Diphascon 7 3 1.4
Ta8 Parachela 3 2 0.2
Ta9 Parachela 1 1 0
Ta10 Diphascon 1 1 0
Ta11 Parachela 6 3 0.7
Ta12 Parachela 1 1 0
Ta13 D. puneceum 6 5 1.4
Ta14 Parachela 1 1 0
Ta15 Parachela 34 3 0
Ta16 Parachela 1 1 0
Ta17 Ramazzottius [2] - < 1.0
Ta18 Ramazzottius [1] [1] 0
Ta19 Parachela 1 1 0
Ta20 Murrayon? [1] [1] 0
Ta21 Parachela 3 1 0
Ta22 Parachela 3 3 0.5
Ta23 Parachela 1 1 0
Ta24 Parachela 3 1 0
Ta25 Hypsibius [2] [1] 0
Ta26 Borealibius [2] [1] 0
Ta27 Parachela 2 2 1.4
Ta28 A. antarcticus 211, 46w [6] 17, 7w [3] 1.6
Ta29 A. antarcticus 5 [8] 3 [1] 1.4
Ta30 Ma. furciger [1] [1] 0
Ta31 Macrobiotus [1] [1] 0
Ta32 Parachela 1 1 0
Ta33 Macrobiotus 1 1 0
Ta34 Macrobiotus [1] [1] 0
Ta35 Macrobiotus 2 2 0.7
Ta36 Macrobiotus 6 3 0.2
Ta37 Macrobiotus 2 1 0
Ta38 Macrobiotus 1 1 0
Ta39 Macrobiotus 1 1 0
Ta40 Macrobiotus 3 2 0.2
Ta41 Macrobiotus [1] [1] 0
Ta42 Macrobiotus 5 [6] 1 [3] 0 [0.7]
Ta43 Macrobiotus [10] [3] 1.8
Ta44 Macrobiotus 6 [25] 4 [3] 0.7
Ta45 Milnesium 3 2 0.5
Ta46 Milnesium 2 1 0
Ta47 Milnesium 1 1 0
Ta48 Mi. tardigradum [2] [2] < 1.0
Ta49 Mi. tardigradum [1] [1] 0
Ta50 Mi. antarcticum [1] [1] 0
Ta51 Milnesium 3 1 0
Ta52 Echiniscoides [29] - < 1.0
Ta53 Echiniscus 13 4 0.5
Ta54 Echiniscus 2 1 0
Ta55 E. blumi [87] - < 1.0
Ta56 E. merokensis [2] - < 1.0
Ta57 E. testudo [9] - < 1.0
Ta58 Echiniscus 9 1 0
Ta59 E. jenningsi 1 1 0
Ta60 Echiniscus 1 1 0
Ta61 Echiniscus 15 1 0
Ta62 Echiniscus 2 1 0
Ta63 Echiniscus 1 1 0
0.05
Eu
Af
KI
MI
TF
SG
SgI
KG
FI
CI
AI
DML
SR
EM
MS
FM
BH
CS
VH
HI
MP
QM
HV
SI
RI
BP
SP

Rotifera

Rotifer molecular diversity

We obtained 526 bdelloid sequences (430 – 661 bp), of which 494 sequences were from Enderby and Wilkes sectors (from 88 soil samples, and 15 net-water samples), ten sequences from DML (from three soil samples), three sequences from FI (from two soil samples), 12 sequences from seven soil samples collected in TF (Table 3), and seven *Philodina* sequences from water samples in RI. A total of 131 unique haplotypes were produced for this study (Fig. 3). Unique haplotypes were used as queries to produce similar hits in GenBank; only four GenBank sequences were found to be above 94.9% similar to at least one of our unique haplotypes (Appendix 4: Table S5). Taken overall, sequences considered for the Bayesian and ML analyses comprised 131 unique haplotypes, four bdelloid GenBank sequences, and two monogonont GenBank sequences the latter used as outgroups (Fig. 3; Appendix 4: Fig. S3, Fig. S4). Other GenBank Blastn hits (top 100 for each lineage) showed maximum sequence similarities between 78 – 91% to a variety of bdelloid genera, including *Philodina*, *Adineta*, *Habrotrocha*, *Pleuretra*, *Macrotrachela*, *Rotaria*, *Abrochtha*, and *Bradyscela* (Appendix 4: Table S5); none of those sequences were included in the final alignment. For practical purposes 47 bdelloid lineages were generated based on the pair-wise p-distance divergence (none of which exceeded 3.6% wld). The bdelloid consensus alignment showed a 3 bp insertion (position 475 - 477bp) of the amino-acid Tyrosine in 23 lineages, and the amino-acid Phenylalanine in two lineages (Fig. 3). We also found that the *COI* was highly AT-rich (A: 24.7%; T: 44.7%; G: 20.2%; C: 10.4%).

Genus Adineta

Seven lineages from the genus *Adineta* were identified from this study; six of which comprised 91 sequences and 29 haplotypes from Wilkes and Enderby samples (Bd1, Bd2, Bd4, Bd8, Bd23, and Bd24) (Fig. 3). Only one *Adineta* lineage (Bd22) from SgI (*Adineta gracilis*), based exclusively on a GenBank sequence (accession no. EF173192), was

included in the analyses and it was found to be similar to Bd23 (4.7 – 5.1% bld). Lineage Bd23 comprised 12 sequences and three haplotypes (2.3% wld) from specimens collected from four regions in the Wilkes and Enderby sectors. The lineages Bd22 – Bd23 formed a well-supported monophyletic group with lineages Bd20 and Bd21 (9.2 – 13% bld; Fig. 3; Appendix 4: Table S2), the latter including four unidentified specimens from Enderby sector. Three more GenBank sequences (accession no. EF173190, EF173191, EF173193) belonging to *Adineta gracilis* from SgI grouped with lineage Bd8; lineage Bd8 also included 16 sequences and three haplotypes (1.4% wld) from soil samples in CS, HI, MP, PB, and SP over 5700 km away from SgI. Lineage Bd24 comprised ten sequences and two haplotypes (3.6% wld; Fig. 3) from HI, MP and SP, 10 km apart. The sister lineage of Bd24, Bd25, comprised two sequences and two haplotypes from unidentified bdelloids from BP. The sequence divergence among the latter two lineages was found to be 11.2 – 12.7% (Appendix 4: Table S2). The three additional *Adineta* lineages (Bd1, Bd2, and Bd4) were grouped in the same clade together with an unidentified bdelloid sequence from FM (Bd3; Fig. 3). The sister lineages, Bd3 and Bd4, diverged by 4% (bld); while sister lineages Bd1 and Bd2 diverged by 10.3 – 12% (bld; Appendix 4: Table S2). Lineage Bd4 included seven sequences and two *Adineta* haplotypes (0.2% wld) collected exclusively at SP. Lineage Bd1 comprised 27 sequences and eleven haplotypes (1.6% wld), and was widely distributed across EA, present in samples from CS to FM (over 2000 km in distance). It's sister lineage Bd2 comprised 19 sequences and eight haplotypes (2.0% wld) from VH, BP, SP, and MS; it was also the only identified *Adineta* lineage that included specimens collected from water samples in BP and SP (Fig. 3). The maximum divergence for the clades Bd1– Bd4 was among Bd1 and Bd3 (14.7 – 15.9% bld; Appendix 4: Table S2).

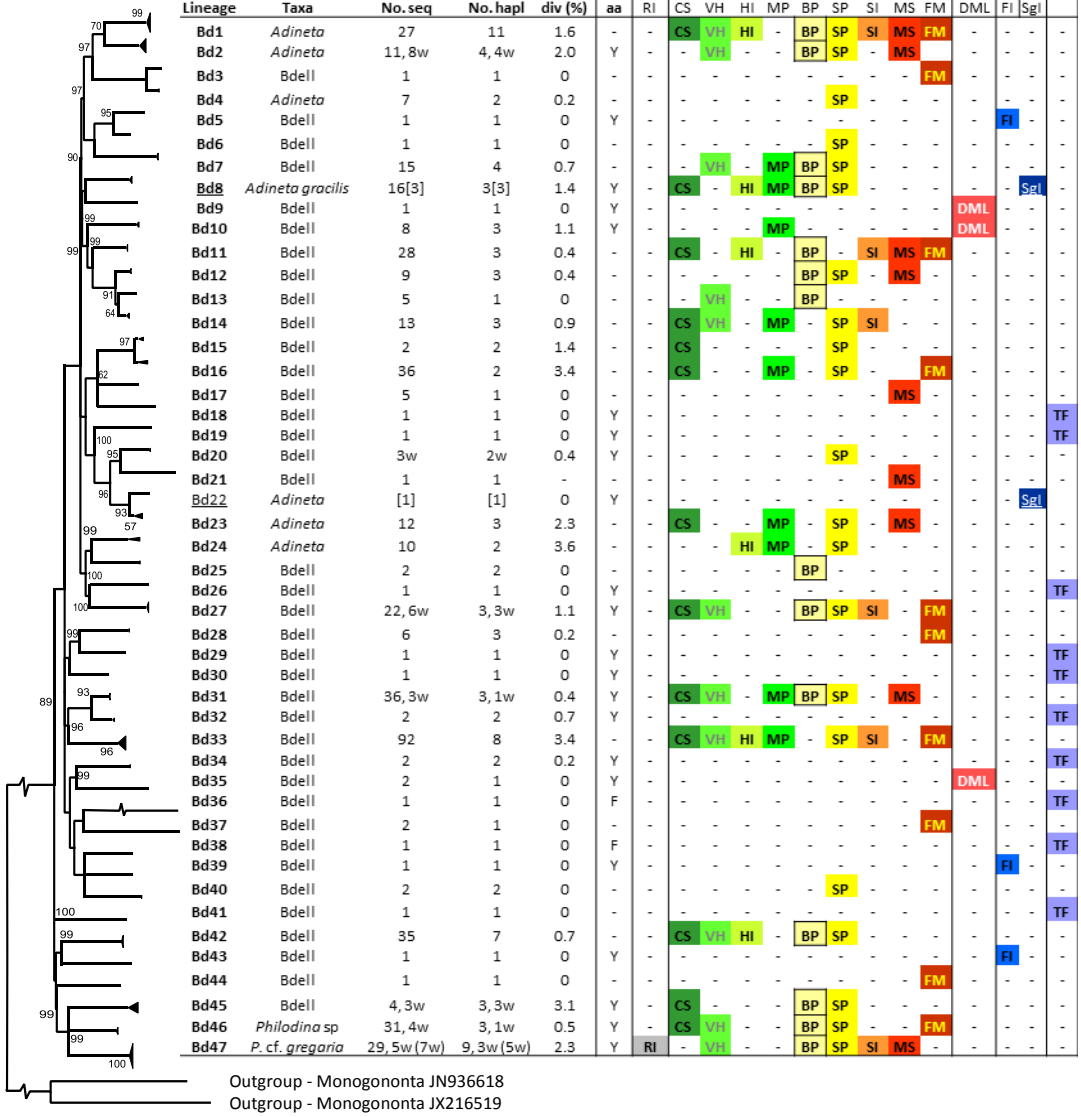
Genus Philodina

Philodina lineages Bd46 and Bd47 shared a common node with Bd45 (13.4 – 17% bld; Appendix 4: Table S2), an unidentified bdelloid lineage that included specimens from EA. Lineage Bd45 comprised seven sequences and six haplotypes (3.1% wld) from soil samples in CS and SP; and from water samples collected in SP and BP. Lineage Bd46

(*Philodina* sp.) revealed low levels of diversity across 35 sequences and four haplotypes (0.5% wld) from soil samples collected over 2000 km apart from Wilkes and Enderby (CS, VH, BP, SP, FM), including two water samples (BP, SP). Lineage Bd47 (*Philodina* cf. *gregaria*) comprised 41 sequences and 17 haplotypes (2.3% wld) collected from samples ~3130 km apart (extending from MS to RI). Sequences grouped within lineage Bd47 corresponded to specimens collected from soil samples in VH, SI, BP, SP, and MS; it included 29 sequences and one haplotype. Sequences from tarn/lakes that also grouped with the lineage Bd47, included five sequences and three haplotypes from VH and BP, and seven sequences and five haplotypes from samples collected on RI.

Unidentified bdelloids

Three *COI* sequences generated from FI in AP, formed three distantly related lineages (Bd5, Bd39, Bd43), diverging by 14 – 15% (Appendix 4: Table S2). The closest association for the AP lineages was observed between Bd5 and a lineage from SP (B32; 7.1% bld). Three other lineages (Bd9, Bd10, and Bd35) comprised bdelloids from Tanngarden (DML); two of those lineages consisted of single haplotypes (Bd9 and B34), and the third (Bd10), comprised two haplotypes and eight sequences (1.1% wld) from specimens also collected in MP (Fig. 3). Overall, we counted 25 lineages that included unidentified bdelloids from EA; one of those lineages (Bd33) was highly represented in EA, and contained the majority of sequences observed for a single lineage (92 sequences and 8 haplotypes; Fig. 3). We also identified a further ten divergent lineages from TF, none of which were from identified bdelloids. The closest similarity involving TF lineages was observed for Bd32 and the lineage Bd31 (5.3 – 6.1% bld). Lineage Bd31 comprised 39 sequences and four haplotypes from specimens collected from soil and water samples in Enderby and Wilkes sectors, in regions as far as 2000 km apart.



Lineage	Taxa	No. seq	No. hapl	div (%)	aa	Sc	Wilkes and Enderby (EA)								Maud	mA		TF
						RI	CS	VH	HI	MP	BP	SP	SI	MS	FM	DML	FI	SgI	
Bd1	*Adineta*	27	11	1.6	-	-	CS	VH	HI	-	BP	SP	SI	MS	FM	-	-	-	-
Bd2	*Adineta*	11, 8w	4, 4w	2.0	Y	-	-	VH	-	-	BP	SP	-	MS		-	-	-	-
Bd3	Bdell	1	1	0	-	-	-	-	-	-	-	-	-	-	FM	-	-	-	-
Bd4	*Adineta*	7	2	0.2	-	-	-	-	-	-	-	SP	-	-	-	-	-	-	-
Bd5	Bdell	1	1	0	Y	-	-	-	-	-	-	-	-	-	-	-	FI	-	-
Bd6	Bdell	1	1	0	-	-	-	-	-	-	-	SP	-	-	-	-	-	-	-
Bd7	Bdell	15	4	0.7	-	-	-	VH	-	MP	BP	SP	-	-	-	-	-	-	-
Bd8	*Adineta gracilis*	16[3]	3[3]	1.4	Y	-	CS	-	HI	MP	BP	SP	-	-	-	-	-	SgI	-
Bd9	Bdell	1	1	0	Y	-	-	-	-	-	-	-	-	-	-	DML	-	-	-
Bd10	Bdell	8	3	1.1	Y	-	-	-	-	MP	-	-	-	-	-	DML	-	-	-
Bd11	Bdell	28	3	0.4	-	-	CS	-	HI	-	BP	-	SI	MS	FM	-	-	-	-
Bd12	Bdell	9	3	0.4	-	-	-	-	-	-	BP	SP	-	MS	-	-	-	-	-
Bd13	Bdell	5	1	0	-	-	-	VH	-	-	BP	-	-	-	-	-	-	-	-
Bd14	Bdell	13	3	0.9	-	-	CS	VH	-	MP	-	SP	SI	-	-	-	-	-	-
Bd15	Bdell	2	2	1.4	-	-	CS	-	-	-	-	SP	-	-	-	-	-	-	-
Bd16	Bdell	36	2	3.4	-	-	CS	-	-	MP	-	SP	-	-	FM	-	-	-	-
Bd17	Bdell	5	1	0	-	-	-	-	-	-	-	-	-	MS	-	-	-	-	-
Bd18	Bdell	1	1	0	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	TF
Bd19	Bdell	1	1	0	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	TF
Bd20	Bdell	3w	2w	0.4	Y	-	-	-	-	-	-	SP	-	-	-	-	-	-	-
Bd21	Bdell	1	1	-	-	-	-	-	-	-	-	-	-	MS	-	-	-	-	-
Bd22	*Adineta*	[1]	[1]	0	Y	-	-	-	-	-	-	-	-	-	-	-	-	SgI	
Bd23	*Adineta*	12	3	2.3	-	-	CS	-	-	MP	-	SP	-	MS	-	-	-	-	-
Bd24	*Adineta*	10	2	3.6	-	-	-	-	HI	MP	-	SP	-	-	-	-	-	-	-
Bd25	Bdell	2	2	0	-	-	-	-	-	-	BP	-	-	-	-	-	-	-	-
Bd26	Bdell	1	1	0	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	TF
Bd27	Bdell	22, 6w	3, 3w	1.1	Y	-	CS	VH	-	-	BP	SP	SI	-	FM	-	-	-	-
Bd28	Bdell	6	3	0.2	-	-	-	-	-	-	-	-	-	-	FM	-	-	-	-
Bd29	Bdell	1	1	0	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	TF
Bd30	Bdell	1	1	0	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	TF
Bd31	Bdell	36, 3w	3, 1w	0.4	Y	-	CS	VH	-	MP	BP	SP	-	MS	-	-	-	-	-
Bd32	Bdell	2	2	0.7	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	TF
Bd33	Bdell	92	8	3.4	-	-	CS	VH	HI	MP	-	SP	SI	-	FM	-	-	-	-
Bd34	Bdell	2	2	0.2	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	TF
Bd35	Bdell	2	1	0	Y	-	-	-	-	-	-	-	-	-	-	DML	-	-	-
Bd36	Bdell	1	1	0	F	-	-	-	-	-	-	-	-	-	-	-	-	-	TF
Bd37	Bdell	2	1	0	-	-	-	-	-	-	-	-	-	-	FM	-	-	-	-
Bd38	Bdell	1	1	0	F	-	-	-	-	-	-	-	-	-	-	-	-	-	TF
Bd39	Bdell	1	1	0	Y	-	-	-	-	-	-	-	-	-	-	-	FI	-	-
Bd40	Bdell	2	2	0	-	-	-	-	-	-	-	SP	-	-	-	-	-	-	-
Bd41	Bdell	1	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	TF
Bd42	Bdell	35	7	0.7	-	-	CS	VH	HI	-	BP	SP	-	-	-	-	-	-	-
Bd43	Bdell	1	1	0	Y	-	-	-	-	-	-	-	-	-	-	-	FI	-	-
Bd44	Bdell	1	1	0	-	-	-	-	-	-	-	-	-	-	FM	-	-	-	-
Bd45	Bdell	4, 3w	3, 3w	3.1	Y	-	CS	-	-	-	BP	SP	-	-	-	-	-	-	-
Bd46	*Philodina* sp	31, 4w	3, 1w	0.5	Y	-	CS	VH	-	-	BP	SP	-	-	FM	-	-	-	-
Bd47	*P. cf. gregaria*	29, 5w (7w)	9, 3w (5w)	2.3	Y	RI	-	VH	-	-	BP	SP	SI	MS	-	-	-	-	-

Outgroup - Monogononta JN936618
Outgroup - Monogononta JX216519

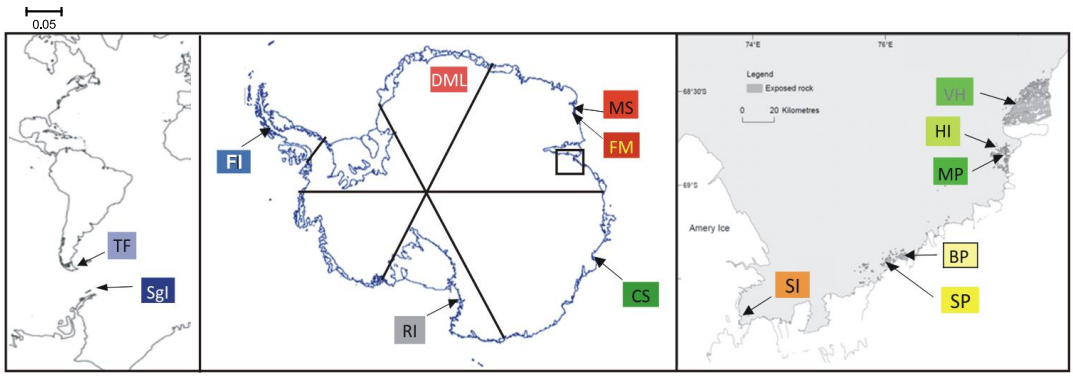


Figure 3 (previous page)**.** ML tree of COI bdelloid rotifer sequences from Antarctica and Tierra del Fuego (TF). Confidence values at nodes were generated from 1,000 bootstrap pseudoreplicates (support values below 50 are not shown). COI lineages are represented from Bd1 to Bd47. Selected outgroup represents two monogononts COI lineages from GenBank. Lineages and regions in bold include sequences from this study. Lineages and regions underlined include sequences gathered from GenBank (number of sequences and haplotypes shown in square brackets). The colour boxes represent the geographic region of sequences, which colour code refers to the maps at the bottom of the figure. List of acronyms: *Philodina* cf. *gregaria* (*P*. cf. *gregaria*); unidentified bdelloid (Bdell); number of sequences included in the specified clade (No. seq); sequences from specimens collected from water samples (w); number of haplotypes in each clade (No. hapl); P-distance percentage within-clade (div %); amino acid (aa), Tyrosine (Y), Phenylalanine (F); Scott sector (Sc); Ross Island (RI); Casey Station (CS); Vestfold Hills (VH); Hop Island (HI); Mather Peninsula (MP); Broknes Peninsula (BP); Stornes Peninsula (SP); Sansom Island (SI); Mawson Station (MS) and Framnes Mountains (FM); Dronning Maud Land (DML); Francis Island (FI); maritime Antarctica (mA); and Signy Island (SgI).

Discussion

Some morphological Antarctic faunal identifications have used references from well-studied regions leading to microfauna been ascribed to previously known species, which suggested cosmopolitan distributions (e.g. Fontaneto et al. 2007). However, other studies highlight the limited morphological variability within species and limited distribution (Pilato and Binda 2001), which opposes the assumption of cosmopolitan species. Microscopic microfauna brings additional problems of a limited suite of morphological characters, which are further exacerbated by contracted specimens, i.e. tardigrades and bdelloids rotifers. With increased sampling in Antarctica and molecular techniques becoming more accessible, the combination of morphological and molecular techniques can be used to explore the Antarctic species geographical distribution and scale of endemicity. Prior to our study, it was not clear how global Antarctic species might be, and although several studies have identified Antarctica species morphologically (e.g. Dastych 1984; Dartnall and Hollowday 1985; Dartnall 2000; De Smet and Gibson 2008; Pilato and Binda 2010) only few have involved DNA data. In our study, those with exclusively Antarctic lineages were most closely aligned with previously reported Antarctica GenBank sequences (with the exception of Ta56; Fig. 2).

A large number of both the Antarctica tardigrades and bdelloids are currently considered endemic, and nematodes, also part of the microfaunal community, are thought

to be formed exclusively by Antarctic endemics (Andrássy 1998). A recent morphological and molecular study of Antarctic nematodes found a combination of locally restricted species with those having broad Antarctic distributions (Velasco-Castrillón and Stevens 2014). Currently, 20 tardigrades species (30.8%) are endemic to the maritime and continental Antarctic, five to Antarctica and sub-Antarctic Islands (7.7%) and three endemic to the sub-Antarctic islands (4.6%) (Table 1). For the rotifer and specifically the bdelloids, five species are considered endemic to Antarctica (16.1%) (Table 2). Species previously reported from Antarctica and considered cosmopolitan, such as *Mi. tardigradum*, have recently been revised (e.g. *Mi. antarcticum* Tumanov, 2006; see: Convey and McInnes 2005), and new forms have been reported as different morpho-species but without description (see Dartnall 1995, 2000; Convey and McInnes 2005). A recent molecular study on Antarctic tardigrades by Czechowski et al. (2012) found operational taxonomic units (OTUs) that indicated potentially new and endemic species within the genera *Macrobiotus* and *Acutuncus*.

If the OTUs (mitochondrial lineages) from our study are viewed as potential new species then this far exceeds the currently recognised morphologically described lists. We found 36 distinct Antarctic bdelloid rotifer COI lineages, which would represent a four-fold increase in the number of species from VH and Larsemann Hills (Enderby sector) compared with reported species for this area (see Dartnall 1995, 2000) (Table 2; Fig. 3). The number of tardigrade COI lineages in our study also expands the level of diversity formally reported for Antarctica (Fig. 2; Table 1; Convey and McInnes 2005). While there have been five Antarctic and sub-Antarctic *Echiniscus* species reported in the literature our study indicated eight *Echiniscus* lineages (Table 1; Fig. 2), though, with the exception of *E. jenningsi*, we are not in a position to identify the other named species. Similarly, while *Mi. tardigradum* sensu lato has been reported from various Antarctic and sub-Antarctic sites and only *Mi. antarcticum* described from the maritime Antarctic, our study revealed five distinct *Milnesium* lineages (Fig. 2; Table 1).

Using the COI gene to establish species boundaries is not an easy task as different thresholds have been previously recognised for different groups. The within-species

sequence divergence for tardigrades (less than 3%) seem to be more conservative than rotifers. Earlier work with tardigrades has shown *Ma. macrocalix* with a 0.3 – 1.0% divergence (Cesari et al. 2009); *Echiniscus testudo* from 0 – 1.28% (Jørgensen et al. 2007); and a study by Faurby et al. (2008) showed within species divergence of 0.2 – 2.9% for *Richtersius coronifer*, and 0 – 0.3% for *Ramazzottius oberhauseri*. Between-species sequence divergence have been observed at 15.3 – 16.3% for *Echiniscus* spp. (Jørgensen et al. 2007), which was similar to the 15.9 – 16.3% for *Macrobiotus* spp. we observed in our study. For bdelloid rotifers, Fontaneto et al. (2011) found sequence divergence within four *Adineta* species ranged from 0.5 – 10.3%, while divergences among species ranged from 1.0 – 23.2%. Other studies have found intra-specific distances within bdelloid species to be on average 1% (Fontaneto et al. 2009) or below 1.1% with among species variation for *Abrochtha* ranging from 8.4 – 15.7% (Birky et al. 2011).

With the circumpolar wind and ocean currents around Antarctica it is the relatively infrequent contrary storms that can provide propagules for colonisation (Ellis-Evans and Walton 1990; McInnes and Pugh 1998). It is therefore possible that many of the Antarctic tardigrades and bdelloids rotifers were present prior to completion of the glaciation of the continent (see Stevens et al. 2006; Convey et al. 2008, 2009), with survival of such endemics in inter glacial maxima refugia (Gibson et al. 2007). This hypothesis of high level Antarctic microfaunal endemism is also expressed in springtails, mites and other microfauna and flora (Chown and Convey 2007; Convey 2010; Stevens and Hogg 2003, 2006; Stevens et al. 2006; Vyverman et al. 2010). Our study showed that the closest sequence similarity for Antarctica tardigrade and bdelloid lineages compared with those from outside the continent were 5.3 – 6.1% bld for bdelloid lineages Bd32 (TF) and Bd31 (EA) (~6600 km apart). Greater biotic similarities have been reported between TF and South America, than from TF to Antarctica (Pugh and Convey 2000). However, under-sampling and vast regions with no data are also likely to have a bias on these microfaunal interpretations. *Adineta gracilis* (Bd8; Fig. 3), a bdelloid rotifer, had the broadest pan-Antarctic distribution extending over 5700 km from maritime Antarctic Signy Island (Graham sector) to continental Antarctic (Enderby sector); though connecting these to dots on a map is not yet possible as there were no examples from Palmer, Scott or Maud

sectors. Also in our study, the tardigrade *Echiniscus* sp. Ta53 was the only lineage with haplotypes in both continental Antarctica (EA) and sub-Antarctica (Kerguelen Island).

Both tardigrades and bdelloids have light-weight dormant propagules that are able to survive desiccation and may be a means of local Antarctic dispersal (e.g. McInnes and Pugh 1998; Sohlenius and Boström 2008), which has also been suggested for some Antarctic nematodes species (Velasco-Castrillón and Stevens 2014). Asexual reproduction described in bdelloids and some tardigrades (Fontaneto et al. 2009; Pilato and Binda 2001) would also allow greater chance of colonising of new environments without the need of sexual mates (as observed for rotifers; Fontaneto et al. 2007). When considering suitable environments for colonisation a study by Velasco-Castrillón et al. (2014) have shown that the presence of tardigrades and rotifers in soil samples occurred at various levels of pH (4.3 – 9.2), moisture content (0.11 – 77%) and E.C. (0.01 – 18.5 dS/m). In our study we found *Acutuncus* cf. *antarcticus* (Ta3) from water samples to tolerate lower ranges in pH (6.4 – 8.3) and E.C. (0.11 – 3.4 dS/m) (Appendix 4: Table S6). We also observed that all 62 tardigrades sequences from water samples fall in only two lineages (*Diphascon* sp. in lineage Ta3, and *Acutuncus* cf. *antarcticus* in lineage Ta28), which were closely aligned with the other terrestrial specimens. *Acutuncus* cf. *antarcticus* (Ta3) was observed to tolerate higher ranges in E.C. and pH than *Diphascon* sp. (Ta28) (Appendix 4: Table S6). Also, *Acutuncus* cf. *antarcticus* (Ta3) was not restricted to the aquatic habitats of 22 tarn/lakes but was present and widespread in soil samples from across 15 regions (three sectors) in continental Antarctica (Fig. 1; Fig. 2). It is important to emphasize the greater diversity of bdelloids, which were represented by seven lineages in 13 tarn/lakes, compared to two tardigrades from 24 tarn/lakes. Only one bdelloid (Bd20) formed a unique tarn/lake lineage; the other six included specimens from both soil and water habitats. This would suggest that bdelloids are generalist and inhabit similar environments.

Using sequence analysis to reveal the biogeographic distribution of Antarctica tardigrade and bdelloids rotifer species, showed that most tardigrade lineages were short-range endemics (within a region), whilst bdelloids indicated a more widespread distribution (within and between sectors). We found our study of molecular COI sequences indicated a greater diversity of Antarctic tardigrades and bdelloids rotifers than had

previously been recognised; even when conservative methods are employed for species delimitation. The within genus uncorrected p-distances values for many of the samples in this study exceed the 3% threshold proposed as species delimitation (Hebert et al. 2003; Cesari et al. 2009). These data provided further insight into the scale of Antarctic micro-invertebrate diversity and, whilst emphasising that our current knowledge is still extremely limited, significantly adds to the hypothesis that these animals are potential Gondwanan relicts.

Acknowledgements

This research was partly funded and supported by the Australian Antarctic Division (http://www.antarctica.gov.au/) Project (ASAC 2355). We would like to thank the University of Adelaide (http://www.sciences.adelaide.edu.au/) for a PhD scholarship to AVC and the South Australian Museum Mawson Trust for providing funding for the Sir Douglas Mawson Doctoral Scholarship (http://www.samuseum.sa.gov.au/). We deeply thank the Australian Antarctic Stations leaders and assistants who contributed to logistics and support of our field work in Antarctica. We also thank Rebecca Stonor, Maria Manjarrez, Pauline Glocke, Tanya Matic (The University of Adelaide) and Federica Colombo for their ongoing laboratory support. We also appreciate the assistance on phylogenetical analysis provided by Mohammad Javidkar. We are grateful to the DNA sequencing agencies BOLD and the Canadian Centre for DNA Barcoding (CCDB). We would like to thank Peter Convey, Paul Geissler (British Antarctic Survey) for providing soil samples from AP and DML, and to Allan Green, Leopoldo Sancho and Ana Pintado (Complutense University of Madrid) for providing soil samples from TF and laboratory facilities and Kirsty Kemp (Zoological Society of London) and the International Graduate Training Course in Antarctic Biology for help with specimen collection.

References

Adams, B. J., R. D. Bardgett, E. Ayres, D. H. Wall, J. Aislabie, S. Bamforth, R. Bargagli, C. Cary, P. Cavacini, L. Connell, P. Convey, J. W. Fell, F. Frati, I. D. Hogg, K. K. Newsham, A. O'Donnell, N. Russell, R. D. Seppelt, and M. I. Stevens. 2006. Diversity and distribution of Victoria Land biota. *Soil Biology and Biochemistry* 38 (10):3003-3018.

Andrássy, I., and J. Gibson. 2007. Nematodes from saline and freshwater lakes of the Vestfold Hills, East Antarctica, including the description of *Hypodontolaimus antarcticus* sp. n. *Polar Biology* 30 (6):669-678.

Andrássy, I. 1998. Nematodes in the Sixth Continent. In *Journal of Nematode Morphlogy and Systematics*, edited by R. Peña Santiago. Jaén: Universidad de Jaén.

Ashton, G. V., M. I. Stevens, M. C. Hart, D. H. Green, M. T. Burrows, E. J. Cook, and K. J. Willis. 2008. Mitochondrial DNA reveals multiple Northern Hemisphere introductions of *Caprella mutica* (Crustacea, Amphipoda). *Molecular Ecology* 17 (5):1293-1303.

Bertolani, R., V. Biserov, L. Rebecchi, and M. Cesari. 2011. Taxonomy and biogeography of tardigrades using an integrated approach: new results on species of the *Macrobiotus hufelandi* group. *Invertebrate Zoology* 8 (1):23-36.

Binda, M. G., and G. Pilato. 2000. *Diphascon (Adropion) tricuspidatum*, a new species of eutardigrade from Antarctica. *Polar Biology* 23 (1):75-76.

Birky Jr, C. W., C. Ricci, G. Melone, and D. Fontaneto. 2011. Integrating DNA and morphological taxonomy to describe diversity in poorly studied microscopic animals: new species of the genus *Abrochtha* Bryce, 1910 (Rotifera: Bdelloidea: Philodinavidae). *Zoological Journal of the Linnean Society* 161 (4):723-734.

Cesari, M., R. Bertolani, L. Rebecchi, and R. Guidetti. 2009. DNA barcoding in Tardigrada: the first case study on *Macrobiotus macrocalix* Bertolani & Rebecchi 1993 (Eutardigrada, Macrobiotidae). *Molecular Ecology Resources* 9 (3):699-706.

Chown, S. L., and P. Convey. 2007. Spatial and temporal variability across life's hierarchies in the terrestrial Antarctic. *Philosophical Transactions of the Royal Society B-Biological Sciences* 362 (1488):2307-2331.

Convey, P. 2010. Terrestrial biodiversity in Antarctica–Recent advances and future challenges. *Polar Science* 4 (2):135-147.

Convey, P., J. A. E. Gibson, C. D. Hillenbrand, D. A. Hodgson, P. J. A. Pugh, J. L. Smellie, and M. I. Stevens. 2008. Antarctic terrestrial life - challenging the history of the frozen continent? *Biological Reviews* 83 (2):103-117.

Convey, P., and S. J. McInnes. 2005. Exceptional tardigrade-dominated ecosystems in Ellsworth Land, Antarctica. *Ecology* 86 (2):519-527.

Convey, P., M. I. Stevens, D. A. Hodgson, J. L. Smellie, C. D. Hillenbrand, D. K. A. Barnes, A. Clarke, P. J. A. Pugh, K. Linse, and S. C. Cary. 2009. Exploring biological constraints on the glacial history of Antarctica. *Quaternary Science Reviews* 28 (27-28):3035-3048.

Costa, F. O., J. R. deWaard, J. Boutillier, S. Ratnasingham, R. T. Dooh, M. Hajibabaei, and P. D. N. Hebert. 2007. Biological identifications through DNA barcodes: the case of the Crustacea. *Canadian Journal of Fisheries and Aquatic Sciences* 64 (2):272-295.

Czechowski, P., C. J. Sands, B. Adams, C. D'Haese, J. Gibson, S. J. McInnes, and M. I. Stevens. 2012. Antarctic Tardigrada: a first step in understanding molecular operational taxonomic units (MOTUs) and biogeography of cryptic meiofauna. *Invertebrate Systematics* 26:526-538.

Dartnall, H. J. G. 1983. Rotifers of the Antarctic and Subantarctic. *Hydrobiologia* 104 (1):57-60.

Dartnall, H. J. G. 1995. Rotifers, and other Aquatic invertebrates, from the Larsemann Hills, Antarctica. *Papers and Proceedings of the Royal Society of Tasmania* 129:17-23.

Dartnall, H. J. G. 2000. A limnological reconnaissance of the Vestfold Hills. *ANARE Reports* 141:1-53.

Dartnall, H. J. G. 2005a. Are Antarctic planktonic rotifers anthropogenic introductions. *Quekett Journal of Microscopy* 40:137-143.

Dartnall, H. J. G. 2005b. Freshwater invertebrates of subantarctic South Georgia. *Journal of Natural History* 39 (37):3321-3342.

Dartnall, H. J. G., and E.D. Hollowday. 1985. Antarctic Rotifers. *Scientific Reports* 100:1-46.

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

Dastych, H. 1989. An annotated list of Tardigrada from the Antarctic. *Entomologische Mitteilungen aus dem Zoologischen Museum Hamburg* 9 (136/137):249-257.

Dastych, 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. 1999. Redescription of the Sub-Antarctic tardigrade *Mopsechiniscus imberbis* (Richters, 1908) (Tardigrda). *Mitt. hamb. zool. Mus. Inst.* 96:21-35.

Dastych, H. 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, and S. J. McInnes. 1994. *Hexapodibius boothi* sp. n., a new species of semi-terrestrial tardigrade from the Maritime Antarctic. *Entomologische Mitteilungen aus dem Zoologischen Museum Hamburg* 11:111-117

Dastych, H., and J. M. Harris. 1995. A new species of the genus *Macrobiotus* from inland nunataks in western Dronning Maud Land, continental Antarctica (Tardigrada). *Entomologische Mitteilungen aus dem Zoologischen Museum Hamburg* 11:175-182.

Dastych, H., G. Ryan, and P. Watkins. 1990. Notes on Tardigrada from western Dronning Maud Land (Antarctica) with a description oftwo new species.

De Smet, W. H, and J. A. E Gibson. 2008. *Rhinoglena kutikovae* n. sp.(Rotifera: Monogononta: Epiphanidae) from the Bunger Hills, East Antarctica: a probable relict species that survived Quaternary glaciations on the continent. *Polar Biology* 31 (5):595-603.

Donner, J. 1972. Report on the finding of Rotifera (Rotataria) from Antarctica. *Polskie Archiwum Hydrobiologii* 19:251-252.

Dougherty, E. C, and L. G. Harris. 1963. Antarctic micrometazoa: fresh-water species in the McMurdo Sound area. *Science* 140 (3566):497-498.

Ellis-Evans, J. C., and D. Walton. 1990. The process of colonization in Antarctic terrestrial and freshwater ecosystems. In *Proceedings of the NIPR Symposium on Polar Biology* 3:151-163.

Faurby, S., K. I. Jönsson, L. Rebecchi, and P. Funch. 2008. Variation in anhydrobiotic survival of two eutardigrade morphospecies: a story of cryptic species and their dispersal. *Journal of Zoology* 275 (2):139-145.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3 (5):294-299.

Fontaneto, D., T. G. Barraclough, K. Chen, C. Ricci, and E. A. Herniou. 2008. Molecular evidence for broad-scale distributions in bdelloid rotifers: everything is not everywhere but most things are very widespread. *Molecular Ecology* 17 (13):3136-3146.

Fontaneto, D., E. A. Herniou, T. G. Barraclough, and C. Ricci. 2007. On the Global Distribution of Microscopic Animals: New Worldwide Data on Bdelloid Rotifers *Zoological Studies* 46 (3):336-346.

Fontaneto, D., N. Iakovenko, I. Eyres, M. Kaya, M. Wyman, and T. G Barraclough. 2011. Cryptic diversity in the genus Adineta Hudson & Gosse, 1886 (Rotifera: Bdelloidea: Adinetidae): a DNA taxonomy approach. *Hydrobiologia* 662 (1):27-33.

Fontaneto, D., M. Kaya, E. A. Herniou, and T. G. Barraclough. 2009. Extreme levels of hidden diversity in microscopic animals (Rotifera) revealed by DNA taxonomy. *Molecular Phylogenetics and Evolution* 53 (1):182-189.

Freckman, D. W., and R. A. Virginia. 1993. Extraction of nematotes from Dry Valley Antarctic soils. *Polar Biology* 13 (7):483-487.

Gibson J. A. E. 2000. Environment of the Bunger Hills. Unpublished report, Australian Antarctic Division, 97 pp.

Gibson, J. A. E, L. Cromer, J. T. Agius, S. J. McInnes, and N. J. Marley. 2007. Tardigrade eggs and exuviae in Antarctic lake sediments: insights into Holocene dynamics and origins of the fauna.

Guidetti, R., R. O. Schill, R. Bertolani, T. Dandekar, and M. Wolf. 2009. New molecular data for tardigrade phylogeny, with the erection of *Paramacrobiotus* gen. nov. *Journal of Zoological Systematics and Evolutionary Research* 47 (4):315-321.

Guil, N., and G. Giribet. 2012. A comprehensive molecular phylogeny of tardigrades—adding genes and taxa to a poorly resolved phylum-level phylogeny. *Cladistics* 28 (1):21-49.

Hajibabaei, M., D. H. Janzen, J. M. Burns, W. Hallwachs, and P. D. N. Hebert. 2006. DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America* 103 (4):968-971.

Hansson, L.A., S. Hylander, H. J. G. Dartnall, S. Lidström, and J.E. Svensson. 2012. High zooplankton diversity in the extreme environments of the McMurdo Dry Valley lakes, Antarctica. *Antarctic Science* 24 (2):131-138.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17 (8):754-755.

Hebert, P. D. N., S. Ratnasingham, and J. R. deWaard. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London Series B-Biological Sciences* 270:S96-S99.

Ivanova, N. V., and C. Grainger. 2006. Pre-made frozen PCR and sequencing plates. *CCDB Advances, Methods Release No* 4.

Ivanova, N. V., J. R. Dewaard, and P. D. N. Hebert. 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes* 6 (4):998-1002.

Jørgensen, A., N. Møbjerg, and R. M. Kristensen. 2007. A molecular study of the tardigrade *Echiniscus testudo* (Echiniscidae) reveals low DNA sequence diversity over a large geographical area. 66 (1s):77-83.

Kaya, M., E. A Herniou, T. G. Barraclough, and D. Fontaneto. 2009. Inconsistent estimates of diversity between traditional and DNA taxonomy in bdelloid rotifers. *Organisms Diversity & Evolution* 9 (1):3-12.

Magalhães, C., M. I. Stevens, S. C. Cary, B. A. Ball, B. C. Storey, D. H. Wall, R. Türk, and U. Ruprecht. 2012. At Limits of Life: Multidisciplinary Insights Reveal

Environmental Constraints on Biotic Diversity in Continental Antarctica. *PLoS One* 7 (9):1-10.

Marshall, D. J., and P. J. A. Pugh. 1996. Origin of the inland Acari of Continental Antarctica, with particular reference to Dronning Maud Land. *Zoological Journal of the Linnean Society* 118 (2):101-118.

McInnes, S. J. 1995a. Taxonomy and ecology of tardigrades from Antarctic lakes. *M. Phil. Open University*:248.

McInnes, S. J. 1995b. Tardigrades from Signy Island, South Orkney Islands, with particular reference to freshwater species. *Journal of natural history* 29 (6):1419-1445.

McInnes, Sandra J. 2010. *Echiniscus corrugicaudatus* (Heterotardigrada; Echiniscidae) a new species from Ellsworth Land, Antarctica. *Polar biology* 33 (1):59-70.

McInnes, S. J., and P. J. A. Pugh. 1998. Biogeography of limno-terrestrial Tardigrada, with particular reference to the Antarctic fauna. *Journal of Biogeography* 25 (1):31-36.

Messing, J. 1993. M13 cloning vehicles: Their contribution to DNA sequencing. *Methods Mol. Biol.* 23:9-22.

Miller, J. D., P. Horne, H. Heatwole, W. R. Miller, and L. Bridges. 1988. A survey of the terrestrial tardigrada of the Vestfold Hills, Antarctica. *Hydrobiologia* 165:197-208.

Miller, W. R., and H. Heatwole. 1995. Tardigrades of the Australian Antarctic Territories - The Mawson coast, East Antarctica. *Invertebrate Biology* 114 (1):27-38.

Miller, W. R., H. Heatwole, R. W. J. Pidgeon, and G. R. Gardiner. 1994. Tardigrades of the Australian Antarctic Territories - The Larsemann Hills, East Antarctica. *Transactions of the American Microscopical Society* 113 (2):142-160.

Miller, W. R., J. D. Miller, and H. Heatwole. 1996. Tardigrades of the Australian Antarctic Territories: the Windmill Islands, East Antarctica. *Zoological Journal of the Linnean Society* 116 (1-2):175-184.

Murray, J. 1910. Part III. Antarctic Rotifera. *British Antarctic Expedition 1907-9, under the command of Sir E.H. Shackleton, c.v.o. Reports on the scientific investigations* 1:41-65.

Nylander, J. A. A. 2004. MrModeltest 2.3.(program distributed by the author) Evolutionary Biology Centre. *Uppsala University, Sweden*.

Opalinski, K. W. 1972. Freshwater fauna and flora in Haswell island(Queen Mary Land, Eastern Antarctica). *Pol Arch Hydrobiol* 19(4):377-381.

Pilato, G., and M. G. Binda. 1999. Three new species of *Diphascon* of the pingue group (Eutardigrada, Hypsibiidae) from Antarctica. *Polar Biology* 21 (5):335-342.

Pilato, G., and M. G. Binda. 2001. Biogeography and limno-terrestrial tardigrades: are they truly incompatible binomials? *Zoologischer Anzeiger-A Journal of Comparative Zoology* 240 (3):511-516.

Pilato, G., and M. G. Binda. 2010. Definition of families, subfamilies, genera and subgenera of the Eutardigrada, and keys to their identification. *Zootaxa* 2404:54.

Pilato, G., S. J. McInnes, and O. Lisi. 2012. *Hebesuncus mollispinus* (Eutardigrada, Hypsibiidae), a new species from maritime Antarctica. *Zootaxa* 3446:60-68.

Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14 (9):817-818.

Powers, L. E., D. W. Freckman, and R. A. Virginia. 1995. Spatial distribution of nematodes in polar desert soils of Antarctica. *Polar Biology* 15 (5):325-333.

Pugh, P. J. A., and P. Convey. 2000. Scotia Arc Acari: antiquity and origin. *Zoological Journal of the Linnean Society* 130 (2):309-328.

Pugh, P. J. A. 1993. A synonymic catalogue of the Acari from Antarctica, the sub-Antarctic Islands and the Southern Ocean. *Journal of Natural History* 27 (2):323-421.

Robeson, M., E. Costello, K. Freeman, J. Whiting, B. Adams, A. Martin, and S. Schmidt. 2009. Environmental DNA sequencing primers for eutardigrades and bdelloid rotifers. *BMC Ecology* 9 (1):25.

Rounsevell, D. E., and P. Horne. 1986. Terrestrial, parasitic and introduced invertebrates of the Vestfold Hills. In *Antarctic Oasis. Terrestrial environments and history of the Vestfold Hills*, edited by J. Pickard. Sydney: Academic Press Australis.

Salomone, N., V. Vignoli, F. Frati, and F. Bernini. 2007. Species boundaries and phylogeography of the "*Euscorpius carpathicus* complex" (Scorpiones: Euscorpiidae) in Italy. *Molecular phylogenetics and evolution* 43 (2):502-514.

Sands, C. J., P. Convey, K. Linse, and S. J. McInnes. 2008a. Assessing meiofaunal variation among individuals utilising morphological and molecular approaches: An example using the Tardigrada. *BMC Ecology* 8:1-11.

Sands, C. J., S. J. McInnes, N. J. Marley, W. P. Goodall-Copestake, P. Convey, and K. Linse. 2008b. Phylum Tardigrada: an "individual" approach. *Cladistics* 24 (6):861-871.

Segers, H. 2007. *Annotated checklist of the rotifers (Phylum Rotifera), with notes on nomenclature, taxonomy and distribution*: Magnolia Press Auckland.

Simons, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. FLOORS. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the entomological Society of America* 87 (6):651-701.

Smykla, J., D. L. Porazinska, N. Iakovenko, K. Janko, W. M. Weiner, W. Niedbała, and M. Drewnik. 2010. Studies on Antarctic soil invertebrates: Preliminary data on rotifers (Rotatoria), with notes on other taxa from Edmonson Point (Northern Victoria Land, Continental Antarctic). *Acta Soc. Zool. Bohem* 74:135-140.

Smykla, J., N. Iakovenko, M. Devetter, and Ł. Kaczmarek. 2012. Diversity and distribution of tardigrades in soils of Edmonson Point (Northern Victoria Land, continental Antarctica). *Czech Polar Reports* 2:61-70.

Sohlenius, B., and S. Boström. 2008. Species diversity and random distribution of microfauna in extremely isolated habitable patches on Antarctic nunataks. *Polar Biology* 31 (7):817-825.

Sohlenius, B., and S. Boström. 2005. The geographic distribution of metazoan microfauna on East Antarctic nunataks. *Polar Biology* 28 (6):439-448.

Sohlenius, B., S. Boström, and A. Hirschfelder. 1995. Nematodes, rotifers and tardigrades from nunataks in Dronning Maud Land, East Antarctica. *Polar Biology* 15 (1):51-56.

Sohlenius, B., S. Boström, and A. Hirschfelder. 1996. Distribution patterns of microfauna (nematodes, rotifers and tardigrades) on nunataks in Dronning Maud Land, East Antarctica. *Polar Biology* 16 (3):191-200.

Sohlenius, B., S. Boström, and K. Ingemar Jönsson. 2004. Occurrence of nematodes, tardigrades and rotifers on ice-free areas in East Antarctica. *Pedobiologia* 48 (4):395-408.

Stevens, M. I., and I. D. Hogg. 2006. The molecular ecology of Antarctic terrestrial and limnetic invertebrates and microbes. In *Trends in Antarctic Terrestrial and Limnetic Ecosystems*: Springer.

Stevens, M. I., P. Greenslade, I. D. Hogg, and P. Sunnucks. 2006. Southern Hemisphere springtails: Could Any Have Survived Glaciation of Antarctica? *Molecular Biology and Evolution* 23 (5):874-882.

Stevens, M. I., and I. D. Hogg. 2003. Long-term isolation and recent range expansion from glacial refugia revealed for the endemic springtail *Gomphiocephalus hodgsoni* from Victoria Land, Antarctica. *Molecular Ecology* 12 (9):2357-2369.

Stevens, M. I., D. Porco, C.A. D'Haese, and L. Deharveng. 2011. Comment on "taxonomy and the DNA barcoding enterprise" by Ebach (2011). *Zootaxa* 2838:85-88.

Sudzuki, M. 1979. On the microfauna of the antarctic region III. Microbiota of the terrestrial interstices. *Memoirs of National Institute of Polar Research. Special issue* 11:104.

Sunnucks, P. 2000. Efficient genetic markers for population biology. *Trends in Ecology & Evolution* 15 (5):199-203.

Suren, A. 1990. Microfauna associated with algal mats in melt ponds of the Ross Ice Shelf. *Polar Biology* 10 (5):329-335.

Swofford, D. L. 2002. PAUP* version 4.0. *Phylogenetic Analysis Using Parsimony (and Other Methods).*

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular biology and evolution* 28 (10):2731-2739.

Tumanov, D. V. 2006. Five new species of the genus *Milnesium* (Tardigrada, Eutardigrada, Milnesiidae). *Zootaxa* 1122:1-23.

Utsugi, K. and Y. Ohyama. 1991. Antarctic Tardigrada II. Molodezhnaya and Mt. Riiser-Larsen areas. *Proc. NIPR Symp. Polar Biology* 161-170.

Utsugi, K., and Y. Ohyama. 1993. Antarctic Tardigrada III. Fildes Peninsula of King George Island. *Polar Biology* 6:139-151.

Velasco-Castrillón, A., M. B. Schultz, F. Colombo, J. A. E. Gibson, K. A. Davies, A. D. Austin, and M. I. Stevens. 2014. Distribution and Diversity of Soil Microfauna from East Antarctica: Assessing the Link between Biotic and Abiotic Factors. *PLoS One.* 9 (1):e87529.

Velasco-Castrillón, A., and M. I Stevens. 2014. Morphological and molecular diversity at a regional scale: A step closer to understanding Antarctic nematode biogeography. *Soil Biology and Biochemistry* 70:272-284.

Vincent, W. F., and M. R. James. 1996. Biodiversity in extreme aquatic environments: lakes, ponds and streams of the Ross Sea sector, Antarctica. *Biodiversity & Conservation* 5 (11):1451-1471.

Vyverman, W., E. Verleyen, A. Wilmotte, D. A. Hodgson, A. Willems, K. Peeters, B. Van de Vijver, A. De Wever, F. Leliaert, and K. Sabbe. 2010. Evidence for widespread endemism among Antarctic micro-organisms. *Polar Science* 4 (2):103-113.

Webster-Brown, J., M. Gall, J. A. E. Gibson, S. Wood, and I. Hawes. 2010. The biogeochemistry of meltwater habitats in the Darwin Glacier region (80 S), Victoria Land, Antarctica. *Antarctic Science* 22 (6):646.

CHAPTER V – General Discussion

As this thesis is presented as a combination of published, accepted and submitted manuscripts to scientific peer-reviewed journals, the general discussion includes a broader discussion of individual chapters, and highlights the future directions on molecular studies to better comprehend limno-terrestrial microfaunal diversity.

Synthesis

This is the first single study to sample a wide range of limno-terrestrial habitats from East Antarctica (Enderby and Wilkes sectors) and to correlate soil geochemistry and other environmental variables with microfaunal diversity using the mitochondrial COI gene. Contrasting trends were observed across a wide spectrum of habitat requirements, with highly endemic taxa restricted to specific environments, to widespread and vastly common taxa able to withstand wide ranges of habitat types (Chapter II). Of special interest are the distributional patterns presented by some of the lineages to specific niches. The ability to withstand 'less-suitable' environments; such as high salinity and ornithogenic soils seems to restrict microfaunal diversity (e.g. Porazinska *et al.*, 2002), possibly reflecting physiological adaptations of organisms to survive in extreme environments. Type of diet has also been reported to play a role in determining species diversity, with microbivorous microfauna being more competitive in nitrogen rich soils than omnivores or predaceous species (e.g. Tenuta & Ferris, 2004; Brown *et al.*, 2004). Soil geochemistry has shown to be a relevant factor to consider when correlating diversity with sampling regions (Chapter II). I have shown that diversity is mostly the result of suitable refugia able to sustain life along with a suite of geochemical factors, more than the geographic region where species were found.

At an Antarctic scale this study has shown high levels of local endemicity for most tardigrade and nematode mitochondrial lineages, reflecting restricted dispersal capabilities and questioning (to some extent) the anhydrobiotic abilities for some of the organisms. Analogous results from various microbial studies (e.g. De Weber *et al.* 2009; Vyverman *et*

al. 2010) also imply that survival of microscopic organisms and occurrence of long distance dispersal events are rare questioning the idea of ubiquitous species (see Fontaneto *et al*., 2008). When considering aeolian transport as a way of colonising new environments we would expect those Antarctic microfauna (that form resistant propagules) that are able to avoid desiccation, osmotic stress and freezing conditions (e.g. McInnes & Pugh, 1998; Ricci *et al.*, 2007) to have higher chances of survival and colonisation. Transport by wind could in theory reduce the probability of geographical isolation and increase the diversity of gene pools (Fontaneto, 2011). Alternatively, the scarcity of suitable habitats for potential colonisers would restrict their geographical distribution. Considering the dispersal mechanisms and the high incidence of endemic lineages to specific sites raises the possibility of vicariance as a cause of speciation (isolation of populations via barriers to dispersal) for locally restricted and endemic lineages. As opposed to this 'strategy' I found that most bdelloids rotifer lineages, the highly common tardigrade *Acutuncus antarcticus* (Ta28) (Chapter IV), and the nematodes *Pl. murrayi* and *S.* cf. *lindsayae* (Chapter III) have a more widespread distribution in comparison to other lineages. The wide distribution range and low sequence divergence could either reflect (i) survival of a species in refugia during the last glacial maxima followed by a low mutation rate; or (ii) ability to withstand long-distance dispersal and having more generalist niche requirements. However, we also need to consider that lack of distributional records for our locally endemic species reflect a lack of rigorous sampling rather than absence of a species.

With this study I have also revealed cases of 'cosmopolitan species' (based on traditional taxonomy) to be complexes of divergent genetic entities, questioning their cosmopolitan 'label'. The process of determining the amount of sequence variation that lineages should have to be considered separate species is still under scrutiny. Studies have reported that the Poisson Tree Process (PTP; Zhang *et al*. 2013) and General Mixed Yules Coalescent (GMYC; Pons *et al*. 2006) models could be an option to estimate those species boundaries (e.g. Fontaneto *et al*., 2009, 2012; Tang *et al.* 2012; Zhang *et al.* 2013). In general, the species delimitation process could be facilitated if lineages are occurring in sympatry (e.g. divergent *Pl.* cf. *frigophilus* lineages from unique samples). The opposite scenario (allopatry) could also be valid to separate those lineages into separate species (e.g.

Pl. cf. *frigophilus* from Antarctica and TF) based on sequence divergence between two morphologically distinct species. When trying to understand sympatric divergence, it would be more evident for asexual species that do not require reproductive isolation; this could explain the cryptic diversity observed for divergent COI lineages (mainly bdelloids) from single soil samples. Parthenogenetic reproduction is not exclusive for rotifers, and it has also been reported for some tardigrades (e.g. Pilato & Binda, 2001; Bertolani, 2010). Organisms that undergo this type of reproduction could result in the absence of gene flow among populations and reduced genetic variability (Jørgensen, 2007) and eventually could lead to speciation (Fontaneto *et al.*, 2012; Birky *et al.*, 2005). Parthenogenesis has mainly been reported for parasitic nematodes (e.g. Castagnone-Sereno, 2006) and more recently for the genus *Plectus* (Adhikari *et al.*, 2010). In my work (Chapter III), absence of male nematodes was observed for the genus *Plectus*, which is in accordance with most studies (e.g. Timm, 1971; Andrássy, 1998; Sohlenius & Boström, 2008); even though presence of isolated *Plectus* males has been reported in the literature (Andrássy, 2008). It is possible that the low sequence divergence within *Plectus* lineages (despite their abundance and widespread distribution) could be the result of parthenogenesis.

I have also compared microfaunal diversity with that from Maud and Scott sectors, and from isolated locations in the Antarctic Peninsula, maritime Antarctica and Tierra del Fuego and from other world-wide locations. Here, I have greatly expanded the current knowledge on diversity levels and have shown potential new undescribed species reflected by divergent mitochondrial lineages (Chapters III, IV). For example, highly divergent mitochondrial lineages (> 5.7% divergence) were observed within the 'species' *Acutuncus antarcticus*, *Pl. murrayi*, *Pl.* cf. *frigophilus*. The molecular phylogenetic analyses revealed that nematode and tardigrade genera, morphologically identified, were monophyletic. Such was not the case for bdelloid rotifers which showed polyphyly for at least one genus (*Adineta*). The difficulty of discerning microfaunal specimens based merely on morphological characters has been discussed widely in the literature (e.g. Andrássy 1998; Floyd et al. 2002; Robeson et al. 2009), which in our case was more evident for bdelloid rotifers.

Genetic markers used to establish diversity have included maternally-inherited mitochondrial and biparentally-inherited nuclear genomes (Stevens & Hogg 2003; Sands *et al.*, 2008; Czechowski *et al.*, 2013). The use of the COI gene has shown to have appropriate resolution for the phylogenetic aspect of this study. This gene is also one of the most widely-used markers in comparative phylogeography and has been adopted as the faunal DNA barcoding gene (e.g., Hebert *et al.*, 2003; Ashton *et al.*, 2008; Prosser *et al*., 2013). So far, little work has been done on species delimitation within the Phyla Tardigrada, Nematoda and Rotifera, with various estimates that varied according to the study and the targeted genera. In general, from data presented here and from previous studies (for nematodes, tardigrades and rotifers), within sequence divergence of 3% or greater for the COI gene has shown to be a reasonable indicator separating morphologically distinct species, (e.g. Jørgensen *et al.*, 2007; Faurby *et al*., 2008; Elasser *et al*., 2009; Fontaneto *et al*., 2009; Kumari *et al*., 2010; Ristau *et al*., 2013). An aspect that needs to be considered is the high divergence shown by lineages within monophyletic genera (up to 30% for tardigrades), in some circumstances exceeding the divergence observed by lineages from different genera (Chapter IV). Although, monophyly was not always resolved for higher ranked taxa, such was the case for the Order Rhabditida (Nematoda) which showed polyphyletic relationships when using nucleotide sequences were subjected to Bayesian and ML analyses, or when performing analyses using amino-acid sequences (Chapter III). With my study I have shown the usefulness of the COI gene in discerning most of the closely related lineages; however caution needs to be taken when trying to resolve deeper crown nodes.

My study has also added to the scientific knowledge of microfaunal diversity from Antarctica and Tierra del Fuego by identifying 18, 43 and 46 distinctive mitochondrial lineages (for nematode, tardigrade and bdelloid rotifer, respectively), the majority of which are likely to represent new species. The mitochondrial DNA barcodes generated for this study now form a preliminary framework for conservation management plans, providing the baseline to assess current diversity and therefore identifying future foreign introductions. This work not only provides new haplotypes and morphology linked to those DNA barcodes but also incorporates microfaunal abundance, distribution and

environmental data making it the first of its kind performed at such a large scale. This is the first Antarctic study that provides COI sequences and distribution records for some of the most common species found in continental Antarctica (*Plectus murrayi, Pl.* cf. *frigophilus, Scottnema* cf. *lindsayae, Halomonhystera* cf. *halophila, H.* cf. *continentalis, Acutuncus antarcticus, Milnesium* cf. *tardigradum, Philodina* cf. *gregaria* and *Adineta* cf. *gracilis*), and can be used as an identification tool for future biogeographic and diversity studies.

Future directions

Research is required not only for flora and free living fauna but also for microbes and parasites. Studies have revealed a considerable and varied microbial diversity (algae, bacterial, cyanobacterial and fungi) in distinct habitats (e.g. Gordon *et al*., 2000; Aislabie *et al.*, 2008; Chong *et al.*, 2009; Pointing *et al.*, 2009; De Weber *et al.* 2009; Cary *et al*., 2010; Vyverman *et al*., 2010; Cowan *et al.* 2011; Peeters *et al.*, 2011) that need to be further examined across Antarctica. Studies on endosymbiotic bacteria (*Wolbachia, Rickettsia* and *Spiroplasma*) have been performed for northern hemisphere arthropods (Goodacre *et al*., 2006) but still need to be undertaken for Antarctic arthropods and other microfauna. Preliminary data indicate that these bacterial types coexist within at least some of the Antarctic arthropod species, information that could be utilized for an unprecedented co-evolutionary study on Antarctic history. Future work needs to involve identification of population origins and colonization routes, established by measures of haplotype and nucleotide diversity, taken into consideration that intraspecific diversity should decrease from sources populations (Avise, 1995; Hewitt, 1996). The relationship between geographical and genetic distances can also be evaluated and estimates of the number of differences between DNA sequences could be used to deduce the ages of population and species divergence events with the use of a molecular clock (De Weber *et al.* 2009; McGaughran *et al*., 2009). To have a better understanding of Antarctic biodiversity it would be necessary to include in the study the total community assemblages, along with the abiotic parameters. This would allow us to comprehend how the communities have

developed and the different requirements that are needed to sustain the various levels, from bacteria to the invertebrates and flora.

The genome of species contains significant indication of events from the past, in particular, patterns of genetic variation within and among populations that could provide information about origins and demography of a species. Understanding species' distributions and genetic diversity is fundamental to comprehend how global environmental changes have and will continue to influence global biodiversity. Without this knowledge we are unable to accurately monitor and protect the Earth's biodiversity.

It is well accepted that multi-gene approach (nuclear and mitochondrial) should be used to determine phylogenetic relationships of organisms in order to establish stronger relationships (Cunningham, 1997). In my study the COI gene showed to be relatively consistent in resolving nodes at species or genus rank, although the resolution for basal nodes in the phylogeny was not always supported by high bootstrap values or posterior probabilities. It could be expected that adding taxa would better resolve the phylogeny, especially for deeper nodes. Results for the present study showed that the amplification success varied according to the geographical area where samples were collected (with higher success rate in East Antarctica). There was a noticeable greater amplification rate for the most abundant microfaunal taxa (bdelloids, tardigrades and nematodes). Although for the nematode genus *Eudorylaimus*, for ciliates, mites, and monogonont rotifers we were not able to retrieve any PCR products. Possibly due to the lack of sequence similarity between primer and DNA binding sites. Which could reflect a fast mutation rate (even at the second codon position), biased substitution and high AT content (Blouin, 2000; Derycke *et al*., 2010). Recently, new developed specific primers for the COI gene (e.g. Prosser *et al*., 2013) have improved considerably the amplification success experienced by some Antarctic taxa when using invertebrate universal primers. The strategy of complementing the COI phylogeny using other molecular DNA markers (e.g. internal transcribed spacer (ITS) region, 28S, 18S, Wingless, and mtDNA COII and 16S) is always attractive and needed to be considered for future studies (e.g. Sands *et al*., 2008; Guidetti *et al*., 2009; Abrams *et al*., 2012) to reinforce the phylogenies for Antarctic biota.

With the advent of next generation sequencing, the possibilities of generating vast amounts of genetic data at a cost-effective way has shown an alternative to traditional Sanger sequencing. The development of 454 sequencing and other sequencing platforms (Solexa, SOLiD) has revealed the power of parallelisation and miniaturisation, increasing the amount of genetic data and decreasing costs (Rothberg & Leamon, 2008). More recently, 'Metabarcoding' using high-throughput sequencing technology has shown to be a viable option to mass-amplify taxonomically informative genes (using universal PCR primers) from vast samples of organisms and from environmental DNA (Ji *et al*., 2013). These authors showed metabarcoded samples to be taxonomically more comprehensive, quicker to produce and less dependent on taxonomical work compared with standard biodiversity data sets. With all these new technologies, the possibility to generate a DNA library for Antarctic microfauna has become more plausible than ever before. Nonetheless, taxonomical expertise is required to be able to assign DNA sequences to a reference library of morphologically identified species. In this study there is still the need to improve the level of taxonomic resolution observed for some of the mitochondrial lineages. This is mainly the case for bdelloid rotifers which were the most divergent group for all Antarctic microfauna, but the most difficult to identify using a morphological approach.

References

Abrams, K., Guzik, M.T., Cooper, S.J.B., Humphreys, W.F., King, R.A., Cho, J.-L. & Austin, A.D. (2012) What lies beneath: molecular phylogenetics and ancestral state reconstruction of the ancient subterranean Australian Parabathynellidae (Syncarida, Crustacea). *Molecular Phylogenetics and Evolution*, **64**, 130-144.

Adhikari, B.N., Tomasel, C.M., Li, G., Wall, D.H. & Adams, B.J. (2010) The antarctic nematode *Plectus murrayi*: An emerging model to study multiple stress survival. *Cold Spring Harbor Protocols*, **2010**, 1199-1204.

Aislabie, J., Jordan, S. & Barker, G. (2008) Relation between soil classification and bacterial diversity in soils of the Ross Sea region, Antarctica. *Geoderma*, **144**, 9-20.

Andrássy, I. (1998) Nematodes in the Sixth Continent. *Journal of Nematode Morphlogy and Systematics* (ed. by R. Peña Santiago), pp. 107-186. Universidad de Jaén, Jaén.

Andrássy, I. (2008) On the male of the Antarctic nematode species, *Plectus murrayi* Yeates, 1970. *Journal of Nematode Morphology and Systematics*, **11**, 87-89.

Ashton, G.V., Stevens, M.I., Hart, M.C., Green, D.H., Burrows, M.T., Cook, E.J. & Willis, K.J. (2008) Mitochondrial DNA reveals multiple Northern Hemisphere introductions of *Caprella mutica* (Crustacea, Amphipoda). *Molecular Ecology*, **17**, 1293-1303.

Avise, J.C. (1995) Molecular markers, natural history and evolution (Review). *Systematic Biology*, **44**, 117-120.

Bertolani, R., Rebecchi, L. & Cesari, M. (2010) A model study for tardigrade identification. *Tools for Identifying Biodiversity: Progress and Problems*, 333-339.

Birky Jr, C.W., Wolf, C., Maughan, H., Herbertson, L. & Henry, E. (2005) Speciation and selection without sex. *Rotifera X*, pp. 29-45. Springer.

Blouin, M. (2000) Brief communication. Neutrality tests on mtDNA: unusual results from nematodes. *Journal of Heredity*, **91**, 156-158.

Brown, I.M., Wharton, D.A. & Millar, R.B. (2004) The influence of temperature on the life history of the Antarctic nematode *Panagrolaimus davidi*. *Nematology*, **6**, 883-890.

Cary, S.C., McDonald, I.R., Barrett, J.E. & Cowan, D.A. (2010) On the rocks: the microbiology of Antarctic Dry Valley soils. *Nat Rev Micro*, **8**, 129-138.

Castagnone-Sereno, P. (2006) Genetic variability and adaptive evolution in parthenogenetic root-knot nematodes. *Heredity*, **96**, 282-289.

Chong, C., Tan, G.A., Wong, R.C., Riddle, M.J. & Tan, I.K. (2009) DGGE fingerprinting of bacteria in soils from eight ecologically different sites around Casey Station, Antarctica. *Polar biology*, **32**, 853-860.

Cowan, D.A., Pointing, S.B., Stevens, M.I., Cary, S.C., Stomeo, F. & Tuffin, I.M. (2011) Distribution and abiotic influences on hypolithic microbial communities in an Antarctic Dry Valley. *Polar biology*, **34**, 307-311.

Cunningham, C.W. (1997) Is congruence between data partitions a reliable predictor of phylogenetic accuracy? Empirically testing an iterative procedure for choosing among phylogenetic methods. *Systematic Biology*, **46**, 464-478.

Czechowski, P., Sands, C.J., Adams, B., D'Haese, C., Gibson, J., McInnes, S.J. & Stevens, M.I. (2012) Antarctic Tardigrada: a first step in understanding molecular operational taxonomic units (MOTUs) and biogeography of cryptic meiofauna. *Invertebrate Systematics*, **26**, 526-538.

De Wever, A., Leliaert, F., Verleyen, E., Vanormelingen, P., Van der Gucht, K., Hodgson, D.A., Sabbe, K. & Vyverman, W. (2009) Hidden levels of phylodiversity in Antarctic green algae: further evidence for the existence of glacial refugia. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 3591-3599.

Derycke, S., Vanaverbeke, J., Rigaux, A., Backeljau, T. & Moens, T. (2010) Exploring the Use of Cytochrome Oxidase c Subunit 1 (COI) for DNA Barcoding of Free-Living Marine Nematodes. *PLoS One*, **5**, e13716.

Elsasser, S.C., Floyd, R., Hebert, P.D.N. & Schulte-Hostedde, A.I. (2009) Species identification of North American guinea worms (Nematoda: *Dracunculus*) with DNA barcoding. *Molecular Ecology Resources*, **9**, 707-712.

Faurby, S., Jönsson, K.I., Rebecchi, L. & Funch, P. (2008) Variation in anhydrobiotic survival of two eutardigrade morphospecies: a story of cryptic species and their dispersal. *Journal of Zoology*, **275**, 139-145.

Floyd, R., Abebe, E., Papert, A. & Blaxter, M. (2002) Molecular barcodes for soil nematode identification. *Molecular Ecology*, **11**, 839-850.

Fontaneto, D., Kaya, M., Herniou, E.A. & Barraclough, T.G. (2009) Extreme levels of hidden diversity in microscopic animals (Rotifera) revealed by DNA taxonomy. *Molecular Phylogenetics and Evolution*, **53**, 182-189.

Fontaneto, D., Iakovenko, N., Eyres, I., Kaya, M., Wyman, M. & Barraclough, T. (2011) Cryptic diversity in the genus *Adineta* Hudson & Gosse, 1886 (Rotifera: Bdelloidea: Adinetidae): a DNA taxonomy approach. *Hydrobiologia*, **662**, 27-33.

Fontaneto, D., Tang, C., Obertegger, U., Leasi, F. & Barraclough, T. (2012) Different Diversification Rates Between Sexual and Asexual Organisms. *Evolutionary Biology*, **39**, 262-270.

Goodacre, S.L., Martin, O.Y., Thomas, C.F.G. & Hewitt, G.M. (2006) *Wolbachia* and other endosymbiont infections in spiders. *Molecular Ecology*, **15**, 517-527.

Gordon, D.A., Priscu, J. & Giovannoni, S. (2000) Origin and phylogeny of microbes living in permanent Antarctic lake ice. *Microbial Ecology*, **39**, 197-202.

Guidetti, R., Schill, R.O., Bertolani, R., Dandekar, T. & Wolf, M. (2009) New molecular data for tardigrade phylogeny, with the erection of *Paramacrobiotus* gen. nov. *Journal of Zoological Systematics and Evolutionary Research*, **47**, 315-321.

Hebert, P.D.N., Ratnasingham, S. & deWaard, J.R. (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270**, S96-S99.

Hewitt, G.M. (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247-276.

Ji, Y., Ashton, L., Pedley, S.M., Edwards, D.P., Tang, Y., Nakamura, A., Kitching, R., Dolman, P.M., Woodcock, P. & Edwards, F.A. (2013) Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. *Ecology letters*, **16**, 1245-1257.

Jørgensen, A., Møbjerg, N. & Kristensen, R.M. (2007) A molecular study of the tardigrade Echiniscus testudo (Echiniscidae) reveals low DNA sequence diversity over a large geographical area. **66**, 77-83.

Kumari, S., Decraemer, W., Luca, F. & Tiefenbrunner, W. (2010) Cytochrome c oxidase subunit 1 analysis of *Xiphinema diversicaudatum*, *X. pachtaicum*, *X. simile* and *X. vuittenezi* (Nematoda, Dorylaimida). *European Journal of Plant Pathology*, **127**, 493-499.

McGaughran, A., Redding, G.P., Stevens, M.I. & Convey, P. (2009) Temporal metabolic rate variation in a continental Antarctic springtail. *Journal of Insect Physiology*, **55**, 129-134.

McInnes, S.J. & Pugh, P.J.A. (1998) Biogeography of limno-terrestrial Tardigrada, with particular reference to the Antarctic fauna. *Journal of Biogeography*, **25**, 31-36.

Peeters, K., Hodgson, D.A., Convey, P. & Willems, A. (2011) Culturable diversity of heterotrophic bacteria in Forlidas Pond (Pensacola Mountains) and Lundström Lake (Shackleton Range), Antarctica. *Microbial ecology*, **62**, 399-413.

Pilato, G. & Binda, M.G. (2001) Biogeography and limno-terrestrial tardigrades: are they truly incompatible binomials? *Zoologischer Anzeiger-A Journal of Comparative Zoology*, **240**, 511-516.

Pointing, S.B., Chan, Y., Lacap, D.C., Lau, M.C.Y., Jurgens, J.A. & Farrell, R.L. (2009) Highly specialized microbial diversity in hyper-arid polar desert. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 19964-2009.

Pons, J., Barraclough, T.G., Gomez-Zurita, J., Cardoso, A., Duran, D.P., Hazell, S., Kamoun, S., Sumlin, W.D. & Vogler, A.P. (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic biology*, **55**, 595-609.

Porazinska, D., Wall, D. & Virginia, R. (2002) Invertebrates in ornithogenic soils at Ross Island, Antarctica. *Polar Biology*, **25**, 569-574.

Prosser, S., Velarde-Aguilar, M., León-Règagnon, V. & Hebert, P. (2013) Advancing nematode barcoding: A primer cocktail for the cytochrome c oxidase subunit I gene from vertebrate parasitic nematodes. *Molecular ecology resources*, **13**, 1108-1115.

Ricci, C., Caprioli, M. & Fontaneto, D. (2007) Stress and fitness in parthenogens: is dormancy a key feature for bdelloid rotifers? *BMC evolutionary biology*, **7**, S9.

Ristau, K., Steinfartz, S. & Traunspurger, W. (2013) First evidence of cryptic species diversity and significant population structure in a widespread freshwater nematode morphospecies (*Tobrilus gracilis*).

Robeson, M., Costello, E., Freeman, K., Whiting, J., Adams, B., Martin, A. & Schmidt, S. (2009) Environmental DNA sequencing primers for eutardigrades and bdelloid rotifers. *BMC Ecology*, **9**, 25.

Rothberg, J.M. & Leamon, J.H. (2008) The development and impact of 454 sequencing. *Nat Biotech*, **26**, 1117-1124.

Sands, C.J., Convey, P., Linse, K. & McInnes, S.J. (2008) Assessing meiofaunal variation among individuals utilising morphological and molecular approaches: An example using the Tardigrada. *BMC Ecology*, **8**, 1-11.

Sohlenius, B. & Boström, S. (2008) Species diversity and random distribution of microfauna in extremely isolated habitable patches on Antarctic nunataks. *Polar Biology*, **31**, 817-825.

Stevens, M.I. & Hogg, I.D. (2003) Long-term isolation and recent range expansion from glacial refugia revealed for the endemic springtail *Gomphiocephalus hodgsoni* from Victoria Land, Antarctica. *Molecular Ecology*, **12**, 2357-2369.

Tang, C.Q., Leasi, F., Obertegger, U., Kieneke, A., Barraclough, T.G. & Fontaneto, D. (2012) The widely used small subunit 18S rDNA molecule greatly underestimates true diversity in biodiversity surveys of the meiofauna. *Proceedings of the National Academy of Sciences*, **109**, 16208-16212.

Tenuta, M. & Ferris, H. (2004) Sensitivity of nematode life-history groups to ions and osmotic tensions of nitrogenous solutions. *Journal of Nematology*, **36**, 85-94.

Timm, R.W. (1971) Antarctic Soil and Freshwater Nematodes from the McMurdo Sound Region. *Proceedings of the Helminthological Society of Washington* **38**, 42-52.

Vyverman, W., Verleyen, E., Wilmotte, A., Hodgson, D.A., Willems, A., Peeters, K., Van de Vijver, B., De Wever, A., Leliaert, F. & Sabbe, K. (2010) Evidence for widespread endemism among Antarctic micro-organisms. *Polar Science*, **4**, 103-113.

Zhang, J., Kapli, P., Pavlidis, P. & Stamatakis, A. (2013) A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, **29**, 2869-2876.

APPENDIX 1

Table S1. Tardigrade species synoyms and possible misidentifications

Name used in Table	Synonyms	Source
Acutuncus antarcticus (Binda & Pilato, 2000)	*Hypsibius arcticus* (Murray, 1907)	Dastych 1991
"	*Hypsibius antarcticus* (Richters, 1904)	Dastych 1989
"	*Macrobiotus antarcticus* (Richters, 1904)	Dastych 1991
"	*Macrobiotus arcticus* Murray, 1910	Dastych 1991
"	*Hypsibius (H.) convergens* (Janetschek, 1962)	Dastych 1991
"	*Hypsibius (H.) dujardini* (Jennings, 1976)	Dastych 1991
"	*Hypsibius (H.) mertoni simoizumii* Sudzuki, 1964	Dastych 1991
"	*Hypsibius simoizumii* (Ramazzotti & Maucci, 1983)	Dastych 1991
"	*Hypsibius mertoni* (Opalinski 1972)	Dastych 1991
Diphascon chilenense langhovdense (Sudzuki, 1964)	*Diphascon chilenense* (Plate 1888)	Dastych 1989; Dastych 1989
Diphascon pingue (Marcus, 1936)	*Diphascon alpinum* (Murray, 1906)	Dastych 1984; Dastych 1989
"	*Diphascon pinguis* (Marcus, 1936)	Dastych 1984
"	*Diphascon (Diphascon) pinguis* (Jennings, 1976)	Dastych 1984
"	*Hypsibius (Diphascon) alpinus* (Jennings, 1976)	Dastych 1984
Echiniscus jenningsi Dastych, 1984	*Echiniscus capillatus* (Jennings 1976)	Dastych 1984; Dastych 1989; McInnes & Ellis-Evans 1987; Usher and Dastych 1987
Hebesuncus schusteri (Dastych, 1984)	*Diphascon conjungens* (Thulin, 1911)	McInnes 2013 pers. comm.
"	*Hebesuncus conjungens* (Thulin, 1911)	McInnes 2013 pers. comm.
"	*Diphascon schusteri* Dastych, 1984	www.invertebrates.si.edu
Isohypsibius papillifer (Murray 1905)	*Macrobiotus papillifer* Murray, 1905	www.eol.org
"	*Hypsibius papillifer* (Murray, 1905)	www.eol.org
Macrobiotus krynauwi Dastych & Harris, 1995	*Macrobiotus furciger* (Murray, 1907)	Sohlenius et al. 2004
"	*Macrobiotus furcatus* (Ehrenberg, 1859)	Dastych 1984; McInnes & Ellis-Evans 1987; Usher & Dastych 1987
"	*Minibiotus furcatus* (Ehrenberg, 1859)	www.itis.gov
Minibiotus weinerorum (Dastych, 1984)	*Macrobiotus weinerorum* Dastych, 1984	www.invertebrates.si.edu
Minibiotus stuckenbergi (Dastych, Ryan & Watkins, 1990)	*Macrobiotus stuckenbergi* Dastych, Ryan & Watkins, 1990	Dastych, 1998.
Pseudechiniscus suillus (Ehrenberg, 1853)	*Echiniscus arctomy* Murray, 1910	Dastych 1984; Dastych 1989
Ramajendas renaudi (Ramazzotti, 1972)	*Hypsibius renaudi* Ramazzotti, 1972	www.itis.gov
"	*Isohypsibius renaudi* (Ramazzotti, 1972)	www.itis.gov
Ramazzottius oberhäuseri (Doyère, 1840)	*Hypsibius (Hypsibius) oberhaeuseri* (Doyère, 1840)	http://www.tmbl.gu.se/
"	*Macrobiotus oberhaeuseri* (Doyère, 1840)	http://www.tmbl.gu.se/
**Diphascon (Diphascon) pingue* ('Variety A') Marcus, 1936	It is a species-group	Dastych 1984, McInnes 1995, Pilato and Binda 1998

Possible misidentifications	
Echiniscus kerguelensis Richters, 1904	Record from SS-SO could be *E. jenningsi* at KGI. Records from EnL could be either *E. jenningsi* or *E. pseudowendti*
Amphibolus volubilus Durante Pasa & Maucci, 1975	It could correspond to *Dactylobiotus*
Diphascon (Diphascon) alpinum Murray, 1906	It could be *Diphascon (Diphascon) langhovdense*
Diphascon (Diphascon) higginsi Binda, 1971	It could probably be *Diphascon (Adropion) greveni*
Diphascon (Diphascon) scoticus Murray, 1905	This is a misidentification and could be *Diphascon (Adropion)* type
Hypsibius cf. *convergens* (Urbanowicz, 1925)	It is probably *Acutuncus*
Isohypsibius saracenus Pilato, 1973	It could be *Acutuncus* or an unnamed *Isohypsibius*
Macrobiotus harmsworthi coronatus (Utsugi, 1991)	It is probably *Macrobioutus blocki* or *M. krynauwi*
Macrobiotus harmsworthi (Barros, 1942)	It is probably *Macrobioutus blocki* or *M. krynauwi*
Macrobiotus montanus Murray, 1910	Identification was based on an egg. The egg could belong to another genus or it could be *M. mottai*

Table S2. List of Rotifer species reported by Segers (2007) for Antarctica (not found in any other literature for continental or maritime Antarctica).

The records in Segers (2007) for Antarctica include: continental Antarctica, maritime Antarctica and sub-Antarctic Islands

Class Monogononta	**Class Bdelloidea**
Brachionus amsterdamensis De Smet, 2001	*Habrotrocha angusticollis angusticollis* (Murray, 1905)
Cephalodella rotunda bryophila (Pawlowski, 1938)	*Habrotrocha crenata crenata* (Murray, 1905)
Ceratotrocha cornigera (Bryce, 1893)	*Habrotrocha pulchra* (Murray, 1905)
Collotheca campanulata (Dobie, 1849)	*Macrotrachela kallosoma* (Schulte, 1954)
Colurella adriatica Ehrenberg, 1831	*Macrotrachela musculosa* (Milne, 1886)
Colurella salina Althaus, 1957	*Mniobia burgeri* Bartoš, 1951
Euchlanis oropha Gosse, 1887	*Philodina jeanneli* de Beauchamp, 1940
Filinia pejleri Hutchinson, 1964	*Philodina plena* (Bryce, 1894)
Keratella kostei Paggi, 1981	
Keratella sancta Russell, 1944	
Lecane closterocerca (Schmarda, 1859)	
Lophocharis oxysternon (Gosse, 1851)	
Microcodon clavus Ehrenberg, 1830	
Mytilina mucronata longicauda Dartnall & Hollowday, 1985	
Notholca hollowdayi Dartnall, 1995	
Notholca squamula (Müller, 1786)	
Notommata cyrtopus cyrtopus Gosse, 1886	
Pourriotia carcharodonta De Smet, 2003	
Rhinoglena frontalis Ehrenberg, 1853	
Trichocerca bidens (Lucks, 1912)	
Trichocerca tigris (Müller, 1786)	

Table S3. Rotifer species synonyms

Name used in Table	Synonyms	Source
Adineta vaga (Davis, 1873)	*Callidina vaga* Davis, 1873	Segers 2007
Habrotrocha angularis (Murray, 1910)	*Callidina angularis* Murray 1910	Segers 2007
Habrotrocha constricta Dujardin, 1841	*Callidina constricta* Dujardin, 1841	Segers 2007
Habrotrocha crenata crenata Murray, 1905	*Mniobia scarlatina f. angulata* Bartoš, 1938	Segers 2007
Habrotrocha pulchra (Murray, 1905)	*Callidina pulchra* Murray, 1905	Segers 2007
Habrotrocha tridens Milne, 1886	*Macrotrachela tridens* Milne, 1886	Segers 2007
Macrotrachela concinna Bryce, 1912	*Callidina concinna* Bryce, 1912	Segers 2007
Macrotrachela constricta Milne, 1886	*Callidina constricta* Dujardin, 1841	W De Smet
Macrotrachela habita (Bryce, 1894)	*Callidina habita* Bryce, 1894	Segers 2007
"	*Mniobia bulbifera* Bartoš, 1939	Segers 2007
"	*Mniobia gibbosa* Bartoš, 1938	Segers 2007
Macrotrachela kallosoma (Schulte, 1954)	*Mniobia kallosoma* Schulte, 1954	Segers 2007
Macrotrachela musculosa (Milne, 1886)	*Callidina musculosa* Milne, 1886	Segers 2007
Macrotrachela quadricornifera quadricornifera Milne, 1886	*Macrotrachela serrulata* Rodewald, 1935	Segers 2007
Mniobia burgeri Bartos, 1951	*Mniobia ostensa* (Donner, 1980)	Segers 2007
Mniobia russeola (Zelinka, 1891)	*Callidina russeola* Zelinka, 1891	Segers 2007
Mniobia symbiotica (Zelinka, 1886)	*Callidina symbiotica* Zelinka, 1886	Segers 2007
Otostephanos torquatus (Bryce, 1913)	*Habrotrocha torquata* Bryce, 1913	Segers 2007
Philodina plena (Bryce, 1894)	*Callidina plena* Bryce, 1894	Segers 2007
Rotaria rotatoria (Pallas, 1766)	*Brachionus rotatorius* Pallas, 1766	Segers 2007
Brachionus angularis Gosse, 1851	*Brachionus angularis orientalis* Sudzuki, 1989	Segers 2007
Brachionus calyciflorus calyciflorus Pallas, 1766	*Brachionus pala* Ehrenberg, 1838	Segers 2007
"	*Brachionus amphiceros* Ehrenberg, 1838	Segers 2007
"	*Brachionus dorcas* Gosse, 1851	Segers 2007
"	*Brachionus gillardi* Hauer, 1966	Segers 2007
"	*Brachionus pala* Ehrenberg, 1838	Segers 2007
"	*Brachionus pala anuraeiformis* Brehm, 1909	Segers 2007
Brachionus bidentatus bidentatus Anderson, 1889	*Brachionus bidentatus* var. *crassispinus* Hauer, 1963	Segers 2007
"	*Brachionus furculatus* Thorpe, 1891	Segers 2007
"	*Brachionus furculatus inermis* Rousselet, 1906	Segers 2007
"	*Brachionus furculatus testudinarius* Jakubski, 1912	Segers 2007
"	*Brachionus furculatus* var. *jirovci* Bartoš, 1946	Segers 2007
Brachionus quadridentatus quadridentatus Hermann, 1783	*Brachionus ancylognathus* Schmarda, 1859	Segers 2007
"	*Brachionus brevispinus* Ehrenberg, 1832	Segers 2007
"	*Brachionus capsuliflorus* Pallas, 1766	Segers 2007
"	*Brachionus cluniorbicularis* Skorikov, 1894	Segers 2007
"	*Brachionus cluniorbicularis isigakiensis* Sudzuki, 1992	Segers 2007
"	*Brachionus rhenanus* Lauterborn, 1893	Segers 2007
Brachionus havanaensis trahea Murray, 1913	*Brachionus trahea* Murray, 1913	Segers 2007
Brachionus urceolaris Müller, 1773	*Brachionus urceolaris semicircularis* Sudzuki, 1989	Segers 2007
Brachionus urceolaris urceolaris Müller, 1773	*Tubipora urceus* Linnaeus, 1758	Segers 2007
Bryceella stylata (Milne, 1886)	*Stephanops stylatus* Milne, 1886	Segers 2007
Cephalodella auriculata (Müller, 1773)	*Vorticella auriculata* Müller, 1773	Segers 2007
"	*Cephalodella prompta* Neiswestnova-Shadina, 1935	Segers 2007
Cephalodella catellina (Müller, 1786)	*Cercaria catellina* Müller, 1786	Segers 2007
"	*Cephalodella armata* Rudescu, 1960	Segers 2007
"	*Cephalodella botezati* Rodewald, 1935	Segers 2007
"	*Cephalodella catellina natans* Bērziņš, 1976	Segers 2007
"	*Cephalodella myersi* Wiszniewski, 1934	Segers 2007
Cephalodella delicata Wulfert, 1937	*Cephalodella eudelicata* Wulfert, 1961	Segers 2007
Cephalodella forficata (Ehrenberg, 1832)	*Notommata forficata* Ehrenberg, 1832	Segers 2007
Cephalodella gibba (Ehrenberg, 1830)	*Furcularia gibba* Ehrenberg, 1830	Segers 2007
"	*Cephalodella microdactyla* Koch-Althaus, 1963	Segers 2007
Cephalodella megalocephala (Glascott, 1893)	*Furcularia megalocephala* Glascott, 1893	Segers 2007
Cephalodella sterea (Gosse, 1887)	*Furcularia sterea* Gosse, 1887	Segers 2007
"	*Cephalodella sterea* exoculis Bērziņš, 1976	Segers 2007
"	*Cephalodella serrata* Wulfert, 1937	Segers 2007
Cephalodella tenuior (Goose, 1886)	*Diaschiza tenuior* Gosse, 1886	Segers 2007

Table S3 (continued)

Name used in Table	Synonyms	Source
Cephalodella ventripes angustior Donner, 1950	*Diaschiza ventripes* Dixon-Nuttall, 1901	Segers 2007
Ceratotrocha cornigera (Bryce, 1893)	Callidina cornigera Bryce, 1893	Segers 2007
Collotheca campanulata (Dobie, 1849)	*Floscularia campanulata* Dobie, 1849	Segers 2007
"	*Collotheca gracilipes* Edmondson, 1939	Segers 2007
"	*Floscularia longicaudata* Hudson, 1883	Segers 2007
Collotheca ornata cornuta (Dobie, 1849)	*Floscularia appendiculata* Leydig, 1854	Segers 2007
Colurella adriatica Ehrenberg, 1831	*Monura bartonia* Gosse, 1887	Segers 2007
"	*Colurus caudatus* Ehrenberg, 1834	Segers 2007
"	*Monura dulcis* Ehrenberg, 1838	Segers 2007
"	*Colurus leptus* Gosse, 1887	Segers 2007
"	*Colurus navalis* Lord, 1884	Segers 2007
Colurella colurus colurus (Ehrenberg, 1830)	*Colurus amblytelus* Gosse, 1886	Segers 2007
"	*Colurus grallator* Gosse, 1887	Segers 2007
"	*Monura loncheres* Gosse, 1887	Segers 2007
"	*Colurella longidigita* Mola, 1930	Segers 2007
"	*Colurus rotundatus* Daday, 1890	Segers 2007
Colurella colurus compressa (Lucks, 1912)	*Colurus compressus* Lucks, 1912	Segers 2007
Encentrum mustela Milne, 1885	*Pleurotrocha mustela* Milne, 1885	Segers 2007
Encentrum permolle Gosse, 1886	*Diglena permollis* Gosse, 1886	Segers 2007
Encentrum uncinatum (Milne, 1886)	*Diglena uncinata* Milne, 1886	Segers 2007
Euchlanis dilatata dilatata Ehrenberg, 1832	*Euchlanis hipposideros* Gosse, 1851	Segers 2007
"	*Euchlanis uniseta* Leydig, 1854	Segers 2007
Euchlanis oropha Gosse, 1887	*Euchlanis parva* Rousselet, 1892	Segers 2007
Filinia pejleri Hutchinson, 1964	*Filinia terminalis kergueleniensis* Lair & Koste, 1984	Segers 2007
Habrotrocha crenata crenata (Murray, 1905)	*Mniobia scarlatina f. angulata* Bartoš, 1938	Segers 2007
Habrotrocha pulchra (Murray, 1905)	*Callidina pulchra* Murray, 1905	Segers 2007
Kellicottia longispina (Kellicott, 1879)	*Anuraea longispina* Kellicott, 1879	Segers 2007
"	*Notholca longispina heterospina* Olofsson, 1917	Segers 2007
"	*Notholca longispina taymirica* Grese, 1955	Segers 2007
Keratella americana Carlin, 1943	*Keratella gracilenta* Ahlstrom, 1943	Segers 2007
"	*Keratella lenzi caudata* Koste, 1972	Segers 2007
Keratella cochlearis Gosse, 1851	*Anuraea cochlearis* Gosse, 1851	Segers 2007
Keratella kostei Paggi, 1981	*Keratella heywoodi* Dartnall, 2005	Segers 2007
Keratella quadrata Müller, 1786	*Brachionus quadratus* Müller, 1786	Segers 2007
Keratella valga (Ehrenberg, 1834)	*Anuraea valga* Ehrenberg, 1834	Segers 2007
Lecane closterocerca (Schmarda, 1859)	*Monostyla closterocerca* Schmarda, 1859	Segers 2007
"	*Monostyla brodskii* Muraveisky, 1935	Segers 2007
"	*Lecane closterocerca amazonica* Koste, 1978	Segers 2007
"	*Monostyla eichsfeldica* Künne, 1926	Segers 2007
"	*Monostyla latvica* Bērziņš, 1943	Segers 2007
"	*Lecane wulferti* Hauer, 1956	Segers 2007
Lecane flexilis Gosse, 1886	*Distyla flexilis* Gosse, 1886	Segers 2007
"	*Cathypna brevis* Murray, 1913	Segers 2007
"	*Lecane glypta* Harring & Myers, 1926	Segers 2007
Lecane latissima Yamamoto, 1955	*Lecane kostei* De Ridder, 1966	Segers 2007
	Cathypna rotundata Olofsson, 1918	Segers 2007
Lecane lunaris (Ehrenberg, 1832)	*Monostyla lunaris* Ehrenberg, 1832	Segers 2007
"	*Monostyla constricta* Murray, 1913	Segers 2007
"	*Lecane lunaris arthrodactyla* Bērziņš, 1982	Segers 2007
"	*Lecane lunaris australis* Bērziņš, 1982	Segers 2007
"	*Monostyla lunaris obserata* Steinecke, 1916	Segers 2007
"	*Monostyla quennerstedti* Bergendal, 1892	Segers 2007
"	*Monostyla sylvatica* Harring, 1913	Segers 2007
"	*Monostyla virga* Harring, 1914	Segers 2007
Lepadella (Lepadella) acuminata (Ehrenberg, 1834)	*Metopidia acuminata* Ehrenberg, 1834	Segers 2007
"	*Lepadella chorea* Bērziņš, 1982	Segers 2007
"	*Lepadella sexcostata* Bartoš, 1955	Segers 2007
Lepadella patella Müller, 1773	*Brachionus patella* Müller, 1773	Segers 2007

Table S3 (continued)

Name used in Table	Synonyms	Source
Lepadella patella oblonga Ehrenberg, 1834	*Squamella oblonga* Ehrenberg, 1834	Segers 2007
"	*Lepadella minor* Koch-Althaus, 1963	Segers 2007
Lepadella triptera (Ehrenberg, 1832)	*Metopidia triptera* Ehrenberg, 1832	Segers 2007
"	*Lepadella alata* Myers, 1934	Segers 2007
"	*Lepadella alona* Wulfert, 1956	Segers 2007
"	*Lepadella clydona* Bērziņš, 1949	Segers 2007
"	*Lepadella crestata* Vasisht & Battish, 1971	Segers 2007
"	*Lepadella deconincki* De Ridder, 1966	Segers 2007
Lindia torulosa Dujardin, 1841	*Notommata roseola* Perty, 1850	Segers 2007
"	*Notommata tardigrada* Leydig, 1854	Segers 2007
Lophocharis oxysternon (Gosse, 1851)	*Metopidia oxysternon* Gosse, 1851	Segers 2007
Macrotrachela kallosoma (Schulte, 1954)	*Mniobia kallosoma* Schulte, 1954	Segers 2007
Macrotrachela musculosa (Milne, 1886)	*Callidina musculosa* Milne, 1886	Segers 2007
Mniobia burgeri Bartoš, 1951	*Mniobia ostensa* Donner, 1980	Segers 2007
Notholca foliacea (Ehrenberg, 1838)	*Anuraea foliacea* Ehrenberg, 1838	Segers 2007
Notholca salina Focke, 1961	*Notholca squamula evensi* Gillard, 1948	Segers 2007
Notholca squamula (Müller, 1786)	*Brachionus squamula* Müller, 1786	Segers 2007
"	*Notholca lapponica* Ruttner Kolisko, 1966	Segers 2007
"	*Notholca striata striata frigida* Rylov, 1922	Segers 2007
Notommata cyrtopus cyrtopus Gosse, 1886	*Notommata carpatica* Rodewald, 1935	Segers 2007
"	*Notommata curvipes* Zawadowsky, 1926	Segers 2007
"	*Notommata distincta* Bergendal, 1892	Segers 2007
"	*Notommata telmata* Harring & Myers, 1922	Segers 2007
Paradicranophorus sordidus Donner, 1968	*Encentrum brevifulcrum* Dartnall, 1997	Segers 2007
Philodina plena (Bryce, 1894)	*Callidina plena* Bryce, 1894	Segers 2007
Proales reinhardti (Ehrenberg, 1834)	*Furcularia reinhardti* Ehrenberg, 1834	Segers 2007
Proales fallaciosa Wulfert, 1937	*Proales tyrphosa* Bērziņš, 1949	Segers 2007
Ptygura crystallina (Ehrenberg, 1834)	*Oecistes crystallinus* Ehrenberg, 1834	Segers 2007
Ptygura melicerta melicerta (Ehrenberg, 1832)	*Oecistes ptygura* Hudson & Gosse, 1886	Segers 2007
"	*Oecistes serpentinus* Gosse, 1886	Segers 2007
"	*Oecistes socialis* Weber, 1888	Segers 2007
Resticula gelida (Harring & Myers, 1922)	*Eosphora gelida* Harring & Myers, 1922	Segers 2007
Rhinoglena fertoeensis (Varga, 1929)	*Rhinops fertoeensis* Varga, 1928	Segers 2007
Rhinoglena frontalis Ehrenberg, 1853	*Rhinops vitrea* Hudson, 1869	Segers 2007
Scaridium longicaudum Müller, 1786	*Trichoda longicauda* Müller, 1786	Segers 2007
Trichocerca bidens (Lucks, 1912)	*Diurella bidens* Lucks, 1912	Segers 2007
"	*Diurella bidens astriata* Rodewald, 1935	Segers 2007
"	*Trichocerca cavia* auct.	Segers 2007
Trichocerca brachyura (Gosse, 1851)	*Monocerca brachyura* Gosse, 1851	Segers 2007
"	*Rattulus palpitatus* Stokes, 1896	Segers 2007
Trichocerca rattus Müller, 1776	*Trichoda rattus* Müller, 1776	Segers 2007
"	*Rattulus carinatus* Lamarck, 1816	Segers 2007
"	*Trichoda criceta* Schrank, 1803	Segers 2007
"	*Trichocerca cristata* Harring, 1913	Segers 2007
"	*Brachionus cylindricus* Schrank, 1776	Segers 2007
"	*Monocerca longicauda* Bory de St. Vincent, 1826	Segers 2007
"	*Trichocerca minor* Fadeew, 1925	Segers 2007
Trichocerca tigris (Müller, 1786)	*Trichoda tigris* Müller, 1786	Segers 2007
"	*Heterognathus macrodactylus* Schmarda, 1859	Segers 2007

Table S4. Nematode species synonyms

Name used in Table	Synonyms	Source
Amblydorylaimus isokaryon (Loof, 1975) Andrássy, 1998	*Eudorylaimus isokaryon* Loof, 1975	Andrássy, 1998
Laimaphelenchus helicosoma (Maslen, 1979) Peneva & Chipev, 1999	*Aphelenchoides helicosoma* Maslen, 1979	Boström 2014, pers. comm.
Calcaridorylaimus signatus (Loof, 1975) Andrássy, 1986	*Mesodorylaimus signatus* Loof, 1975	Boström 2014, pers. comm.
Ceratoplectus armatus (Butschli, 1873) Andrássy, 1984	*Plectus armatus* Butschli, 1873	http://www.gbif.org/
"	*Plectus arctus* Truskova, 1976	http://www.gbif.org/
Coomansus gerlachei (de Man, 1904) Jairajpuri & Khan, 1977	*Mononchus gerlachei* de Man, 1904	Andrássy, 1998
"	*Clarkus gerlachei* (de Man, 1904) Jairajpuri, 1970	Andrássy, 1998
"	*Coomansus intestinus* apud Andrássy (1993)	Andrássy, 1998
Eudorylaimus antarcticus (Steiner, 1916) Yeates, 1970	*Dorylaimus antarcticus* Steiner, 1916	Andrássy, 1998, 2008; Adams et al. 2006
"	*Antholaimus antarcticus* (Steiner, 1916) Thorne & Swanger, 1936	Andrássy, 1998, 2008
Eudorylaimus glacialis Andrássy, 1998	*Eudorylaimus antarcticus* apud Yeates, 1970 & Loof, 1975	Andrássy, 2008
Eudorylaimus quintus Andrássy, 2008	*Eudorylaimus antarcticus* Timm, 1971	Andrássy, 2006
Eutobrilus antarcticus Tsalolikhin, 1981	*Raritobrilus antarcticus* (Tsaloliknim, 1981)	Gagarin, 2009
Geomonhystera antarcticola Andrássy, 1998	*Monhystera villosa* apud Timm, 1971	Andrássy, 1998
Geomonhystera villosa (Butschli, 1873) Andrássy, 1981	*Monhystera villosa* Butschli, 1873	Andrássy, 1981
"	*Monhystera insignis* Cobb, 1893	Andrássy, 1981
"	*Monhystera impetuosa* Cobb, 1904	Andrássy, 1981
"	*Monhystera mali* Fuchs, 1938	Andrássy, 1981
"	*Monhystera paravillosa* Meyl, 1954	Andrássy, 1981
Halomonhystera antarctica (Cobb, 1914) Andrássy, 2006	*Monhystera antarctica* Cobb, 1914	Andrássy, 2006
"	*Geomonhystera antarctica* (Cobb, 1914) Jacobs, 1987	Andrássy, 2006
Halomonhystera disjuncta (Bastian, 1865) Andrássy, 2006	*Monhystera disjuncta* Bastian, 1865	Andrássy, 2006
"	*Geomonhystera disjuncta (*Bastian, 1865) Jacobs, 1987	Andrássy, 2006
"	*Monhystera ambigua* Bastian, 1865	Andrássy, 2006
"	*Monhystera vivipara* Allgén, 1929	Andrássy, 2006
"	*Desmolaimus viviparus* Allgén, 1929	Andrássy, 2006
"	*Monhystera paraambigua* Allgén, 1933	Andrássy, 2006
"	*Monhystera paradisjuncta* De Coninck, 1943	Andrássy, 2006
"	*Geomonhystera paradisjuncta* (De Coninck, 1943) Jacobs, 1987	Andrássy, 2006
Halomonhystera uniformis (Cobb, 1914) Andrássy, 2006	*Monhystera uniformis* Cobb, 1914	Andrássy, 2006
"	*Geomonhystera uniformis* (Cobb, 1914) Jacobs, 1987	Andrássy, 2006
"	*Monhystera barentsi* Steiner, 1916	Andrássy, 2006
Plectus antarcticus de Man, 1904	*Plectus (Plectoides) antarcticus* de Man, 1904	Andrássy, 1998
Plectus murrayi Yeates, 1970	*Plectus belgicae* Steiner, 1916	Andrássy, 1998
"	*Plectus antarcticus* (Kirjanova, 1958; Timm, 1971; Yeates, 1979; Kito et al., 1991; Vinciguerra, 1994; Heyns, 1995)	Andrássy, 1998
"	*Plectus acuminatus* apud (Bostrom, 1995, 1997)	Andrássy, 1998
Rhyssocolpus paradoxus (Loof, 1975) Andrássy, 1986	*Eudorylaimus paradoxus* (Loof, 1975)	http://www.gbif.org/

APPENDIX 2

Table S1. Location, abiotic parameters and meiofauna abundance for 109 samples from East Antarctica

Sample	Gr	Ele (m)	Coordinates South	Coordinates East	Collection date	Categ	EC *dS/m*	Org C%	P *mg/kg*	NO_3 *p.p.m*	NH_3 *p.p.m*	Moi %	pH	Nem/ gdw	Tard/ gdw	Rot/ gdw	Cil/ gdw	Mit/ gdw	Abun / gdw
SP-04	5	4	-69.40	76.09	22/01/2010	al-cy	0.88	9.88	69	3.4	372.6	77.05	5.9	2.79	672.1	934.2	0.00	exu	1609
MP-06	5	70	-68.85	77.94	1/02/2010	al-cy	0.23	2.07	177.2	3.4	98.1	29.15	5.1	0.00	23.71	707.3	0.68	0.00	731.7
SI-02	5	15	-69.71	73.75	3/02/2010	moss	0.14	2.69	43.9	10.8	20.1	1.22	5.4	0.00	140.8	483.9	0.00	0.00	624.7
SI-03	5	15	-69.71	73.75	3/02/2010	moss	0.48	2.39	123.5	3.4	222	20.01	6.4	115.2	12.00	360.6	41.4	0.00	529.3
VH-07	5	15	-68.60	77.96	14/02/2010	al-cy	0.25	0.09	8.9	6.8	15	18.96	8.2	0.00	75.83	397.2	0.00	0.00	473.0
SI-01	5	20	-69.71	73.75	3/02/2010	moss	0.13	3.33	82	3.4	117	12.56	7.3	5.49	30.74	434.8	0.00	0.00	471.0
BP-10	4	68	-69.39	76.38	18/01/2010	inorg	0.02	0.28	28.2	3.4	5.1	6.42	5.2	0.00	0.00	303.9	0.00	0.03	304.0
CS-08	8	30	-66.28	110.52	24/12/2009	moss	0.66	3.48	57.4	3.4	28.5	19.55	5.6	28.39	80.00	124.7	0.00	0.01	233.1
L-Isl1-02	8	21	-69.41	76.00	23/01/2010	moss	0.13	1.46	113.9	3.4	14.1	25.85	5.2	0.65	49.49	156.7	0.00	0.00	206.9
VH-10	5	6	-68.50	78.08	28/02/2010	al-cy	3.02	1.08	67.2	6.8	31.2	24.44	7.6	27.63	4.66	99.74	2.54	0.00	134.6
MS-06	8	16	-67.60	62.87	15/02/2010	lichen	0.07	2.22	86.2	12.2	16.8	0.66	6.0	6.76	71.22	52.47	0.00	0.00	130.5
VH-21	5	47	-68.58	78.24	3/02/2010	al-cy	3.5	5.48	1.9	4.1	7.8	58.39	6.7	0.00	16.15	22.30	85.6	0.00	124.0
HI-10	5	32	-68.83	77.68	29/01/2010	al-cy	0.57	2.84	74.9	3.4	24.9	36.35	5.6	0.00	31.23	80.50	5.03	0.00	116.8
MP-05	8	70	-68.85	77.94	1/02/2010	moss	0.06	0.79	38.4	3.4	4.8	12.06	6.5	14.93	11.35	89.02	0.10	exu	115.4
SP-06	8	10	-69.40	76.09	22/01/2010	moss	0.15	2.9	88.7	3.4	63.9	18.57	6.3	3.73	31.49	58.36	0.25	0.64	94.46
HI-04	7	15	-68.83	77.69	28/01/2010	moss	0.29	0.27	53.3	3.4	10.5	5.45	5.2	0.76	3.81	66.17	0.00	0.00	70.74
CS-13	8	34	-66.28	110.53	24/12/2009	moss	0.37	8.4	108.3	3.4	42.3	67.08	4.7	0.61	8.26	41.43	0.00	0.00	50.30
CS-10	8	25	-66.28	110.52	24/12/2009	moss	0.45	6.14	60.7	3.4	35.1	19.4	5.4	16.50	10.12	21.84	0.00	0.12	48.59
HI-05	8	15	-68.83	77.70	28/01/2010	al-cy	48.1	0.71	51.5	3.4	9.6	16.6	6.7	0.05	3.61	43.88	0.00	0.29	47.83
HI-01	5	35	-68.82	77.71	27/01/2010	al-cy	0.94	0.77	60.5	41.5	43.5	1.88	6.0	0.00	0.00	44.76	1.69	0.00	46.45
CS-06	7	31	-66.28	110.52	24/12/2009	moss	0.13	1.14	32.7	3.4	21.3	8.75	5.5	10.04	0.79	27.18	0.00	0.00	38.01
VH-14	8	60	-68.57	78.48	10/03/2010	moss	0.41	3.64	122.1	6.8	57.3	1.14	6.1	0.16	0.00	28.12	0.00	0.00	28.28
VH-09	11	6	-68.50	78.08	28/02/2010	al-cy	1.11	0.18	19.4	6.1	14.4	14.09	8.0	26.64	0.00	1.21	0.12	0.00	27.97
MS-04	8	5	-67.60	62.86	12/02/2010	al-cy	0.25	0.38	10.8	3.4	6.6	3.79	6.7	0.00	17.88	9.27	0.00	0.00	27.15
CS-05	7	30	-66.28	110.52	24/12/2009	moss	0.04	0.54	34.8	3.4	24.6	6.31	5.3	0.10	1.28	22.29	0.00	0.00	23.66
VH-01	7	25	-68.48	78.42	14/01/2010	inorg	0.08	2.37	27.5	3.4	60.6	26.74	6.7	2.89	3.99	16.22	0.00	0.00	23.10
CS-11	7	34	-66.28	110.53	24/12/2009	inorg	0.28	8.49	99.4	4.7	31.5	63.27	4.7	0.71	4.79	12.52	0.00	0.00	18.02
L-Isl1-04	7	21	-69.41	76.00	23/01/2010	moss	0.03	2.4	27.1	3.4	11.4	24.18	4.8	1.27	0.95	14.77	0.00	0.11	17.09
MS-03	7	24	-67.60	62.87	12/02/2010	moss	0.08	0.42	15.4	12.9	8.4	8.76	6.0	3.55	8.88	4.10	0.00	0.00	16.53
L-Isl1-01	7	21	-69.41	76.00	23/01/2010	inorg	0.26	1.85	114.2	3.4	14.7	22.96	4.6	3.71	2.18	10.22	0.00	0.00	16.11
HI-06	9	14	-68.83	77.71	28/01/2010	al-cy	0.81	0.97	4.2	4.1	27.9	28.62	6.9	13.11	0.00	0.10	0.00	0.00	13.21
CS-12	7	34	-66.28	110.53	24/12/2009	al-cy	0.6	9.6	84.4	3.4	123.6	69.41	5.8	0.78	3.66	8.63	0.00	0.00	13.08
MS-07	7	8	-67.60	62.87	15/02/2010	inorg	0.07	0.71	40.6	11	63.6	11.05	6.2	0.91	10.78	1.29	0.00	0.00	12.98
SP-07	7	41	-69.40	76.09	22/01/2010	moss	0.02	0.5	5.2	3.4	8.7	9.22	5.9	0.40	1.32	7.14	0.00	1.19	10.05
HI-11	7	21	-68.83	77.67	29/01/2010	al-cy	0.08	0.52	184.3	3.4	77.1	11.51	6.1	0.00	3.98	4.88	0.00	0.00	8.86
L-Isl2-02	7	27	-69.37	76.14	23/01/2010	inorg	0.07	0.67	14.7	3.4	36.9	14.59	5.8	0.28	3.37	4.68	0.00	0.00	8.34
SP-03	7	44	-69.40	76.10	22/01/2010	moss	0.03	1	15.6	3.4	28.8	0.28	5.8	0.01	2.93	4.72	0.00	exu	7.66
MP-02	9	65	-68.86	77.94	1/02/2010	lichen	0.06	1.3	24	3.4	11.1	9.24	6.3	2.73	0.00	3.92	0.00	0.00	6.65
BP-12	7	1.6	-69.38	76.38	19/01/2010	inorg	0.09	0.58	7.3	3.4	5.1	20.17	5.8	0.05	5.61	0.41	0.00	0.00	6.08
SP-05	7	5	-69.40	76.09	22/01/2010	moss	0.4	6.76	65.6	3.4	13.8	42.83	4.7	0.07	2.31	3.31	0.14	0.00	5.82
VH-18	9	23	-68.60	78.29	3/02/2010	al-cy	3.5	0.24	2	6.8	4.8	5.33	6.7	1.65	0.00	4.01	0.00	0.00	5.66
CS-04	7	31	-66.28	110.54	24/12/2009	inorg	0.06	1.68	150	5.4	101.4	13.27	4.8	0.60	1.95	2.67	0.00	0.00	5.22
VH-06	9	28	-68.61	77.95	14/02/2010	inorg	0.24	0.15	9.1	6.1	12.9	15.45	8.1	2.48	0.00	2.09	0.21	0.04	4.83
BP-02	7	54	-69.38	76.38	16/01/2010	inorg	0.03	0.1	8.4	3.4	18.6	11.86	6.4	1.70	0.07	2.76	0.00	0.00	4.53
L-Isl2-04	7	27	-69.37	76.14	23/01/2010	inorg	0.44	0.95	4.7	3.4	4.8	18.45	4.3	0.17	0.78	2.94	0.00	0.00	3.90
VH-02	9	17	-68.64	78.30	14/01/2010	inorg	0.1	0.22	11.6	3.4	9.1	10.75	6.1	3.80	0.00	0.00	0.00	0.00	3.80
CS-01	7	28.4	-66.28	110.53	24/12/2009	moss	0.12	2.08	92.2	3.4	27.9	18.18	5.5	0.15	1.32	1.27	0.83	0.10	3.67
BP-03	7	16	-69.39	76.39	16/01/2010	inorg	0.04	0.19	7.8	3.4	7.2	11.84	6.3	0.16	2.48	1.02	0.00	0.00	3.66
SP-09	7	9	-69.43	76.04	23/01/2010	inorg	0.04	0.14	44.6	18.4	15.6	7.7	6.9	0.61	1.58	1.39	0.00	0.00	3.58
FM-03	9	480	-67.78	62.79	14/02/2010	inorg	0.03	0	26	8.1	6.6	1.59	6.8	2.65	0.00	0.87	0.00	0.00	3.53
MS-01	7	4	-67.60	62.87	11/02/2010	al-cy	0.05	0.45	49.3	16.9	8.4	2.9	6.3	0.14	0.10	3.05	0.00	0.00	3.30
MP-01	9	70	-68.86	77.94	1/02/2010	moss	0.06	0.55	5.2	3.4	5.4	1.5	6.5	1.22	0.00	1.69	0.24	exu	3.15
VH-15	9	47	-68.57	78.48	10/03/2010	moss	0.13	1.94	21	4.7	35.4	2.13	6.7	2.90	0.00	0.10	0.00	0.07	3.08
MS-02	7	16	-67.60	62.87	11/02/2010	moss	0.09	3.66	79.1	4.7	12.9	33.46	5.5	1.89	0.63	0.32	0.02	0.00	2.86
VH-11	10	6	-68.50	78.08	28/02/2010	inorg	0.38	0.18	12.1	7.5	6.9	5.85	6.1	0.15	0.02	2.06	0.00	0.55	2.78
FM-02	7	490	-67.78	62.79	14/02/2010	inorg	0.11	0.05	22.3	12.2	7.5	6.53	7.6	0.06	0.06	2.23	0.09	0.00	2.44
VH-20	9	25	-68.60	78.24	3/02/2010	moss	0.27	0.13	1.4	5.4	5.1	8.47	6.5	2.36	0.00	0.01	0.00	0.00	2.37
HI-03	11	23	-68.83	77.68	28/01/2010	inorg	4.39	0.63	310.5	3.4	118.5	15.55	8.2	2.02	0.00	0.00	0.25	0.00	2.27
CS-03	7	31	-66.28	110.54	24/12/2009	moss	0.06	3.13	171.2	3.4	57	21.23	5.1	0.05	1.12	1.02	0.00	0.00	2.18
HI-15	7	14	-68.83	77.71	31/01/2010	inorg	0.14	0.29	10.1	3.4	13.5	15.1	6.7	0.28	0.05	1.32	0.33	0.00	1.98
BP-07	12	30	-69.39	76.35	17/01/2010	inorg	0.08	0.78	2.3	3.4	6.6	18.11	6.6	0.44	0.00	1.39	0.00	0.00	1.83
VH-19	7	25	-68.60	78.24	3/02/2010	moss	0.05	0.17	5.1	3.4	12	5.78	6.0	0.82	0.50	0.48	0.00	0.00	1.79
VH-05	7	15	-68.51	78.51	6/02/2010	inorg	0.16	0.01	41	14.2	17.4	0.91	7.8	0.16	0.01	1.56	0.00	0.00	1.74
HI-02	12	31	-68.82	77.70	27/01/2010	inorg	0.1	0.45	97.8	3.4	6	1.81	7.2	0.00	0.00	1.63	0.00	0.00	1.63
MP-03	10	45	-68.86	77.93	1/02/2010	moss	0.03	0.16	8.5	3.4	16.5	2.37	7.1	1.23	0.00	0.31	0.00	0.04	1.58
VH-17	9	66	-68.60	78.35	3/02/2010	moss	0.02	0.48	8.7	3.4	4.5	2.25	6.9	1.37	0.00	0.19	0.00	0.00	1.57
L-Isl2-03	7	27	-69.37	76.14	23/01/2010	inorg	0.22	0.52	8.5	3.4	4.8	16.3	5.8	0.73	0.22	0.55	0.00	0.00	1.49
FM-06	12	460	-67.77	62.82	14/02/2010	inorg	0.01	0.01	18.7	8.8	15	1.29	5.7	0.01	0.00	1.46	0.00	0.00	1.47
BP-01	10	31	-69.39	76.38	16/01/2010	al-cy	0.08	0.2	32.3	3.4	6.9	10.72	5.4	0.03	0.01	1.28	0.00	0.03	1.36
VH-08	10	50	-68.63	78.41	19/02/2010	inorg	0.26	0.17	11.3	3.4	33	8.51	7.9	0.27	0.00	0.86	0.00	0.11	1.25
HI-13	11	12	-68.83	77.73	30/01/2010	inorg	0.04	0.07	6.3	3.4	7.2	15.18	7.5	0.28	0.00	0.73	0.19	0.00	1.20
CS-09	7	25	-66.28	110.52	24/12/2009	moss	0.09	3.14	36.8	4.1	21.9	15.79	4.6	0.57	0.34	0.19	0.00	0.00	1.10
L-Isl1-03	7	21	-69.41	76.00	23/01/2010	moss	0.03	0.28	40.9	3.4	5.7	1.54	5.8	0.41	0.08	0.55	0.00	0.00	1.04

Table S1 (continued)

Sample	Gr	Ele (m)	Coordinates South	Coordinates East	Collection date	Categ	EC *dS/m*	Org C%	P *mg/kg*	NO_3 *p.p.m*	NH_3 *p.p.m*	Moi %	pH	Nem/ gdw	Tard/ gdw	Rot/ gdw	Cil/ gdw	Mit/ gdw	Abun / gdw
BP-05	9	6	-69.39	76.35	17/01/2010	inorg	0.06	0.23	3.5	3.4	5.4	13.86	6.5	0.51	0.00	0.46	0.00	0.00	0.98
VH-16	7	20	-68.60	78.36	1/02/2010	inorg	0.27	0.17	4.8	3.4	5.1	6.37	9.0	0.77	0.10	0.02	0.00	0.00	0.89
VH-13	11	15	-68.66	77.88	4/03/2010	inorg	1.52	0.28	66.1	19	69.3	6.89	7.3	0.70	0.00	0.04	0.09	0.00	0.83
SP-02	7	40	-69.40	76.10	21/01/2010	inorg	0.02	0.06	13.9	3.4	8.1	7.06	6.2	0.15	0.09	0.47	0.00	0.00	0.71
SP-01	10	40	-69.43	75.99	21/01/2010	inorg	0.04	0.06	3.9	3.4	4.5	4.76	5.5	0.03	0.00	0.50	0.00	0.13	0.66
L-Isl1-06	6	21	-69.41	76.01	23/01/2010	moss	0.02	0.6	9.5	3.4	5.1	1.08	5.9	0.00	0.00	0.65	0.00	0.00	0.65
MP-07	9	80	-68.85	77.94	1/02/2010	moss	0.05	0.6	5.2	3.4	4.5	0.25	6.5	0.06	0.00	0.52	0.00	exu	0.58
FM-05	12	460	-67.77	62.82	14/02/2010	inorg	0.04	0.01	12.5	23	12.9	4.77	5.8	0.00	0.00	0.57	0.00	0.00	0.57
CS-14	6	4.2	-66.28	110.54	24/12/2009	inorg	0.16	1.29	107.4	3.4	9	16.43	4.5	0.00	0.00	0.51	0.00	exu	0.51
BP-06	7	40	-69.39	76.35	17/01/2010	inorg	0.03	0.21	5.3	3.4	23.7	12.06	8.0	0.02	0.05	0.41	0.00	0.00	0.48
VH-22	6	47	-68.58	78.24	3/02/2010	inorg	0.13	0.01	2.5	4.1	5.4	9.42	7.5	0.00	0.03	0.43	0.00	0.00	0.46
BP-08	3	60	-69.40	76.38	18/01/2010	inorg	0.03	0.08	3.2	3.4	5.7	6.93	6.1	0.42	0.01	0.00	0.00	0.00	0.43
SP-08	9	59	-69.40	76.12	22/01/2010	inorg	0.03	0.11	4.3	3.4	6.3	12.67	6.2	0.13	0.00	0.15	0.00	0.06	0.33
L-Isl1-05	9	21	-69.41	76.01	23/01/2010	moss	0.01	0.17	15.9	3.4	5.7	0.11	6.1	0.14	0.00	0.16	0.00	0.00	0.30
CS-07	2	44	-66.28	110.52	24/12/2009	moss	0.23	3.6	169.3	3.4	18.9	27.73	5.7	0.00	0.00	0.00	0.00	0.29	0.29
VH-03	3	5	-68.51	78.51	6/02/2010	inorg	0.03	0.1	9.9	3.4	30.9	5.23	7.6	0.27	0.00	0.00	0.00	0.00	0.27
L-Isl1-07	10	21	-69.41	76.01	23/01/2010	inorg	0.04	0.01	4.2	3.4	4.5	1.78	6.5	0.12	0.03	0.04	0.00	0.04	0.23
BP-09	3	69	-69.39	76.38	18/01/2010	inorg	0.02	0.05	6.6	3.4	5.1	8.04	6.0	0.20	0.00	0.00	0.00	0.00	0.20
BP-04	12	60	-69.39	76.39	16/01/2010	inorg	0.06	0.06	5	3.4	6	7.62	6.9	0.03	0.00	0.15	0.00	0.00	0.18
MP-04	7	44	-68.85	77.94	1/02/2010	inorg	0.03	0.01	5.2	3.4	5.1	13.71	6.1	0.05	0.05	0.08	0.00	0.00	0.17
L-Isl2-01	3	27	-69.37	76.14	23/01/2010	inorg	0.22	0.11	4.6	3.4	4.2	1.26	6.7	0.13	0.00	0.00	0.00	exu	0.13
BP-14	12	46	-69.39	76.33	20/01/2010	inorg	0.02	0.11	3.2	3.4	6	1.14	7.2	0.08	0.00	0.05	0.00	0.00	0.13
HI-14	3	36	-68.83	77.74	30/01/2010	inorg	0.02	0.01	3.6	3.4	5.4	0.44	7.8	0.12	0.00	0.00	0.00	0.00	0.12
BP-11	11	0	-69.38	76.40	18/01/2010	inorg	0.05	0.05	4.3	3.4	17.7	13.38	6.9	0.03	0.00	0.03	0.03	0.00	0.10
FM-01	3	490	-67.78	62.79	14/02/2010	inorg	0.07	0.28	43.8	8.2	16.2	1.85	6.1	0.08	0.00	0.00	0.00	0.01	0.09
VH-04	6	15	-68.51	78.51	6/02/2010	inorg	4.05	0.32	249.6	100.2	120	8.35	7.8	0.00	0.01	0.08	0.00	0.00	0.09
BP-13	12	25	-69.39	76.32	20/01/2010	inorg	0.06	0.08	4.5	3.4	4.8	11.71	7.2	0.00	0.00	0.08	0.00	0.00	0.08
CS-02	12	28.4	-66.28	110.53	24/12/2009	inorg	0.03	0.17	146.1	3.4	31.5	7.64	5.3	0.00	0.00	0.04	0.00	0.01	0.05
VH-12	6	4	-68.66	77.87	4/03/2010	moss	18.5	1.73	469	548.5	345	25.24	7.5	0.00	0.00	0.05	0.00	0.00	0.05
HI-08	12	10	-68.82	77.70	28/01/2010	inorg	0.33	0.83	9.8	3.4	7.8	14.24	9.2	0.01	0.01	0.01	0.00	0.00	0.03
FM-04	6	470	-67.78	62.79	14/02/2010	inorg	3.66	0.61	39.6	1163	12.3	6.87	6.5	0.00	0.01	0.02	0.00	0.00	0.03
HI-09	1	33	-68.83	77.69	29/01/2010	inorg	0.1	0.03	9.2	3.4	7.2	10.9	8.6	0.01	0.00	0.00	0.00	0.00	0.01
HI-07	0	15	-68.83	77.70	28/01/2010	inorg	0.37	0.37	242.1	40.1	153	7.12	6.3	0.00	0.00	0.00	0.00	0.00	0.00
HI-12	0	14	-68.83	77.72	29/01/2010	inorg	0.08	0.01	18	3.4	7.2	0.81	7.2	0.00	0.00	0.00	0.00	0.00	0.00
HI-16	0	25	-68.83	77.68	31/01/2010	inorg	1.66	0.57	217.3	142.8	117	10.43	5.0	0.00	0.00	0.00	0.00	0.00	0.00
MS-05	0	16	-67.60	62.86	15/02/2010	inorg	0.96	0.15	63.7	17.7	9.9	1.87	6.1	0.00	0.00	0.00	0.00	0.00	0.00

Samples were sorted by total meiofauna abundance. Samples acronyms: Larsemann Hills-Broknes Peninsula (BP), Larsemann Hills - Stornes Peninsula (SP), Sansom Island (SI), Vestfold Hills (VH), Casey Station (CS), Hop Island (HI), Larsemann Islands (L-Isl), Mather Peninsula (MP), Mawson Station (MS), and Framnes Mountains (FM). Variables acronyms: Elevation (Elev), Vegetation content (Cont), algae-cyanobacteria (al-cy), soil with no visible photosynthetic material (inorg), electric conductivity (EC), phosphorous (P), moisture (Moi), gdw (grams of dry weight of soil), nematodes (Nem), tardigrades (Tard), bdelloid rotifers (Rot), ciliates (Cil), mites (Mit), only mite exuviae (exu), and total abundance (Abun).

Table S2. Measurements and de Man's ratios for *Plectus murrayi* and *P. frigophilus* females from East Antarctica compared to other regions from various studies

Plectus	Region	N	Body length (µm)	Tail length (µm)	Width (µm)	Esoph. length (µm)	De Man's ratios 'a'	'b'	'c'
*P.*cf. *murrayi*	**CS**	5	800-920	85-110	28-38	165-225	22.1-28.9	3.8-4.8	7.4-9.4
*P.*cf. *murrayi*	**VH**	5	810-910	100-110	28-36	190-200	24.6-28.9	4.1-4.7	8.1-9.0
*P.*cf. *murrayi*	**HI, MP**	6	810-1080	80-110	33-44	170-250	22.3-28.4	4.3-4.8	7.8-10.8
*P.*cf. *murrayi*	**BP, SP**	7	800-1000	90-110	28-48	170-220	20-31.7	4.0-5.5	8.5-10.6
*P.*cf. *murrayi*	**MS-FM**	5	810-880	95-105	37-44	200-220	20.0-21.9	3.9-4.2	8.1-9.1
P. murrayi	Gondwana (VL)[1]	16	817 ± 12	97 ± 2	31 ± 1	184 ± 1	26.5 ± 0.3	4.4 ± 0.1	8.5 ± 0.2
P. murrayi	Dry Valley (VL) [2]	10	750 - 840	-	-	-	24 - 28	3.8 - 4.4	7.6 - 8.8
P. murrayi	Soya coast (EA)[3]	10	810-935	104-114	31-38	168-221	22.8-27.5	3.9-5.2	7.8-8.8
P. murrayi	Marble Point (VL)[4]	25	600-820	-	-	-	15.2-24.8	4.7-6.0	6.5-9.1
P. murrayi	Strand Moraines (VL)[4]	16	683-882	-	-	-	18.6-31.5	4.6-5.5	6.6-8.3
P. murrayi	Bunger Hills (EA)[5]	34	650-1000	98-128	31-52	151-200	16.2-23.7	3.7-5.2	6.1-8.6
*P.*cf. *frigophilus*	**CS**	1	1430	120	52	320	27.5	4.5	11.9
*P.*cf. *frigophilus*	**BP, SP**	4	1380-2050	120-140	50-55	280-420	26.5-37.3	4.1-4.9	9.9-14.6
*P.*cf. *frigophilus*	**FM**	1	1400	130	45	360	31.1	3.9	10.8
P. frigophilus	McMurdo Sound[2]	10	1350-1720	-	-	-	24-33	4.9-5.4	9.9-11
P. frigophilus	Edmonson Point (VL)[2]	5	1600-1820	-	-	-	23-24	4.7-5.0	11-Dec
P. frigophilus	Soya coast (EA)[3]	4	1455-1700	137-161	50-62	310-357	26.1-29.1	4.5-4.8	9.3-11.4
P. frigophilus	Bunger Hills (EA)[5]	25	1190-1580	120-160	38-56	290-350	25.5-33	4.0-4.8	9.2-10.9
P. frigophilus	Marble Point (VL)[6]	10	1400-1990	-	-	-	22.2-32.5	4.4-5.2	9.7-12.3
P. frigophilus	Strand Moraines (VL)[6]	10	1540-2060	-	-	-	25.7-30.0	4.5-5.1	10.5-13.5
P. frigophilus	Obruchev Hills (EA)[7]	3	1360-1887	-	-	-	23-28	4.8-5.1	10.6-12.5

Measurements and ratios for *Plectus murrayi* and *P. frigophilus* female populations for the current study (in bold) compared to other regions across Antarctica. [1] shows data from Raymond, 2010 ('mean ± SE' are given for Gondwana populations); [2] indicates data from descriptions by Andrássy, 1998; [3] data from species description by Kito et al., 2008; [4] indicates data taken from Yeates, 1970; [5] shows measurements from EA by Yeates, 1979; [6] indicates measurements and ratios from Timm, 1971; and [7] shows the original measurements and ratios by Kirjanova, 1958. Gaps indicate data not available. Acronyms: Casey Station (CS), Vestfold Hills (VH), Hop Island (HI), Mather Peninsula (MP), Larsemann Hills-Broknes Peninsula (BP), Larsemann Hills - Stornes Peninsula (SP), Mawson Station (MS), Framnes Mountains (FM), East Antarctica (EA), Victoria Land (VL). De Man's ratios: 'a': total body length / maximum body diameter; 'b': total body length / total esophagus length; 'c': total body length / tail length.

References

Andrássy I (1998) Nematodes in the Sixth Continent. In: Peña Santiago R, editor. Journal of Nematode Morphlogy and Systematics. Jaén: Universidad de Jaén. pp. 107-186.

Kirjanova ES (1958) Antarctic specimens of freshwater nematodes of the genus *Plectus bastian* (Nematoda, Plectidae). Information Bulletin of the Soviet Antarctic Expedition 3: 101-103.

Kito K, Ohyama Y (2008) Rhabditid nematodes found from a rocky coast contaminated with treated waste water of Casey Station in East Antarctica, with a description of a new species of *Dolichorhabditis* Andrássy, 1983 (Nematoda: Rhabditidae). Zootaxa 1850: 43-52.

Raymond M (2010) Cold-temperature adaptation in nematodes from the Victoria Land Coast, Antarctica. Dunedin: University of Otago. 1-312 p.

Timm RW (1971) Antarctic Soil and Freshwater Nematodes from the McMurdo Sound Region. Proceedings of the Helminthological Society of Washington 38: 42-52.

Yeates GW (1970) Two Terrestrial Nematodes from the McMurdo Sound Region, Antarctica, with a Note on *Anaplectus arenicola* Killick, 1964. Journal of Helminthology 44: 27-34.

Yeates GW (1979) Terrestrial nematodes from the Bunger Hills and Gaussberg, Antarctica. New Zealand Journal of Zoology 6: 641-643.

Table S3. Pearson correlation matrix for 109 sites and the most relevant environmental and biotic variables

	content	Elev	lnEC	lnC	lnP	lnNO3	lnNH3	lnMoist	lnpH	FS	Nem	Plectus	Eudor	Scott	Tard	Rot	Cil	Acar	lnNem_ab	lnTard_ab	lnRot_ab	lnCil_ab	lnAb_t
content	1.00	**-.194***	0.04	**.545****	**.244***	-0.12	0.17	-0.01	**-.290****	**.322****	0.06	**.265****	0.04	-0.17	**.210***	**.279****	0.08	0.18	**.257****	**.368****	**.426****	0.13	**.457****
Elev	**-.194***	1.00	-0.08	**-.239***	-0.02	**.319****	-0.11	**-.211***	0.02	**-.241***	-0.02	-0.07	-0.09	0.11	-0.15	-0.04	-0.02	0.00	-0.13	**-.206***	-0.10	-0.03	-0.13
lnEC	0.04	-0.08	1.00	**.304****	**.338****	**.481****	**.383****	**.318****	0.15	0.07	-0.17	-0.11	-0.17	**-.258****	0.04	-0.02	**.231***	-0.05	-0.07	0.08	0.07	.274**	0.08
lnC	**.545****	**-.239***	**.304****	1.00	**.548****	-0.04	**.531****	**.477****	**-.478****	**.264****	-0.01	**.323****	**-.222***	**-.425****	**.375****	**.232***	0.15	0.08	**.207***	**.577****	**.500****	**.219***	**.533****
lnP	**.244***	-0.02	**.338****	**.548****	1.00	**.344****	**.712****	**.216***	**-.334****	**.214***	**-.241***	0.02	**-.471****	**-.386****	**.202***	-0.04	0.13	-0.01	-0.08	**.306****	**.237***	0.13	0.18
lnNO3	-0.12	**.319****	**.481****	-0.04	**.344****	1.00	**.319****	-0.09	0.11	0.10	**-.350****	**-.236***	**-.189***	-0.09	-0.08	-0.07	-0.02	**-.207***	**-.315****	-0.16	-0.17	-0.03	**-.296****
lnNH3	0.17	-0.11	**.383****	**.531****	**.712****	**.319****	1.00	**.350****	-0.09	0.16	-0.09	0.07	**-.321****	**-.334****	0.16	0.00	0.18	-0.07	0.06	**.308****	**.223***	**.190***	**.222***
lnMoist	-0.01	**-.211***	**.318****	**.477****	**.216***	-0.09	**.350****	1.00	**-.205***	-0.09	0.06	0.19	-0.17	**-.334****	**.369****	0.16	**.248****	-0.07	0.17	**.419****	**.262****	**.264****	**.313****
lnpH	**-.290****	0.02	0.15	**-.478****	**-.334****	0.11	-0.09	**-.205***	1.00	-0.12	0.13	**-.265****	0.18	**.276****	**-.259****	-0.13	0.14	-0.14	0.02	**-.341****	**-.298****	0.12	**-.234***
FS	**.322****	**-.241***	0.07	**.264****	**.214***	0.10	0.16	-0.09	-0.12	1.00	-0.05	0.13	0.05	-0.10	0.10	0.07	0.11	0.05	0.17	0.18	0.17	0.06	**.228***
Nem	0.06	-0.02	-0.17	-0.01	**-.241***	**-.350****	-0.09	0.06	0.13	-0.05	1.00	**.633****	**.354****	**.327****	0.09	0.07	0.01	0.02	**.795****	0.06	0.01	-0.04	0.17
Plectus	**.265****	-0.07	-0.11	**.323****	0.02	**-.236***	0.07	0.19	**-.265****	0.13	**.633****	1.00	**.218***	**-.236***	**.265****	0.12	-0.09	0.01	**.576****	**.310****	**.230***	-0.09	**.326****
Eudoryl	0.04	-0.09	-0.17	**-.222***	**-.471****	**-.189***	**-.321****	-0.17	0.18	0.05	**.354****	**.218***	1.00	**.413****	**-.230***	-0.03	-0.10	**.212***	**.323****	**-.259****	**-.215***	-0.07	-0.07
Scott	-0.17	0.11	**-.258****	**-.425****	**-.386****	-0.09	**-.334****	**-.334****	**.276****	-0.10	**.327****	**-.236***	**.413****	1.00	**-.253****	-0.06	-0.07	0.11	0.18	**-.314****	**-.261****	-0.09	-0.19
Tard	**.210***	-0.15	0.04	**.375****	**.202***	-0.08	0.16	**.369****	**-.259****	0.10	0.09	**.265****	**-.230***	**-.253****	1.00	**.386****	0.01	-0.10	0.17	**.861****	**.522****	0.08	**.477****
Rot	**.279****	-0.04	-0.02	**.232***	-0.04	-0.07	0.00	0.16	-0.13	0.07	0.07	0.12	-0.03	-0.06	**.386****	1.00	0.10	0.03	0.16	**.363****	**.712****	0.11	**.517****
Cil	0.08	-0.02	**.231***	0.15	0.13	-0.02	0.18	**.248****	0.14	0.11	0.01	-0.09	-0.10	-0.07	0.01	0.10	1.00	-0.04	0.10	0.06	0.17	**.918****	**.224***
Acar	0.18	0.00	-0.05	0.08	-0.01	**-.207***	-0.07	-0.07	-0.14	0.05	0.02	0.01	**.212***	0.11	-0.10	0.03	-0.04	1.00	0.07	-0.05	0.07	-0.02	0.08
lnNem_ab	**.257****	-0.13	-0.07	**.207***	-0.08	**-.315****	0.06	0.17	0.02	0.17	**.795****	**.576****	**.323****	0.18	0.17	0.16	0.10	0.07	1.00	**.224***	**.216***	0.09	**.415****
lnTard_ab	**.368****	**-.206***	0.08	**.577****	**.306****	-0.16	**.308****	**.419****	**-.341****	0.18	0.06	**.310****	**-.259****	**-.314****	**.861****	**.363****	0.06	-0.05	**.224***	1.00	**.701****	0.16	**.702****
lnRot_ab	**.426****	-0.10	0.07	**.500****	**.237***	-0.17	**.223***	**.262****	**-.298****	0.17	0.01	**.230***	**-.215***	**-.261****	**.522****	**.712****	0.17	0.07	**.216***	**.701****	1.00	**.263****	**.880****
lnCil_ab	0.13	-0.03	**.274****	**.219***	0.13	-0.03	**.190***	**.264****	0.12	0.06	-0.04	-0.09	-0.07	-0.09	0.08	0.11	**.918****	-0.02	0.09	0.16	**.263****	1.00	**.315****
lnAbund_t	**.457****	-0.13	0.08	**.533****	0.18	**-.296****	**.222***	**.313****	**-.234***	**.228***	0.17	**.326****	-0.07	-0.19	**.477****	**.517****	**.224***	0.08	**.415****	**.702****	**.880****	**.315****	1.00

Variables preceded by 'ln' were subjected to log (x+0.1) transformation (after Nielsen et al., 2011; Knox et al., 2012). Numbers in bold indicate correlation significant at 0.01 level (**) and 0.05 level (*). Abbreviation as following: vegetation (content), elevation (Elev), electric conductivity (EC), organic Carbon (C), moisture (Moist), fine sediment (FS), nematodes (Nem), *Eudorylaimus* (Eudor), *Scottnema* (Scott), tardigrades (Tard), rotifers (Rot), ciliates (Cil), mites (Mit), abundance (ab), total meiofauna (t). Headings: Plectus, Eudor, Scott, Tard, Rot, Cil and Mit show Pearson values for an original matrix of presence-absence. Type I error is an important aspect when conducting multiple comparison tests (Peres-Neto, 1999). We do not correct probability values for our Pearson correlation since standard corrections like Holm's sequential Bonferroni correction are found to be extremely conservative (Moran, 2003; Peres-Neto et al., 2003). Peres-Neto (1999) also suggests not using the Bonferroni test with a large number of comparisons given that the alpha value would be too small increasing the type II errors. Ellison & Gotelli (2004) also suggest that adjusting the alpha value (significance level) is not recommended. A similar study (type of data/analyses) to ours that also uses Pearson correlations does not undertake any corrections for the reasons we cite above (Cannone et al., 2008). Here we do not apply a correction for multiple comparisons.

References

Cannone N., Wagner D, Hubberten, HW, Guglielmin M (2008) Biotic and abiotic factors influencing soil properties across a latitudinal gradient in Victoria Land, Antarctica. Geoderma 144: 50-65.

Ellison GN, Gotelli N (2004) A primer of ecological statistics. Sinauer, Sunderland, Massachusetts, USA.

Knox MA, Hogg ID, Pilditch CA, Lörz A-N, Nodder SD (2012) Abundance and diversity of epibenthic amphipods (Crustacea) from contrasting bathyal habitats. Deep Sea Research Part I: Oceanographic Research Papers 62: 1-9.

Moran MD (2003) Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos 100: 403-405.

Nielsen UN, Wall DH, Li G, Toro M, Adams BJ, et al. (2011) Nematode communities of Byers Peninsula, Livingston Island, maritime Antarctica. Antarctic Science 23: 349-357.

Peres-Neto PR (1999) How many statistical tests are too many? The problem of conducting multiple ecological inferences revisited. Marine Ecology Progress Series 176: 303-306.

Peres-Neto PR, Jackson DA, Somers KM (2003) Giving meaningful interpretation to ordination axes: assessing loading significance in principal component analysis. Ecology 84: 2347-2363.

APPENDIX 3

Preparation of nematode slides

Some of the nematodes were fixed for morphological measurements using 3% formalin at 60°C as described by Hooper (1986). Nematodes were initially placed in a watchglass and warm formalin was added to kill the live nematodes to retain their original shape. After two weeks excess formalin in the watchglass was removed and replaced with Solution I (Glycerol 1%; 30%-Ethanol 99%) and left half full for one week. Solution I was then removed and replaced with Solution II (Glycerol 5%; 95%-Ethanol 95%) and half covered with a glass lid to allow the alcohol to evaporate for one week. Specimens were then mounted in pure glycerol.

Permanents mounts were made using the wax-ring method (Hooper, 1986). Paraffin wax was melted in a glass petri-dish on a hot plate at 60°C. A 1.5cm in diameter metal loop was introduced in the hot paraffin and afterwards placed in the centre of a clean slide. A drop of glycerol was placed within the wax circle formed by the paraffin and the nematodes transferred onto the drop. Nematodes were well distributed around the drop and sunk to the bottom. A round coverslip was placed over the wax ring, making sure there were no visible air bubbles inside (Raymond, 2010). The slide with the ring was placed on a hot plate at 45-60°C for a few seconds until the wax ring was melted, and then removed to a cold surface. At this stage the wax ring will reappeared again and which seals the coverslip to the slide. Nail hardener was placed around the ring to ensure the proper sealing process. Digital images of the slides were taken with a Nikon camera (DF100, Japan) attached to a Nikon compound phase contrast microscope (Eclipse 50i, Japan) at 100x, 200x and 400x magnification.

References

Hooper, D.J., 1986. Handling, fixing, staining and mounting nematodes, Southey, J.F. (Editor), Laboratory Methods for Work with Plant and Soil Nematodes. Her Majesty's Stationary Office, London, pp. 34-38.

Raymond, M., 2010. Cold-Temperature Adaptation in Nematodes from the Victoria Land Coast, Antarctica. University of Otago, Dunedin, pp. 1-312.

De Man's ratios considered for nematodes' morphometrics (after Fortuner, 1990).

a = total body length / maximum body diameter

b = total body length / total esophagus length

c = total body length / tail length

V = distance from head to vulva x 100 / total body length

Reference

Fortuner, R., 1990. Ratios and Indexes in Nematode Taxonomy. Nematologica 36, 205-216.

DNA, PCR and sequencing protocols

DNA extraction, PCR, and COI sequencing were performed by the Canadian Centre for DNA Barcoding (CCDB) at the Biodiversity Institute of Ontario, University of Guelph, using standard laboratory protocols (Ivanova *et al*.2006; Ivanova & Grainger 2006). Total DNA was extracted from the entire individual and the mitochondrial COI gene amplified with different sets of primers. Amplification used a cocktail of primers that included three forward NemF1 (5'-CRACWGTWAATCAYAARAATATTGG-3') + NemF2 (5'-ARAGATCTAATCATAAAGATATYGG-3') + NemF3 (5'-ARAGTTCTAATCATAARGATATTGG-3') and three reverse (NemR1 (5'-AAACTTC WGGRTGACCAAAAAATCA-3') + NemR2 (5'-AWACYTCWGGRTGMCCAAAA AAYCA-3') + NemR3 (5'-AAACCTCWGGATGACCAAAAAATCA-3') primer sequences mixed in a 1:1:1 ratio (Prosser *et al.* 2013) tailed with modified M13 sequences (after Messing 1993) were used as described in Ivanova *et al*. (2007).

PCR products were amplified on the thermocycler (Mastercycler® ep gradient, Eppendorf®) under the following conditions: 1 min at 94 °C for initial denaturation, 5 cycles of 94 °C for 40 sec, annealing at 45 °C for 40 sec, and extension at 72 °C for 1 min, followed by 35 cycles at 94 °C for 40 sec, 40 sec at 51°C, and 1 min at 72 °C, with a final extension of 72 °C for 5 min. The 12.5 µl PCR reaction mix for one reaction includes 2 µl of DNA template, 6.25 µl of 10% trehalose, 2 µl of ultrapure water, 1.25 µl of 10X PCR buffer for Platimum *Taq* (Invitrogen™), 0.625 µl of 50 mM $MgCl_2$ (Invitrogen™), 0.0625 µl of 10 mM dNTPs (New England Biolabs®), 0.06 µl of Taq DNA Polymerase (Platinum®) and 0.125 µl of each 10 µM primer (Invitrogen™). The PCR products were checked by electrophoresis on a 2% agarose E-gel® stained with Ethidium bromide. PCR amplification products were cleaned-up following the Sephadex® protocol and sequenced in a 3730xl DNA Analyzer (Applied Biosystems; Ivanova & Grainger 2006).

References

Ivanova, N., Grainger, C., 2006. Pre-made frozen PCR and sequencing plates. CCDB Advances, Methods Release No 4.

Ivanova, N.V., Dewaard, J.R., Hebert, P.D., 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes 6, 998-1002.

Ivanova, N.V., Zemlak, T.S., Hanner, R.H., Hebert, P.D.N., 2007. Universal primer cocktails for fish DNA barcoding. Molecular Ecology Notes 7, 544-548.

Messing, J., 1993. M13 cloning vehicles: Their contribution to DNA sequencing. Methods Mol. Biol. 23, 9-22.

Prosser, S., Velarde-Aguilar, M., León-Règagnon, V., Hebert, P., 2013. Advancing nematode barcoding: A primer cocktail for the cytochrome c oxidase subunit I gene from vertebrate parasitic nematodes. Molecular Ecology Resources 13, 1108-1115.

Table S1 Mitochondrial lineages, measurements and de Man's ratios for 32 *Scottnema* cf. *lindsayae* specimens (adult and juvenile combined) collected across Sector 2.

Clade	Region	Body length (µm)	Tail length (µm)	Body width (µm)	de Man's ratios	
					a	c
N18	**BP**	590	48	34	17.4	12.3
N18	**BP**	410	32	28	14.6	12.8
N18	**BP**	700	50	38	18.4	14.0
N18	**SP**	680	45	33	20.6	15.1
N18	**SP**	500	40	30	16.7	12.5
N18	**SP**	500	38	30	16.7	13.2
N18	**SP**	580	42	34	17.1	13.8
N18	**HI**	670	45	33	20.3	14.9
N18	**HI**	450	35	28	16.1	12.9
N18	**HI**	480	35	30	16.0	13.7
N18	**HI**	500	33	30	16.7	15.2
N18	**HI**	520	35	30	17.3	14.9
N18	**HI**	520	35	30	17.3	14.9
N18	**MP**	740	50	45	16.4	14.8
N18	**MP**	650	48	35	18.6	13.5
N18	**MP**	600	45	30	20.0	13.3
N18	**VH**	600	48	45	13.3	12.5
N18	**VH**	650	45	34	19.1	14.4
N18	**VH**	640	45	42	15.2	14.2
N18	**VH**	530	40	33	16.1	13.3
N18	**VH**	600	51	35	17.1	11.8
N18	**VH**	640	45	33	19.4	14.2
N19	**DML**	650	45	40	16.3	14.4
N19	**DML**	650	45	35	18.6	14.4
N20	**FM**	500	42	30	16.7	11.9
N20	**FM**	650	44	34	19.1	14.8
N20	**FM**	580	44	33	17.6	13.2
N20	**FM**	500	44	33	15.2	11.4
N20	**FM**	430	33	28	15.4	13.0
N21	**FM**	440	40	30	14.7	11
-	**FM**	550	42	35	15.7	13.1
-	**FM**	520	40	35	14.9	13

Abbreviation as following: Broknes Peninsula (BP), Stornes Peninsula (SP), Hop Island (HI), Mather Peninsula (MP), Vestfold Hills (VH), Dronning Maud Land (DML), and Framnes Mountains (FM).

Table S2 Morphological measurements and de Man's ratios for 33 *Plectus murrayi* female specimens collected from Sector 2.

Region	Age	Body length (µm)	Tail length (µm)	Body width (µm)	Oesophagus length (µm)	Head-vulva (µm)	de Man's ratios			
							a	b	c	V
MS	a	880	105	44	210	420	20	4.2	8.4	47.7
MS	a	860	95	40	220	420	21.5	3.9	9.1	47.7
MS	a	850	95	40	220	425	21.3	3.9	8.9	48.3
MS	a	850	105	40	210	420	21.3	4	8.1	47.7
MS	a	810	95	37	200	380	21.9	4.1	8.5	43.2
MP	a	1080	100	38	250	530	28.4	4.3	10.8	60.2
MP	j	810	90	35	170	-	23.1	4.8	9	-
MP	j	760	95	35	180	-	21.7	4.2	8	-
HI	a	980	100	44	-	480	22.3	-	9.8	-
HI	a	800	80	33	-	390	24.2	-	10	44.3
HI	a	860	110	36	180	410	23.9	4.8	7.8	46.6
HI	a	810	90	33	180	390	24.5	4.5	9	44.3
SP	a	800	90	35	200	400	22.9	4	8.9	45.5
SP	a	950	90	30	220	380	31.7	4.3	10.6	43.2
SP	a	920	100	42	-	420	21.9	-	9.2	47.7
SP	j	690	90	26	170	-	26.5	4.1	7.7	-
SP	a	1000	110	42	220	480	23.8	4.5	9.1	54.5
SP	a	960	110	48	210	440	20	4.6	8.7	50
SP	j	600	80	22	180	-	27.3	3.3	7.5	-
SP	a	800	90	28	-	380	28.6	-	8.9	43.2
BP	a	940	110	34	170	440	27.6	5.5	8.5	50
VH	a	810	100	28	200	390	28.9	4.1	8.1	44.3
VH	j	730	80	26	145	-	28.1	5	9.1	-
VH	a	880	100	33	190	430	26.7	4.6	8.8	48.9
VH	a	900	100	33	190	420	27.3	4.7	9	47.7
VH	a	860	100	35	-	400	24.6	-	8.6	45.5
VH	a	910	110	36	200	425	25.3	4.6	8.3	48.3
CS	a	840	105	38	220	410	22.1	3.8	8	46.6
CS	j	780	80	26	-	-	30	-	9.8	-
CS	a	920	100	38	225	460	24.2	4.1	9.2	52.3
CS	a	800	85	30	165	370	26.7	4.8	9.4	42
CS	a	810	110	28	180	380	28.9	4.5	7.4	43.2
CS	a	830	90	34	-	400	24.4	-	9.2	45.5

Abbreviation as following: Mawson Station (MS), Mather Peninsula (MP), Hop Island (HI), Stornes Peninsula (SP), Broknes Peninsula (BP), Vestfold Hills (VH), Casey Station (CS), adult female (a) and juvenile female (j).

Table S3 Mitochondrial lineages, measurements and de Man's ratios for 33 *Plectus* cf. *frigophilus* female specimens collected from Sector 2, and two from TF.

Region	Clade	s/w	Age	Body length (µm)	Tail length (µm)	body width (µm)	Oesophagus length (µm)	de Man's ratios		
								a	b	c
CS	N12	soil	j?	1080	105	46	-	23.5	-	10.3
BP	N12	water	a	1700	160	70	-	24.3	-	10.6
BP	N12	water	a	1800	160	65	380	27.7	4.7	11.3
BP	N12	water	a	1500	130	50	320	30.0	4.7	11.5
BP	N12	water	a	1400	120	45	270	31.1	5.2	11.7
BP	N12	water	a	1700	160	65	330	26.2	5.2	10.6
BP	N12	water	a	1700	165	50	350	34.0	4.9	10.3
BP	N12	water	a	1200	110	45	260	26.7	4.6	10.9
SP	N12	soil	a	1380	120	52	340	26.5	4.1	11.5
SP	N12	soil	a	1380	130	50	320	27.6	4.3	10.6
SP	N12	water	a	1600	150	60	360	26.7	4.4	10.7
SP	N12	water	a	1900	170	60	390	31.7	4.9	11.2
SP	N12	water	a	1600	160	60	320	26.7	5.0	10.0
SP	N12	water	a	1500	130	60	340	25.0	4.4	11.5
SP	N12	water	a	1550	160	55	310	28.2	5.0	9.7
SP	N12	water	a	1600	160	60	330	26.7	4.8	10.0
CS	N11	soil	a	1430	120	52	320	27.5	4.5	11.9
BP	N11	water	a	1700	140	55	350	30.9	4.9	12.1
BP	N11	water	a	1600	140	55	330	29.1	4.8	11.4
BP	N11	water	a	1400	130	40	290	35.0	4.8	10.8
BP	N11	water	a	1700	150	50	350	34.0	4.9	11.3
BP	N11	water	j?	1100	100	45	260	24.4	4.2	11.0
SP	N11	soil	a	2050	140	55	420	37.3	4.9	14.6
SP	N11	soil	a	1380	140	52	280	26.5	4.9	9.9
SP	N11	water	a	1600	140	50	350	32.0	4.6	11.4
SP	N11	water	a	1300	130	40	280	32.5	4.6	10.0
SP	N11	water	a	1550	140	55	310	28.2	5.0	11.1
SP	N11	water	a	1600	150	50	320	32.0	5.0	10.7
SP	N11	water	a	1400	130	55	300	25.5	4.7	10.8
SP	N11	water	a	1500	120	50	300	30.0	5.0	12.5
SP	N11	water	a	1300	150	45	290	28.9	4.5	8.7
FM	N11	soil	j?	930	110	33	240	28.2	3.9	8.5
FM	N11	soil	a	1400	130	45	360	31.1	3.9	10.8
TF	N22	soil	a	970	-	38	-	25.5	-	-
TF	N23	soil	a	900	-	38	-	23.7	-	-

Abreviation as following: Casey Station (CS), Broknes Peninsula (BP), Stornes Peninsula (SP), Framnes Mountains (FM), Tierra del Fuego (TF), possible female juvenile (j?) and adult female (a).

Table S4 Geographic location, measurements and de Man's ratios for 44 specimens from the genus *Eudorylaimus* collected from Sector 2, compared with *E. quintus*, *E. sextus*, *E. glacialis E. nudicaudatus*, and *E. shirasei* populations across Antarctica from three studies.

Species /gender /(reference)	*Region*	*Sector*	*N*	*Body length (μm)*	*Tail length (μm)*	*body width (μm)*	*Oesophagus length (μm)*	*supplements*	*de Man's ratios* a	b	c
♂	**BP**	2	-	1600	38	40	330	-	40.0	4.8	42.1
♂	**BP**	2	-	1720	36	35	360	8	49.1	4.8	47.8
♀	**BP**	2	-	1450	35	35	340	-	41.4	4.3	41.4
♀	**BP**	2	-	1200	30	35	-	-	34.3	-	40.0
♂	**SP**	2	-	1500	38	43	-	9	34.9	-	39.5
♂	**SP**	2	-	1600	38	40	360	8	40.0	4.4	42.1
♂	**SP**	2	-	1550	38	40	-	8	38.8	-	40.8
j?	**SP**	2	-	1150	32	36	280	-	31.9	4.1	35.9
j	**SP**	2	-	1200	38	42	-	-	28.6	-	31.6
j?	**SP**	2	-	1080	38	42	-	-	25.7	-	28.4
j	**SP**	2	-	1150	30	35	300	-	32.9	3.8	38.3
j	**SP**	2	-	1000	35	35	-	-	28.6	-	28.6
j?	**HI**	2	-	1220	42	33	260	-	37.0	4.7	29.0
j?	**HI**	2	-	1080	31	38	260	-	28.4	4.2	34.8
♀	**HI**	2	-	1350	38	42	320	-	32.1	4.2	35.5
E. nudicaudatus, or *E. shirasei* ♂	**HI**	2	-	2150	40	45	360	-	47.8	6.0	53.8
E. nudicaudatus, or E. shirasei ♀	**HI**	2	-	2320	40	42	400	-	55.2	5.8	58.0
j?	**MP**	2	-	1100	42	45	325	-	24.4	3.4	26.2
♀	**MP**	2	-	1280	40	42	350	-	30.5	3.7	32.0
♀	**MP**	2	-	1520	36	38	350	-	40.0	4.3	42.2
♀	**MP**	2	-	1500	50	36	350	-	41.7	4.3	30.0
♂	**MP**	2	-	1400	38	42	-	11	33.3	-	36.8
E. cf. *quintus* ♂	**MP**	2	-	1600	37	40	310	10	40.0	5.2	43.2
E. nudicaudatus, or *E. shirasei* ♂	**MP**	2	-	2400	62	45	400	12	53.3	6.0	38.7
E. cf. *quintus* ♀	**MP**	2	-	1800	50	43	360	-	41.9	5.0	36.0
E. cf. *sextus* ♂	**VH**	2	-	1400	36	35	340	8	40.0	4.1	38.9
E. cf. *sextus* ♀	**VH**	2	-	1340	38	40	330	-	33.5	4.1	35.3
E. cf. *sextus* ♀	**VH**	2	-	1800	50	40	380	-	45.0	4.7	36.0
E. cf. *sextus* ♂	**VH**	2	-	1500	35	45	350	8	33.3	4.3	42.9
E. cf. *sextus* ♀	**VH**	2	-	1600	45	45	420	-	35.6	3.8	35.6
E. cf. *sextus* ♂	**VH**	2	-	1700	40	45	380	9	37.8	4.5	42.5
E. cf. *sextus* ♂	**VH**	2	-	1400	40	40	-	9	35.0	-	35.0
E. cf. *sextus* ♂	**VH**	2	-	1300	38	35	360	8	37.1	3.6	34.2
E. cf. *sextus* ♂	**VH**	2	-	1350	36	35	-	7	38.6	-	37.5
j?	**VH**	2	-	1100	40	33	300	-	33.3	3.7	27.5
E. cf. *sextus* ♀	**VH**	2	-	1300	50	38	310	-	34.2	4.2	26.0
E. cf. *sextus* ♂	**VH**	2	-	1750	47	41	370	9	42.7	4.7	37.2
E. cf. *sextus* ♂	**VH**	2	-	1380	43	33	380	7	41.8	3.6	32.1
E. cf. *sextus* ♀	**VH**	2	-	1470	50	40	370	-	36.8	4.0	29.4
E. cf. *sextus* ♀	**VH**	2	-	1550	50	40	380	-	38.8	4.1	31.0
j?	**VH**	2	-	1100	36	36	-	-	30.6	-	30.6
♂	**VH**	2	-	1120	52	50	290	8	22.4	3.9	21.5
E. cf. glacialis ♂	**MS**	2	-	2100	50	45	430	-	46.7	4.9	42.0
E. cf. glacialis ♂	**MS**	2	-	1700	50	45	320	8	37.8	5.3	34.0
E. quintus ♀ (1)	FM	2	3	1.76-2.05	-	-	-	-	36-38	4.4-5.2	44-51
E. quintus ♂ (1)	FM	2	3	1.60-1.94	-	-	-	9-11	36-40	5.3-5.4	44-45
E. sextus ♀ (1)	VH	2	3	1.26-1.80	-	-	-	-	29-34	4.3-5.2	38-46
E. sextus ♂ (1)	VH	2	5	1.20-1.72	-	-	-	8-10	32-42	4.2-4.9	40-47
E. glacialis ♀ (1)	FM	2	3	1.7-1.86	-	-	-	-	35-37	4.4-5.1	39-43
E. glacialis ♂ (1)	FM	2	2	1.46-1.67	-	-	-	8	35-37	4.4-4.6	35-39
E. glacialis ♀ (3)	VL	3	5	1.47-1.71	-	-	-	-	34-42	3.4-5.6	35-50
E. glacialis ♂ (3)	VL	3	10	1.36-1.83	-	-	-	-	34-50	3.8-5.1	31-40
E. nudicaudatus ♀ (1)	DML	1	6	1.40-2.08	-	-	-	-	32-46	3.7-5.0	32-46
E. nudicaudatus ♂ (1)	DML	1	8	1.77-2.20	-	-	-	range	42-49	4.0-4.7	36-44
E. shirasei ♀ (2)	PC	1	7	1.54-2.20	42-61	-	-	-	31-39	4.0-5.6	30-40
E. shirasei ♂ (2)	PC	1	6	1.35-1.94	40-54	-	-	-	33-42	3.7-5.2	33-36
E. shirasei ♀ (2)	MV	2	2	2.40-2.67	56-62	-	-	-	37-39	5.1-6.0	43
E. shirasei ♂ (2)	MV	2	2	2.34-2.50	52-59	-	-	-	45	5.1-5.2	40-48

Abbreviation as following: Broknes Peninsula (BP), Stornes Peninsula (SP), Hop Island (HI), Mather Peninsula (MP), Vestfold Hills (VH), Mawson Station (MS), Victoria Land (VL), Dronning Maud Land (DML), Prince Olav Coast (POC), Mount Vechernyaya (MtV), female (♀), male (♂), juvenile (j), possible female juvenile (j?), and number of measured nematodes (N). References from three studies are shown in parenthesis in the first column: (1) Andrássy, 1998; (2) Kito *et al*., 1996; (3) Yeates *et al*., 1970. Gaps indicate data not available

References

Andrássy, I., 1998. Nematodes in the Sixth Continent, In: Peña Santiago, R. (Ed.), Journal of Nematode Morphlogy and Systematics. Universidad de Jaén, Jaén, pp. 107-186.

Kito, K., Shishida, Y., Ohyama, Y., 1996. New species of the genus *Eudorylaimus* Andrassy, 1959 (Nematoda: Qudsianematidae) from East Antarctica. Polar Biology 16, 163-169.

Yeates, G.W., 1970. Two Terrestrial Nematodes from the McMurdo Sound Region, Antarctica, with a Note on *Anaplectus arenicola* Killick, 1964. Journal of Helminthology 44, 27-34.

Table S5 Mitochondrial lineages, measurements and de Man's ratios for 16 adult *Halomonhystera* specimens from Broknes Peninsula (BP), Hop Island (HI), and Vestfold Hills (VH).

Species	Clade	soil/water	Region	Body length (um)	Tail length (um)	Body width (um)	de Man's ratios a	de Man's ratios c
*H.*cf. *halophila*	N15	water	HI	1600	120	50	32.0	13.3
*H.*cf. *halophila*	N15	water	HI	1400	95	45	31.1	14.7
*H.*cf. *halophila*	N15	water	HI	1400	90	45	31.1	15.6
*H.*cf. *continentalis*	N16a	soil	BP	650	65	25	26.0	10.0
*H.*cf. *continentalis*	N16b	water	HI	750	75	30	25.0	10.0
*H.*cf. *continentalis*	N16b	water	HI	670	70	25	26.8	9.6
*H.*cf. *continentalis*	N17	soil	**VH**	660	66	28	23.6	10.0
*H.*cf. *continentalis*	N17	soil	**VH**	470	62	22	21.4	7.6
*H.*cf. *continentalis*	N17	soil	**VH**	710	60	28	25.4	11.8
*H.*cf. *continentalis*	N17	soil	**VH**	700	62	28	25.0	11.3
*H.*cf. *continentalis*	N17	soil	**VH**	520	55	20	26.0	9.5
*H.*cf. *continentalis*	N17	soil	**VH**	650	70	35	18.6	9.3
*H.*cf. *continentalis*	-	soil	**HI**	790	-	-	-	-
*H.*cf. *continentalis*	-	soil	**HI**	720	62	26	27.7	11.6
*H.*cf. *continentalis*	-	soil	**HI**	510	50	22	23.2	10.2
*H.*cf. *continentalis*	-	soil	**HI**	750	68	28	26.8	11.0

Table S6 Pearson's product-moment correlation coefficients for log_{10} transformed abiotic variables (electric conductivity, pH and moisture) versus presence-absence of nematode taxa for soil and water samples from Sector 2.

		logEC	logpH	logMoist	*Plectus murrayi*	*Plectus frigophilus*	*Scottnema* cf. *lindsayae*	*Eudorylaimus* spp	*Halomonhystera* spp	cf. Panagrolaimidae
log Electric Conductivity (salinity)	Pearson Correlation	1	.442**	.607**	-.424**	.027	-.406**	-.280**	.663**	.044
	Sig. (2-tailed)		.000	.000	.000	.766	.000	.002	.000	.634
	N	121	121	121	121	121	121	121	121	121
log pH	Pearson Correlation	**.442****	1	.257**	-.538**	.105	.043	-.016	.442**	.042
	Sig. (2-tailed)	.000		.005	.000	.251	.642	.865	.000	.648
	N	121	121	121	121	121	121	121	121	121
log Moisture percentage	Pearson Correlation	**.607****	**.257****	1	-.446**	.518**	-.553**	-.375**	.345**	-.043
	Sig. (2-tailed)	.000	.005		.000	.000	.000	.000	.000	.637
	N	121	121	121	121	121	121	121	121	121
Plectus murrayi	Pearson Correlation	**-.424****	**-.538****	**-.446****	1	-.443**	-.105	.235**	-.438**	.010
	Sig. (2-tailed)	.000	.000	.000		.000	.251	.010	.000	.916
	N	121	121	121	121	121	121	121	121	121
Plectus frigophilus	Pearson Correlation	.027	.105	.518**	**-.443****	1	-.239**	-.188*	-.265**	-.073
	Sig. (2-tailed)	.766	.251	.000	.000		.008	.039	.003	.428
	N	121	121	121	121	121	121	121	121	121
Scottnema* cf. *lindsayae	Pearson Correlation	**-.406****	.043	**-.553****	-.105	**-.239****	1	.416**	-.135	-.066
	Sig. (2-tailed)	.000	.642	.000	.251	.008		.000	.141	.471
	N	121	121	121	121	121	121	121	121	121
***Eudorylaimus* spp**	Pearson Correlation	**-.280****	-.016	**-.375****	**.235****	**-.188***	**.416****	1	-.271**	-.074
	Sig. (2-tailed)	.002	.865	.000	.010	.039	.000		.003	.417
	N	121	121	121	121	121	121	121	121	121
***Halomonhystera* spp**	Pearson Correlation	**.663****	**.442****	**.345****	**-.438****	**-.265****	-.135	**-.271****	1	-.061
	Sig. (2-tailed)	.000	.000	.000	.000	.003	.141	.003		.505
	N	121	121	121	121	121	121	121	121	121
cf. Panagrolaimidae	Pearson Correlation	.044	.042	-.043	.010	-.073	-.066	-.074	-.061	1
	Sig. (2-tailed)	.634	.648	.637	.916	.428	.471	.417	.505	
	N	121	121	121	121	121	121	121	121	121

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

No correction for multiple comparisons was performed as studies have shown that it is not suitable (Peres-Neto, 1999; Moran, 2003; Peres-Neto *et al.*, 2003).

References

Moran, M.D., 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos 100, 403-405.

Peres-Neto, P.R., 1999. How many statistical tests are too many? The problem of conducting multiple ecological inferences revisited. Marine ecology progress series 176, 303-306.

Peres-Neto, P.R., Jackson, D.A., Somers, K.M., 2003. Giving meaningful interpretation to ordination axes: assessing loading significance in principal component analysis. Ecology 84, 2347-2363.

Table S7 Estimates of Sequence Divergence (p-distances) among 43 haplotypes. The number of base differences per site between sequences are shown. The analysis involved 43 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 438 nucleotide positions in the final alignment. Analyses were conducted in MEGA5 (Tamura *et al*., 2011).

		Clade	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	N1_FN397753_Rhabditida_sp																						
2	N2_FN397790_Rhabditida_sp	I	0.091																				
3	N3_MSNEM055-AVC288_01-Panagrol-HI	I	0.100	0.098																			
4	N4_HM627507_Panagrolaimus_paetzoldi	I	0.116	0.116	0.116																		
5	N5_FN397788_Rhabditida_sp	II	0.153	0.153	0.164	0.144																	
6	N6_FN397766_Rhabditida_sp_China	II	0.158	0.164	0.160	0.153	0.146																
7	N7_FN397792_Rhabditida_sp	II	0.180	0.185	0.171	0.160	0.144	0.146															
8	N8_MSNEM043-AVC492_03-Panagrol-MS	II	0.187	0.183	0.180	0.189	0.162	0.160	0.164														
9	N9_HM627505_Plectus_aquatiliis_Netherl	III	0.290	0.295	0.258	0.267	0.281	0.299	0.281	0.295													
10	N10_MSNEM372|AVC256_Nem04-P_murrayi	III	0.274	0.274	0.251	0.258	0.274	0.276	0.295	0.301	0.164												
11	N10_MSNEM171|AVC296_Nem11-P_murrayi	III	0.274	0.274	0.251	0.258	0.274	0.276	0.295	0.301	0.164	0.000											
12	N10_MSNEM168|AVC296_Nem08-P_murrayi	III	0.274	0.274	0.251	0.258	0.274	0.276	0.295	0.301	0.164	0.000	0.000										
13	N10_MSNEM336|AVC14_Nem06-P_murrayi	III	0.274	0.274	0.251	0.258	0.274	0.276	0.295	0.301	0.164	0.000	0.000	0.000									
14	N10_MSNEM161_AVC239_01_P_murrayi	III	0.274	0.274	0.251	0.258	0.274	0.276	0.295	0.301	0.164	0.000	0.000	0.000	0.000								
15	N10_MSNEM305|AVC42_Nem06-P_murrayi	III	0.274	0.274	0.251	0.258	0.274	0.276	0.295	0.301	0.164	0.000	0.000	0.000	0.000	0.000							
16	N10_MSNEM040|AVC514_Nem05-P_murrayi	III	0.274	0.274	0.251	0.258	0.274	0.276	0.295	0.301	0.164	0.000	0.000	0.000	0.000	0.000	0.000						
17	N10_MSNEM275|AVC6_Nem3-P_murrayi	III	0.276	0.276	0.253	0.260	0.276	0.279	0.297	0.304	0.167	0.002	0.002	0.002	0.002	0.002	0.002	0.002					
18	N10_MSNEM264|AVC93_Nem7-P_murrayi	III	0.274	0.272	0.251	0.258	0.274	0.274	0.295	0.301	0.164	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.005				
19	N23_MSNEM813_PIA_S31_Nem05_Plectus	III	0.283	0.272	0.251	0.263	0.288	0.290	0.283	0.297	0.176	0.153	0.153	0.153	0.153	0.153	0.153	0.153	0.155	0.153			
20	N22_MSNEM811_PIA_S31_Nem03_Plectus	III	0.283	0.276	0.251	0.263	0.290	0.288	0.290	0.301	0.176	0.148	0.148	0.148	0.148	0.148	0.148	0.148	0.151	0.148	0.014		
21	N11_MSNEM329-AVC504_2-Pl_frigophilus	III	0.292	0.292	0.272	0.274	0.299	0.306	0.299	0.324	0.169	0.151	0.151	0.151	0.151	0.151	0.151	0.151	0.153	0.151	0.082	0.087	
22	N12_MSNEM760_AVC76_05_Pl_frigophilus	III	0.285	0.272	0.242	0.253	0.272	0.281	0.276	0.299	0.162	0.137	0.137	0.137	0.137	0.137	0.137	0.137	0.139	0.137	0.059	0.068	0.080
23	N12_MSNEM743AVC162_04-Pl_frigophilus	III	0.283	0.269	0.240	0.251	0.269	0.279	0.274	0.297	0.164	0.135	0.135	0.135	0.135	0.135	0.135	0.135	0.137	0.135	0.057	0.066	0.082
24	N12_MSNEM742|AVC162_03-Pl_frigophilus	III	0.283	0.272	0.242	0.253	0.269	0.279	0.276	0.297	0.167	0.137	0.137	0.137	0.137	0.137	0.137	0.137	0.139	0.137	0.059	0.068	0.084
25	N13_MSNEM598_Rhyssocol paradoxus_AP	IV	0.361	0.372	0.368	0.381	0.368	0.372	0.370	0.363	0.413	0.413	0.413	0.413	0.413	0.413	0.413	0.413	0.411	0.413	0.429	0.429	0.438
26	N14_MSNEM629_Dorylaimidae_AP	IV	0.345	0.345	0.317	0.317	0.358	0.336	0.349	0.388	0.365	0.365	0.365	0.365	0.365	0.365	0.365	0.365	0.363	0.365	0.365	0.363	0.372
27	N24_MSNEM1031-Pint-C32_01_cf Plectidae	IV	0.356	0.361	0.358	0.358	0.379	0.361	0.372	0.374	0.418	0.381	0.381	0.381	0.381	0.381	0.381	0.381	0.381	0.379	0.409	0.411	0.420
28	N15_MSNEM562_AVC261_06_Halomonhyst	V	0.345	0.340	0.336	0.331	0.345	0.338	0.356	0.347	0.352	0.358	0.358	0.358	0.358	0.358	0.358	0.358	0.361	0.358	0.388	0.381	0.372
29	N16b_MSNEM552|AVC286_05-Halomonhyst	V	0.333	0.329	0.329	0.322	0.331	0.333	0.342	0.342	0.349	0.356	0.356	0.356	0.356	0.356	0.356	0.356	0.358	0.356	0.390	0.384	0.374
30	N16a_MSNEM142|AVC105_03-Halomonhyst	V	0.331	0.326	0.326	0.317	0.331	0.336	0.342	0.340	0.345	0.354	0.354	0.354	0.354	0.354	0.354	0.354	0.356	0.354	0.388	0.381	0.377
31	N17_MSNEM753_AVC272_04_Halomonhyst	V	0.321	0.312	0.324	0.319	0.343	0.345	0.345	0.341	0.341	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.353	0.348	0.376	0.369	0.362
32	N17_MSNEM470|MBS017_06-Halomonhyst	V	0.326	0.320	0.331	0.324	0.345	0.342	0.342	0.338	0.352	0.358	0.358	0.358	0.358	0.358	0.358	0.358	0.361	0.356	0.381	0.374	0.374
33	N17_MSNEM564|AVC311_01-Halomonhyst	V	0.326	0.322	0.331	0.324	0.345	0.345	0.342	0.338	0.352	0.358	0.358	0.358	0.358	0.358	0.358	0.358	0.361	0.358	0.381	0.374	0.374
34	N17_MSNEM473|MBS018_02-Halomonhyst	V	0.326	0.320	0.331	0.324	0.345	0.342	0.342	0.338	0.349	0.358	0.358	0.358	0.358	0.358	0.358	0.358	0.361	0.356	0.379	0.372	0.372
35	N17_MSNEM465-MBS017_06-Halomonhyst	V	0.326	0.320	0.331	0.324	0.345	0.342	0.342	0.338	0.349	0.358	0.358	0.358	0.358	0.358	0.358	0.358	0.361	0.356	0.379	0.372	0.372
36	N17_MSNEM471|MBS017_07-Halomonhyst	V	0.326	0.320	0.331	0.324	0.345	0.342	0.342	0.338	0.349	0.358	0.358	0.358	0.358	0.358	0.358	0.358	0.361	0.356	0.379	0.372	0.372
37	N18_MSNEM394|AVC247_08-Scottnema	VI	0.379	0.384	0.397	0.386	0.409	0.402	0.420	0.411	0.411	0.393	0.393	0.393	0.393	0.393	0.393	0.393	0.395	0.390	0.404	0.397	0.406
38	N18_MSNEM291-AVC85_06-Scottnema-EA	VI	0.374	0.379	0.393	0.381	0.409	0.397	0.420	0.406	0.409	0.390	0.390	0.390	0.390	0.390	0.390	0.390	0.393	0.388	0.402	0.395	0.406
39	N18_MSNEM265|AVC93_08-Scottnema	VI	0.379	0.384	0.397	0.386	0.409	0.402	0.420	0.411	0.409	0.390	0.390	0.390	0.390	0.390	0.390	0.390	0.393	0.388	0.402	0.395	0.404
40	N19_MSNEM585_Cdh63_03_DML_Scottne	VI	0.381	0.381	0.400	0.388	0.406	0.397	0.418	0.409	0.409	0.390	0.390	0.390	0.390	0.390	0.390	0.390	0.393	0.388	0.404	0.397	0.409
41	N21_MSNEM094-AVC503_04-Scottnema-FM	VI	0.379	0.386	0.395	0.386	0.409	0.404	0.416	0.411	0.411	0.386	0.386	0.386	0.386	0.386	0.386	0.386	0.388	0.386	0.400	0.393	0.402
42	N20_MSNEM386-AVC505_10-Scottnema-FM	VI	0.374	0.379	0.390	0.381	0.406	0.397	0.416	0.416	0.406	0.384	0.384	0.384	0.384	0.384	0.384	0.384	0.386	0.381	0.395	0.388	0.400
43	N25_MSNEM828-12_PIA-S-5-1_Criconemat	VII	0.349	0.377	0.352	0.356	0.356	0.377	0.370	0.370	0.384	0.390	0.390	0.390	0.390	0.390	0.390	0.390	0.388	0.390	0.377	0.377	0.388

Table S7 (continued)

		Clade	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
23	N12_MSNEM743AVC162_04-Pl_frigophilus	III	0.002																				
24	N12_MSNEM742|AVC162_03-Pl_frigophilus	III	0.005	0.002																			
25	N13_MSNEM598_Rhyssocol paradoxus_AP	IV	0.418	0.416	0.416																		
26	N14_MSNEM629_Dorylaimidae_AP	IV	0.352	0.349	0.352	0.247																	
27	N24_MSNEM1031-Pint-C32_01_cf Plectidae	IV	0.406	0.404	0.406	0.409	0.393																
28	N15_MSNEM562_AVC261_06_Halomonhyst	V	0.372	0.370	0.370	0.397	0.393	0.434															
29	N16b_MSNEM552|AVC286_05-Halomonhyst	V	0.372	0.370	0.370	0.393	0.397	0.434	0.023														
30	N16a_MSNEM142|AVC105_03-Halomonhyst	V	0.370	0.368	0.368	0.395	0.397	0.436	0.023	0.005													
31	N17_MSNEM753_AVC272_04_Halomonhyst	V	0.365	0.362	0.362	0.400	0.417	0.441	0.041	0.022	0.017												
32	N17_MSNEM470|MBS017_06-Halomonhyst	V	0.372	0.370	0.370	0.397	0.413	0.436	0.041	0.023	0.018	0.002											
33	N17_MSNEM564|AVC311_01-Halomonhyst	V	0.372	0.370	0.370	0.397	0.413	0.438	0.041	0.023	0.018	0.005	0.002										
34	N17_MSNEM473|MBS018_02-Halomonhyst	V	0.370	0.368	0.368	0.400	0.416	0.438	0.043	0.025	0.021	0.005	0.002	0.005									
35	N17_MSNEM465-MBS017_06-Halomonhyst	V	0.370	0.368	0.368	0.400	0.416	0.438	0.043	0.025	0.021	0.005	0.002	0.005	0.000								
36	N17_MSNEM471|MBS017_07-Halomonhyst	V	0.370	0.368	0.368	0.400	0.416	0.438	0.043	0.025	0.021	0.005	0.002	0.005	0.000	0.000							
37	N18_MSNEM394|AVC247_08-Scottnema	VI	0.402	0.400	0.402	0.470	0.452	0.447	0.427	0.425	0.427	0.417	0.416	0.418	0.416	0.416	0.416						
38	N18_MSNEM291-AVC85_06-Scottnema-EA	VI	0.400	0.397	0.400	0.470	0.452	0.447	0.432	0.429	0.432	0.422	0.420	0.422	0.420	0.420	0.420	0.005					
39	N18_MSNEM265|AVC93_08-Scottnema	VI	0.400	0.397	0.400	0.468	0.450	0.447	0.429	0.427	0.429	0.420	0.418	0.420	0.418	0.418	0.418	0.005	0.005				
40	N19_MSNEM585_Cdh63_03_DML_Scottne	VI	0.404	0.402	0.404	0.466	0.454	0.447	0.432	0.429	0.432	0.422	0.420	0.422	0.420	0.420	0.420	0.011	0.011	0.011			
41	N21_MSNEM094-AVC503_04-Scottnema-FM	VI	0.397	0.395	0.397	0.468	0.450	0.445	0.432	0.429	0.432	0.424	0.422	0.420	0.422	0.422	0.422	0.014	0.018	0.018	0.021		
42	N20_MSNEM386-AVC505_10-Scottnema-FM	VI	0.395	0.393	0.395	0.466	0.450	0.447	0.434	0.432	0.434	0.424	0.422	0.425	0.422	0.422	0.422	0.018	0.018	0.018	0.016	0.014	
43	N25_MSNEM828-12_PIA-S-5-1_Criconemat	VII	0.379	0.377	0.377	0.416	0.406	0.429	0.429	0.429	0.427	0.427	0.422	0.422	0.420	0.420	0.420	0.443	0.441	0.441	0.450	0.443	0.441

Reference

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28, 2731-2739.

Table S8 List of GenBank accession numbers, sequence IDs and isolate IDs for each of the nematode haplotypes. Region, habitat and collection date are also shown in the table.

GenBank accession number	GenBank sequence_ID	GenBank Isolate_ID	Lineage	Taxon	Region	Habitat	Collection date
KJ124184 - KJ124187	Seq1 - Seq4	N3_01 - N3_04	N3	cf. Panagrolaimidae	HI	soil	2010
KJ124188	Seq5	N8_01	N8	cf. Panagrolaimidae	MS	soil	2010
KJ124189	Seq6	N10_01	N10	*Plectus murrayi*	SP	soil	2010
KJ124190 - KJ124289	Seq7 - Seq106	N10_02 - N10_101	N10	*Plectus murrayi*	CS, VH, HI, SP, BP, SI, MS, FM	soil - water	2009 - 2010
KJ124290	Seq107	N10_102	N10	*Plectus murrayi*	HI	soil	2010
KJ124291 - KJ124351	Seq108 - Seq168	N10_103 - N10_163	N10	*Plectus murrayi*	CS, VH, HI, BP, SI	soil	2009 - 2010
KJ124352	Seq169	N10_164	N10	*Plectus murrayi*	SP	soil	2010
KJ124353 - KJ124381	Seq170 - Seq198	N10_165 - N10_193	N10	*Plectus murrayi*	VH, HI, MP, BP, SP, MS	soil	2010
KJ124382	Seq199	N10_194	N10	*Plectus murrayi*	MS	soil	2010
KJ124383	Seq200	N10_195	N10	*Plectus murrayi*	CS	soil	2009
KJ124384 - KJ124396	Seq201 - Seq213	N10_196 - N10_208	N10	*Plectus murrayi*	CS, VH, BP, SP	soil	2009 - 2010
KJ124397 - KJ124430	Seq214 - Seq247	N11_01 - N11_34	N11	*Plectus* cf. *frigophilus*	CS, BP, SP, FM	soil - water	2009 - 2010
KJ124431 - KJ124464	Seq248 - Seq281	N12_01 - N12_34	N12	*Plectus* cf. *frigophilus*	CS, BP, SP	soil	2009 - 2010
KJ124465	Seq282	N12_35	N12	*Plectus* cf. *frigophilus*	SP	soil	2010
KJ124466, KJ124467	Seq283, Seq284	N12_36, N12_37	N12	*Plectus* cf. *frigophilus*	SP	soil	2010
KJ124468	Seq285	N13_01	N13	Dorylaimida	AP	soil	2007
KJ124469	Seq286	N14_01	N14	Dorylaimida	AP	soil	2007
KJ124470 - KJ124473	Seq287 - Seq290	N15_01 - N15_04	N15	*H.* cf. *halophila*	HI	water	2010
KJ124474	Seq291	N16a_01	N16a	*H.* cf. *continentalis*	BP	soil	2010
KJ124475, KJ124476	Seq292, Seq293	N16b_01, N16b_02	N16b	*H.* cf. *continentalis*	HI	water	2010
KJ124477	Seq294	N17_01	N17	*H.* cf. *continentalis*	VH	soil	2010
KJ124478	Seq295	N17_02	N17	*H.* cf. *continentalis*	VH	water	2010
KJ124479	Seq296	N17_03	N17	*H.* cf. *continentalis*	VH	soil	2010
KJ124480	Seq297	N17_04	N17	*H.* cf. *continentalis*	VH	soil	2010
KJ124481	Seq298	N17_05	N17	*H.* cf. *continentalis*	HI	water	2010
KJ124482, KJ124483	Seq299, Seq300	N17_06, N17_07	N17	*H.* cf. *continentalis*	VH	soil	2010
KJ124484	Seq301	N22_01	N22	*Plectus* cf. *frigophilus*	TF	soil	2009
KJ124485	Seq302	N23_01	N23	*Plectus* cf. *frigophilus*	TF	soil	2009
KJ124486	Seq303	N24_01	N24	Plectidae	TF	soil	2009
KJ124487 - KJ124490	Seq304 - Seq307	N18_01 - N18_04	N18	*Scottnema* cf. *lindsayae*	SP	soil	2010
KJ124491, KJ124492	Seq308, Seq309	N18_05, N18_06	N18	*Scottnema* cf. *lindsayae*	BP, SP	soil	2010
KJ124493	Seq310	N18_07	N18	*Scottnema* cf. *lindsayae*	BP	soil	2010
KJ124494 - KJ124539	Seq311 - Seq356	N18_08 - N18_53	N18	*Scottnema* cf. *lindsayae*	VH, HI, MP, BP, SP	soil	2010
KJ124540, KJ124541	Seq357, Seq358	N19_01, N19_02	N19	*Scottnema* cf. *lindsayae*	DML	soil	2009
KJ124542 - KJ124549	Seq359 - Seq366	N20_01 - N20_08	N20	*Scottnema* cf. *lindsayae*	FM	soil	2010
KJ124550	Seq367	N21_01	N21	*Scottnema* cf. *lindsayae*	FM	soil	2010
KJ124551 - KJ124554	Seq368 - Seq371	N25_01 - N25_04	N25	Criconematidae	TF	soil	2009

Acronyms list: Casey Station (CS), Vestfold Hills (VH), Hop Island (HI), Mather Peninsula (MP), Stornes Peninsula (SP), Broknes Peninsula (BP), Sansom Island (SI), Mawson Station (MS), Framnes Mountains (FM), Dronning Maud Land (DML), Antarctic Peninsula (AP) and Tierra del Fuego (TF). Lineage number corresponds to those defined in Fig. 1. Detailed specimen records, images, collection data, voucher specimen information, sequence information, including trace files and primer details are accessible on the BOLD webpage in the ANTAR (Antarctic Invertebrates) project.

Table S9 List of 121 soil and water samples from Sector 2 showing nematode taxa and the abiotic parameters: Electric conductivity (EC), pH and Moisture (%).

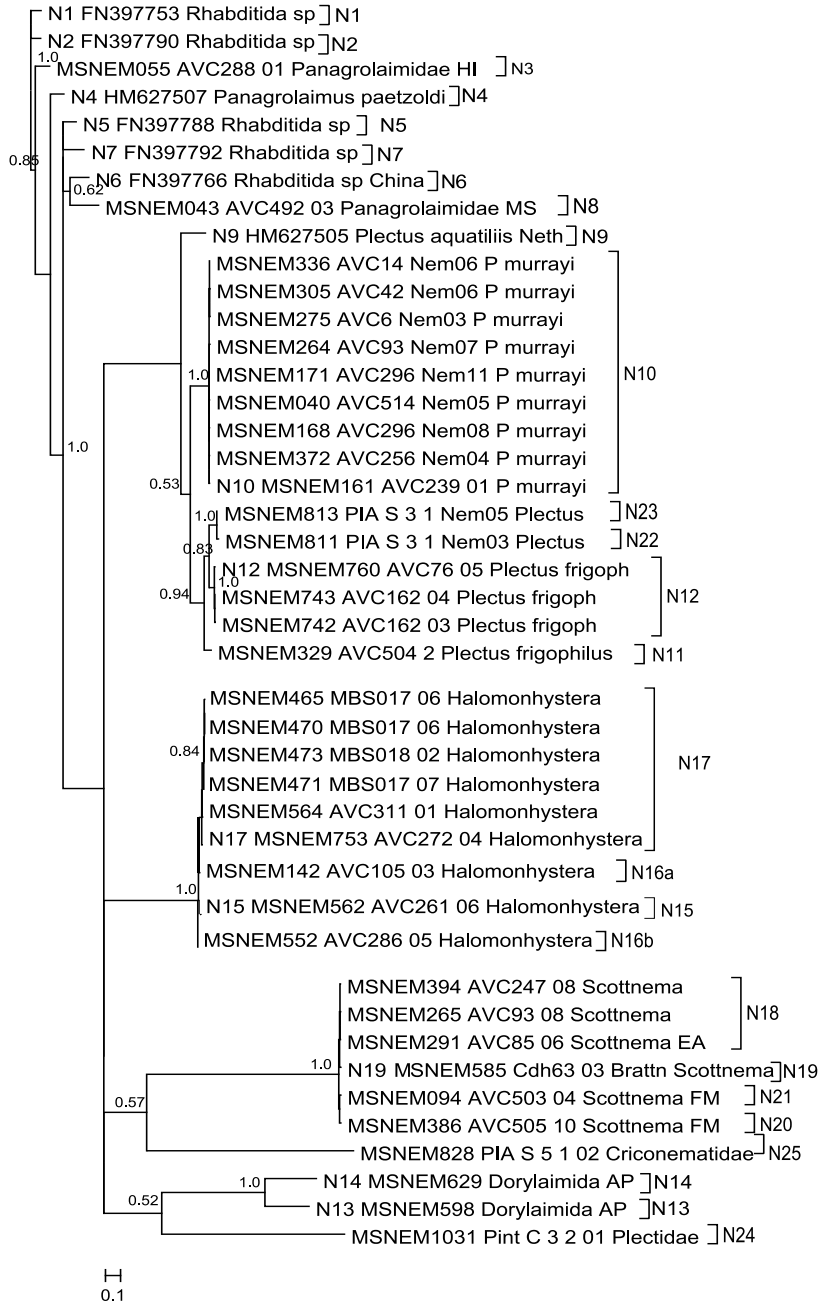
Sample	Region	Plectus murrayi	Plectus frigophilus	Scottn	Eudor	Halom	Panagr	EC (d S/m)	pH	Moisture (%)
AVC107	BP	0	0	0	0	1	0	1.798	7.55	100
AVC261	HI	0	0	0	0	1	0	45.9	9.16	100
AVC272	HI	0	0	0	0	1	0	14.42	8.19	100
AVC278	HI	0	0	0	0	1	0	2.34	8.44	100
AVC286	HI	0	0	0	0	1	0	6.86	8.73	100
AVC311	HI	0	0	0	0	1	0	6.84	7.37	100
AVC316	HI	0	0	0	0	1	0	13.23	7.61	100
AVC337	HI	0	0	0	0	1	0	12.31	8.05	100
AVC361	HI	0	0	0	0	1	0	23.4	7.97	100
AVC365	HI	0	0	0	0	1	0	81.5	7.63	100
AVC368	HI	0	0	0	0	1	0	15.39	7.7	100
AVC372	HI	0	0	0	0	1	0	21	8.03	100
AVC386	HI	0	0	0	0	1	0	17.79	7.23	100
AVC393	HI	0	0	0	0	1	0	6.95	7.58	100
AVC400	HI	0	0	0	0	1	0	20.4	8.56	100
AVC403	HI	0	0	0	0	1	0	10.71	7.82	100
AVC410	HI	0	0	0	0	1	0	18.19	7.08	100
AVC110	BP	0	1	0	0	0	0	1.701	4.89	100
AVC119	BP	0	1	0	0	0	0	0.987	7.42	100
AVC127	BP	0	1	0	0	0	0	0.344	7.3	100
AVC150	BP	0	1	0	0	0	0	0.444	7.36	100
AVC158	SP	0	1	0	0	0	0	0.1544	6.7	100
AVC162	SP	0	1	0	0	0	0	0.521	7.47	100
AVC168	SP	0	1	0	0	0	0	0.243	7.81	100
AVC172	SP	0	1	0	0	0	0	0.1885	7.6	100
AVC176	SP	0	1	0	0	0	0	0.1948	7.3	100
AVC189	SP	0	1	0	1	0	0	0.232	7.04	100
AVC199	SP	0	1	0	0	0	0	0.703	7.17	100
AVC205	SP	0	1	0	0	0	0	0.1359	7.44	100
AVC222	SP	0	1	0	0	0	0	0.107	6.95	100
AVC226	SP	0	1	0	0	0	0	0.358	6.92	100
AVC230	SP	0	1	0	0	0	0	0.545	7.27	100
AVC236	SP	0	1	0	0	0	0	0.517	7.43	100
AVC241	SP	0	1	0	0	0	0	0.948	7.9	100
AVC254	SP	0	1	0	0	0	0	0.238	7	100
AVC34	BP	0	1	0	0	0	0	0.942	7.22	100
AVC45	BP	0	1	0	0	0	0	0.376	6.69	100
AVC61	BP	0	1	0	0	0	0	0.315	7.05	100
AVC66	BP	0	1	0	0	0	0	0.329	6.83	100
AVC70	BP	0	1	0	0	0	0	0.258	7.8	100
AVC76	BP	0	1	0	0	0	0	0.238	7.12	100
AVC94	BP	0	1	0	1	0	0	0.356	6.48	100
AVC466	VH	0	0	0	1	0	0	9.89	8.61	100
AVC95	BP	1	0	0	0	0	0	0.1476	6.9	100
AVC188	SP	0	1	0	0	0	0	0.88	5.88	77.05
AVC16	CS	1	0	0	0	0	0	0.6	5.8	69.41
AVC17	CS	1	0	0	0	0	0	0.37	4.7	67.08
AVC15	CS	0	1	0	0	0	0	0.28	4.7	63.27
AVC488	MS	1	0	0	0	0	0	0.09	5.5	33.46
AVC310	HI	1	0	0	1	0	0	0.81	6.9	28.62
AVC22	VH	1	0	0	0	0	0	0.08	6.68	26.74
AVC243	SP	1	0	0	0	0	0	0.13	5.2	25.85
MBS018	VH	0	0	0	0	1	0	3.02	7.6	24.44
AVC246	SP	1	0	0	0	0	0	0.03	4.8	24.18
AVC242	SP	1	0	0	0	0	0	0.26	4.6	22.96
AVC3	CS	1	0	0	0	0	0	0.06	5.05	21.23
AVC111	BP	1	1	0	0	0	0	0.09	5.8	20.17
AVC482	SI	1	0	0	0	0	0	0.48	6.4	20.01
AVC11	CS	1	0	0	0	0	0	0.66	5.6	19.55
AVC14	CS	1	0	0	0	0	0	0.45	5.4	19.4
AVC208	SP	1	0	0	0	0	0	0.15	6.3	18.57
AVC256	SP	1	0	0	0	0	0	0.44	4.3	18.45
AVC1	CS	1	0	0	0	0	0	0.12	5.5	18.18
AVC73	BP	1	0	0	0	0	0	0.08	6.6	18.11
AVC304	HI	1	0	0	0	0	0	48.1	6.7	16.6
AVC253	SP	1	0	0	1	0	0	0.22	5.8	16.3

Table S9 (continued)

Sample	Region	Plectus murrayi	Plectus frigophilus	Scottn	Eudor	Halom	Panagr	EC (d S/m)	pH	Moisture (%)
AVC13	CS	1	0	0	0	0	0	0.09	4.6	15.79
AVC288	HI	0	0	0	0	0	1	4.39	8.24	15.55
MBS008	VH	0	0	1	1	0	0	0.24	8.1	15.45
AVC390	HI	0	0	1	0	1	0	0.04	7.5	15.18
AVC405	HI	1	0	0	1	0	0	0.14	6.72	15.1
MBS017	VH	0	0	0	0	1	0	1.11	8	14.09
AVC62	BP	1	0	0	1	0	0	0.06	6.5	13.86
AVC439	MP	1	0	0	0	0	0	0.03	6.1	13.71
AVC105	BP	0	0	0	0	1	0	0.05	6.88	13.38
AVC4	CS	1	0	0	0	0	0	0.06	4.8	13.27
AVC227	SP	0	0	1	1	0	0	0.03	6.2	12.67
AVC477	SI	1	0	0	0	0	0	0.13	7.3	12.56
AVC445	MP	1	0	0	1	0	0	0.06	6.5	12.06
AVC68	BP	1	0	0	0	0	0	0.03	8	12.06
AVC41	BP	0	0	1	0	0	0	0.03	6.41	11.86
AVC42	BP	1	1	1	1	0	0	0.04	6.3	11.84
AVC517	MS	1	0	0	1	0	0	0.07	6.17	11.05
AVC28	VH	1	0	1	1	0	0	0.1	6.13	10.75
AVC426	MP	1	0	0	1	0	0	0.06	6.32	9.24
AVC210	SP	1	0	0	1	0	0	0.02	5.9	9.22
AVC492	MS	1	0	0	0	0	1	0.08	6	8.76
AVC7	CS	1	0	0	0	0	0	0.13	5.5	8.75
MBS015	VH	0	0	1	1	0	0	0.26	7.9	8.51
AVC469	VH	0	0	1	1	0	0	0.27	6.5	8.47
AVC93	BP	1	0	1	1	0	0	0.02	6	8.04
AVC239	SP	1	0	1	0	0	0	0.04	6.92	7.7
AVC44	BP	0	0	1	0	0	0	0.06	6.9	7.62
AVC 173	SP	1	0	0	0	0	0	0.02	6.15	7.06
AVC85	BP	1	0	1	1	0	0	0.03	6.1	6.93
MBS022	VH	1	0	0	0	0	0	1.52	7.3	6.89
AVC504	FM	1	0	0	0	0	0	0.11	7.6	6.53
AVC451	VH	1	0	1	1	0	0	0.27	9	6.37
AVC6	CS	1	0	0	0	0	0	0.04	5.3	6.31
MBS019	VH	0	0	1	0	1	0	0.38	6.1	5.85
AVC468	VH	0	0	1	1	0	0	0.05	6.03	5.78
AVC296	HI	1	0	0	0	0	0	0.29	5.22	5.45
AVC467	VH	1	0	0	1	0	0	3.5	6.7	5.33
MBS003	VH	1	0	0	0	0	0	0.03	7.59	5.23
AVC432	MP	1	0	0	1	0	0	0.03	7.06	2.37
AVC457	VH	1	0	1	1	0	0	0.02	6.93	2.25
MBS028	VH	1	0	0	1	0	0	0.13	6.7	2.13
AVC503	FM	0	0	1	0	0	0	0.07	6.11	1.85
AVC249	SP	0	0	1	1	0	0	0.04	6.5	1.78
AVC505	FM	0	0	1	0	0	0	0.03	6.8	1.59
AVC245	SP	1	0	0	0	0	0	0.03	5.84	1.54
AVC425	MP	1	0	1	1	0	0	0.06	6.5	1.5
AVC508	FM	1	0	0	0	0	0	0.01	5.7	1.29
AVC250	SP	1	0	0	1	0	0	0.22	6.7	1.26
AVC147	BP	0	0	1	0	0	0	0.02	7.21	1.14
MBS027	VH	1	0	0	0	0	0	0.41	6.1	1.14
MBS007	VH	0	0	1	0	0	0	0.16	7.8	0.91
AVC514	MS	1	0	0	0	0	0	0.07	6	0.66
AVC394	HI	0	0	1	0	0	0	0.02	7.8	0.44
AVC450	MP	1	0	1	1	0	0	0.05	6.5	0.25
AVC247	SP	0	0	1	1	0	0	0.01	6.1	0.11

Abbreviation as following: Mawson Station (MS), Mather Peninsula (MP), Hop Island (HI), Stornes Peninsula (SP), Broknes Peninsula (BP), Vestfold Hills (VH), Casey Station (CS), *Scottnema* cf. *lindsayae* (Scott), *Eudorylaimus* ssp (Eudor), *Halomonhystera* spp (Halom), and cf. Panagrolaimidae (Panagr).

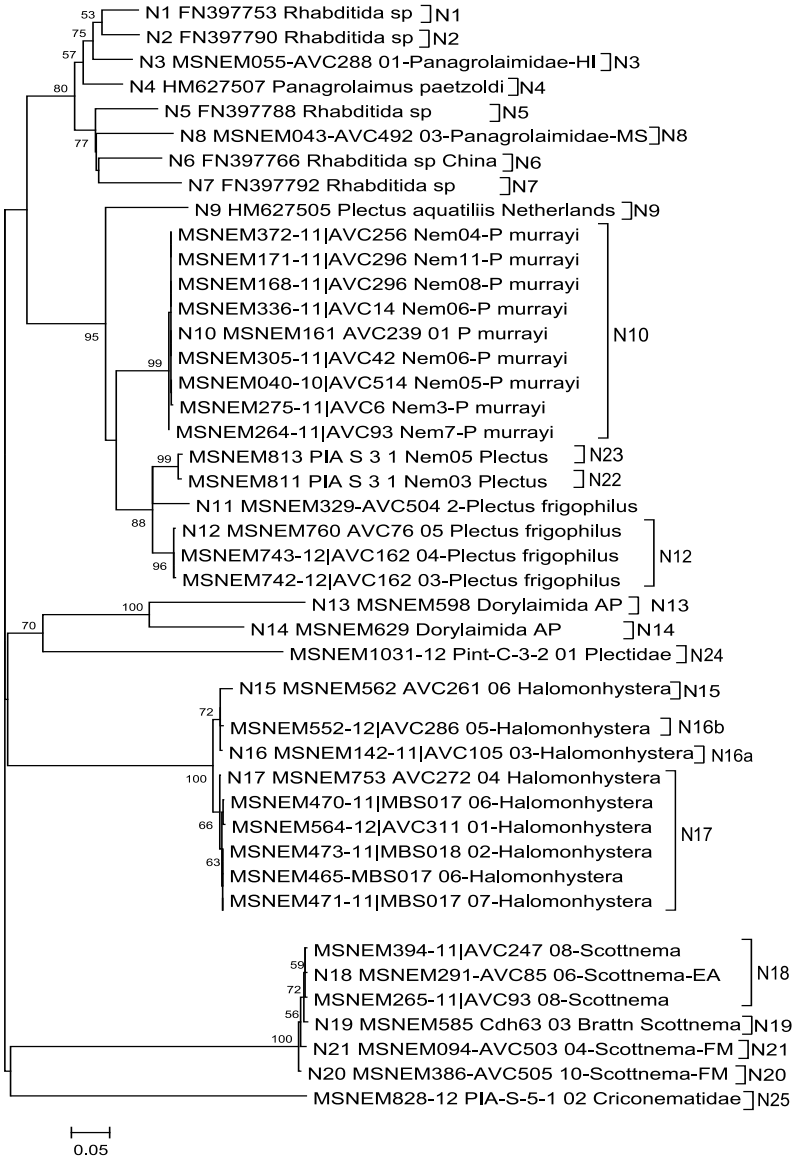
Fig. S1 Bayesian consensus tree based on mtDNA (COI) dataset from 43 haplotypes, implemented in MRBAYES (Huelsenbeck & Ronquist, 2001) under a GTR+Γ model. Numbers at nodes indicate posterior probabilities. Numbers N1 – N25 correspond to the established mitochondrial lineages.



Reference

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754-755.

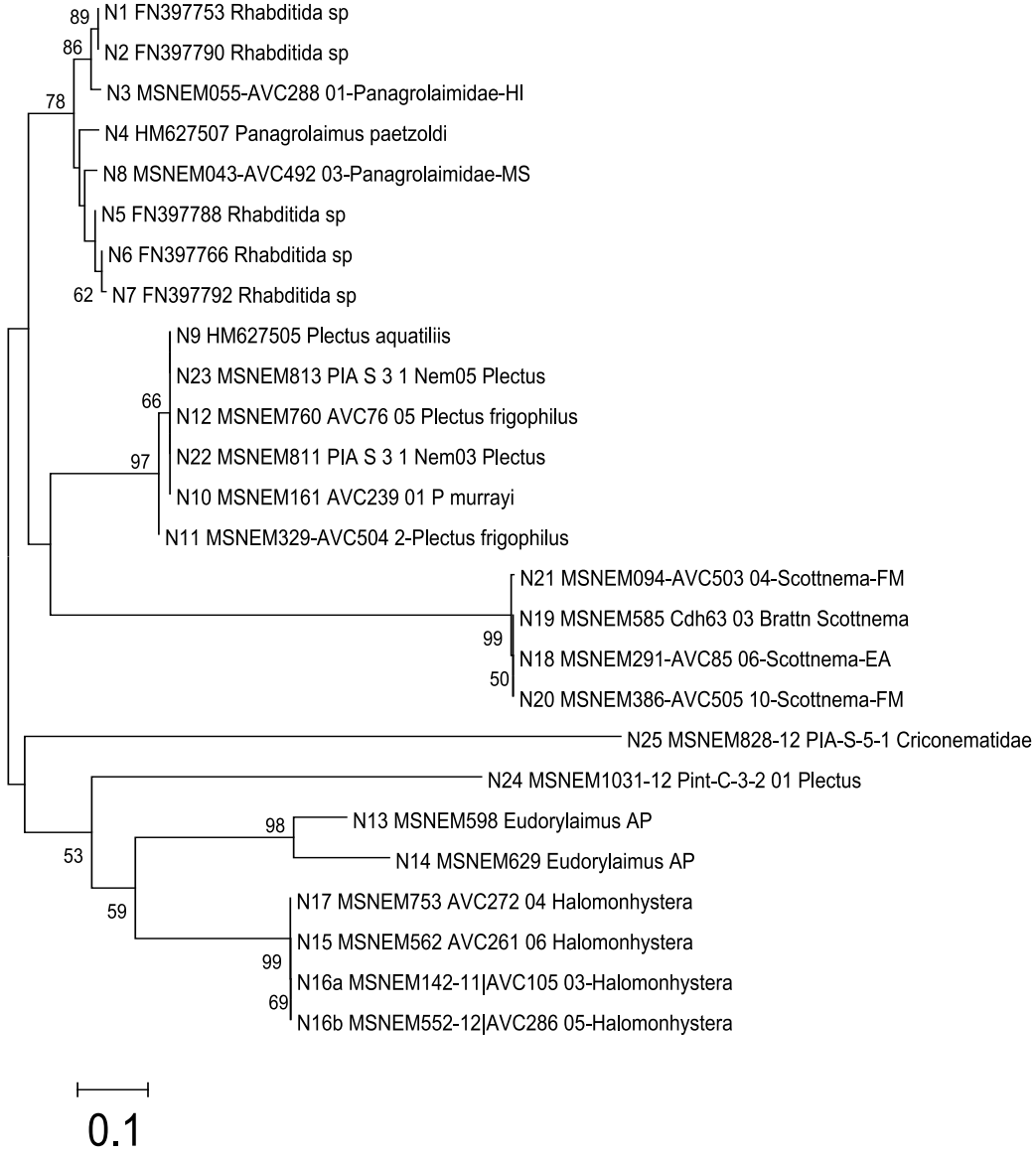
Fig. S2 ML tree based on mtDNA (COI) dataset from 43 nematodes haplotypes. ML bootstrap values (1000 replicas) >50% are shown next to the nods. The analysis was performed in MEGA5 (Tamura *et al.*, 2011) under a GTR+Γ model of evolution. Numbers N1 – N25 correspond to the established mitochondrial lineages.



Reference

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28, 2731-2739.

Fig. S3 ML consensus tree (1000 bootstrap replicas) of amino acid sequences for a representative sequence for each of the 26 COI lineages, based on JTT+Γ model of substitution with invariant sites were created using the program MEGA5 (Tamura *et al.*, 2011). Only bootstrap values >50% are shown.



Reference

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28, 2731-2739.

APPENDIX 4

Fig. S1. ML tree based on mtDNA (COI) dataset from 130 tardigrade COI haplotypes. ML bootstrap values (1000 replicates) >50% are shown next to the nods. The analysis was performed in MEGA5 using a GTR+I+Γ model of evolution. Numbers Ta1 – Ta63 correspond to the established mitochondrial lineages used in the main manuscript and in Fig. 2.

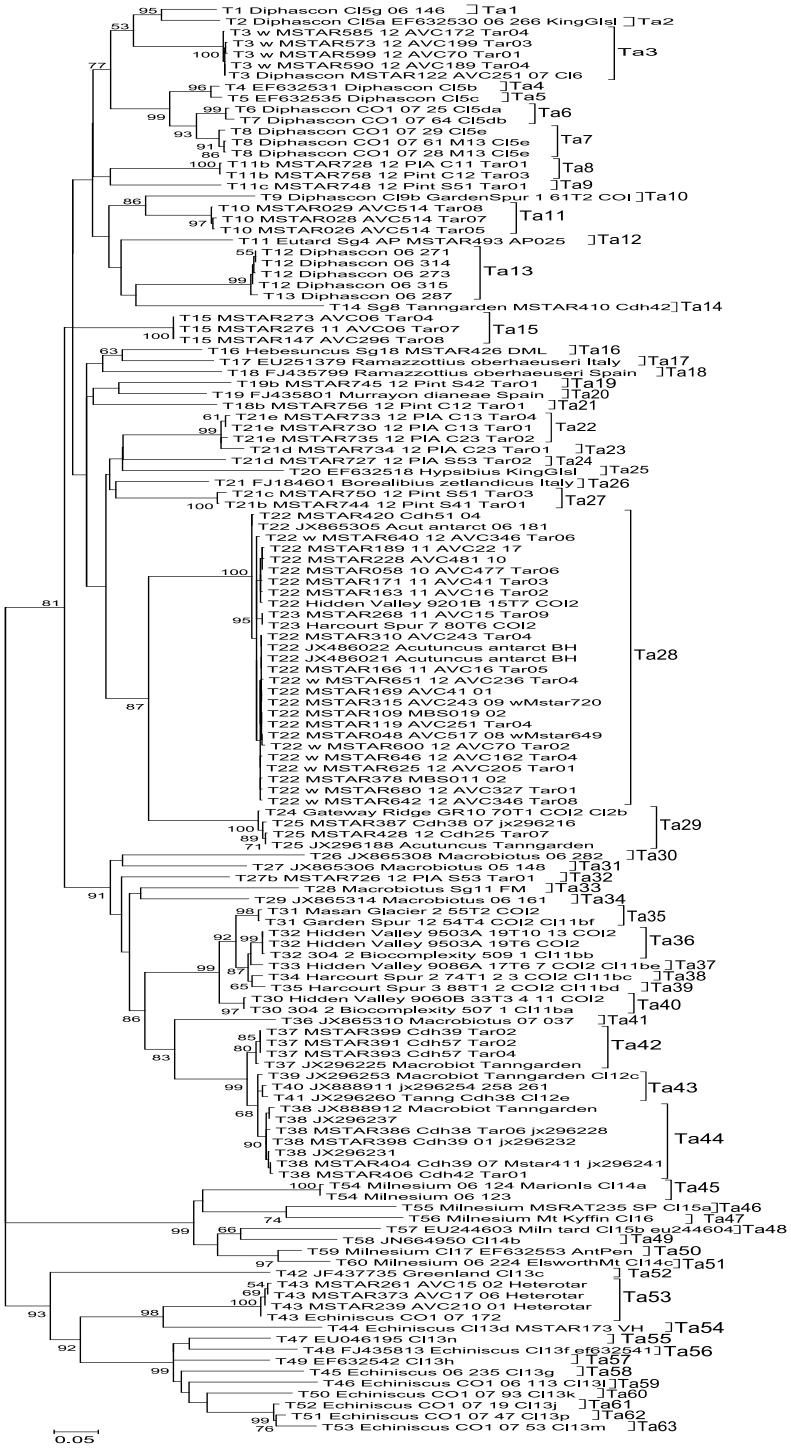


Fig. S2. Bayesian consensus tree based on COI dataset from 130 tardigrade haplotypes, implemented in MrBayes using a GTR+I+Γ model. Numbers at nodes indicate posterior probabilities. Numbers Ta1-Ta63 correspond to the established mitochondrial lineages used in the main manuscript and in Fig. 2.

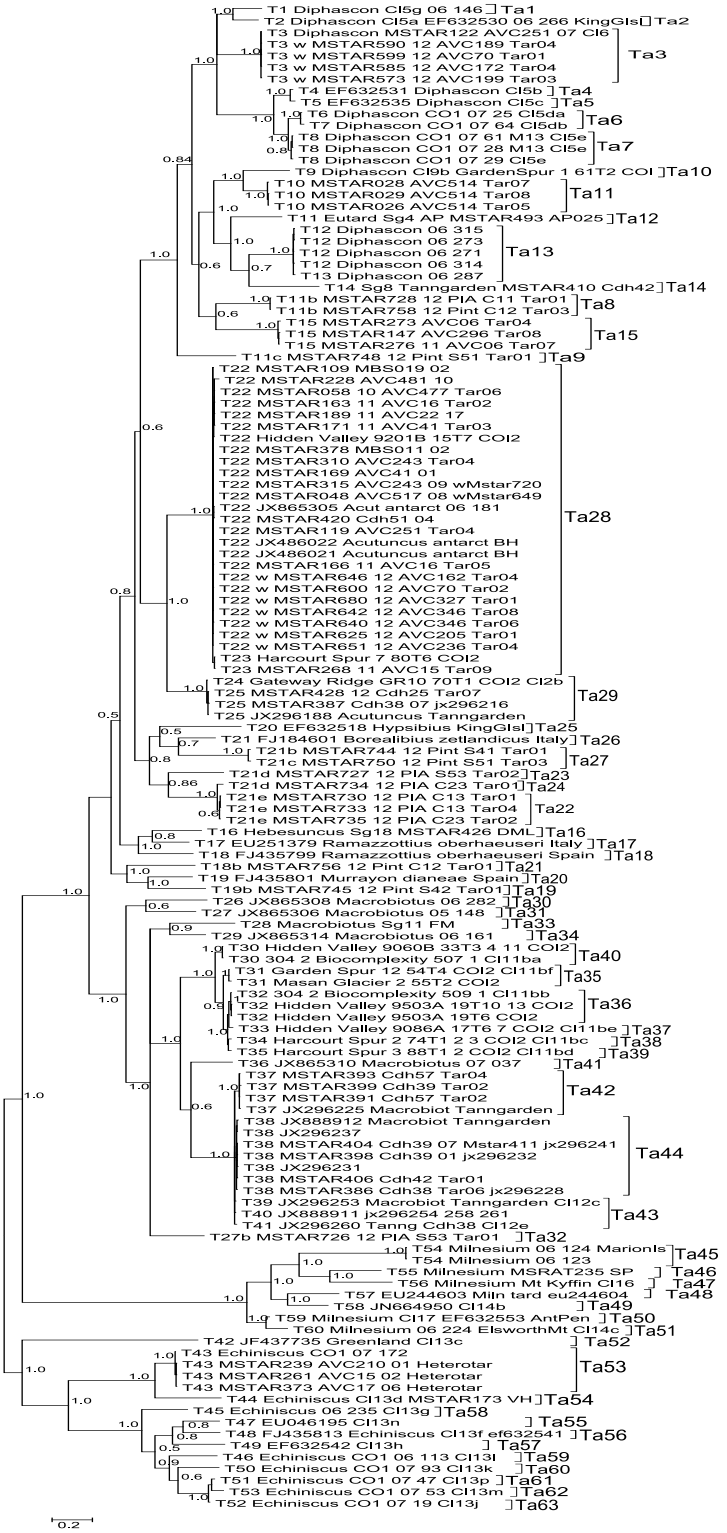


Fig. S3. ML tree based on mtDNA (COI) dataset from 137 rotifer haplotypes. ML bootstrap values (1000 replicas) >50% are shown next to the nods. The analysis was performed in MEGA5 using a GTR+I+Γ model of evolution. Numbers Bd1 – Bd47 correspond to the established mitochondrial lineages for bdelloid rotifers used in the main manuscript and in Fig. 3. Two monogonont haplotypes were used as Outgroup.

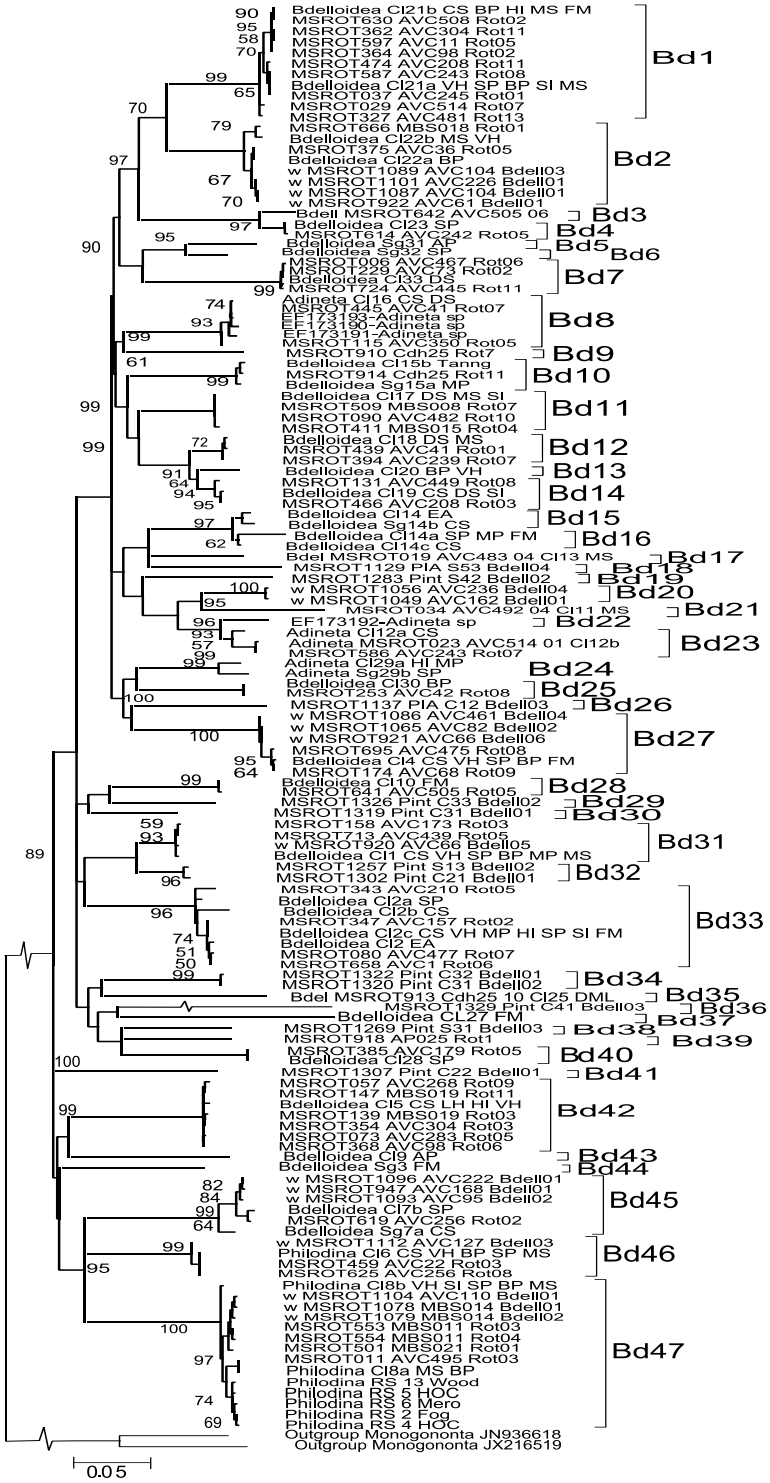


Fig. S4. Bayesian consensus tree based on COI dataset from 137 rotifer haplotypes, implemented in MrBayes using a GTR+I+Γ model. Numbers at nodes indicate posterior probabilities. Numbers Bd1 – Bd47 correspond to the established mitochondrial lineages for bdelloid rotifers used in the main manuscript and in Fig. 3. Two monogonont haplotypes were used as Outgroup.

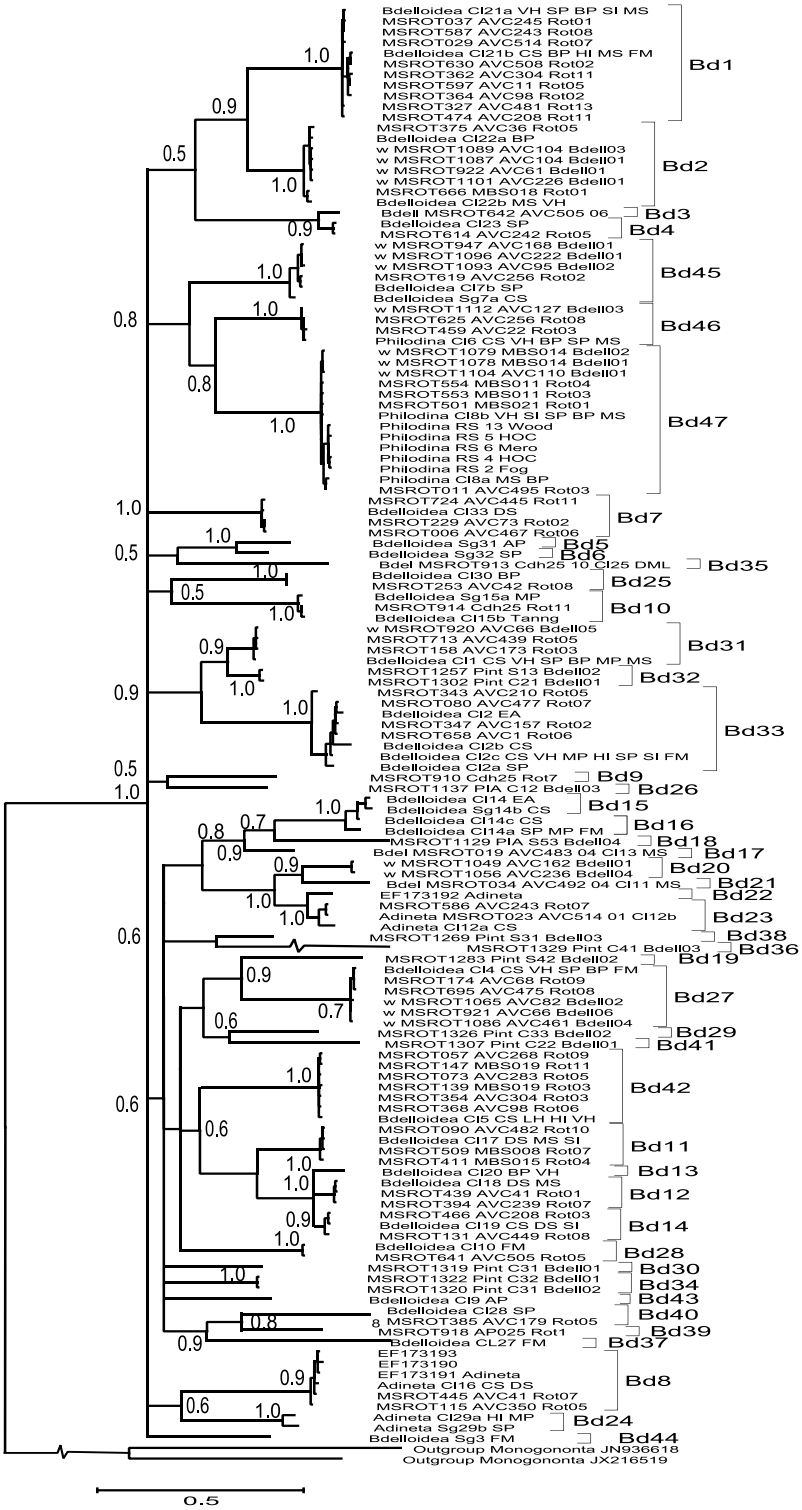


Table S1. Pair-wise distance comparison matrix for COI haplotypes from 130 tardigrades showing all 63 established mitochondrial lineages used in the main manuscript and in Fig. 2

Lineage		Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
Ta1	1	T1_Diphascon_Cl5g_06_146																																																					
Ta2	2	T2_Diphascon_Cl5a_EF632530_266_KG	0.12																																																			
Ta3	3	T3_Diphascon_MSTAR122_AVC251_07	0.17	0.17																																																		
Ta3	4	T3_w_MSTAR590_12_AVC189_Tar04	0.17	0.17	0.00																																																	
Ta3	5	T3_w_MSTAR599_12_AVC70_Tar01	0.17	0.17	0.00	0.00																																																
Ta3	6	T3_w_MSTAR585_12_AVC172_Tar04	0.16	0.16	0.00	0.00	0.00																																															
Ta3	7	T3_w_MSTAR573_12_AVC199_Tar03	0.17	0.17	0.00	0.00	0.00	0.00																																														
Ta4	8	T4_EF632531_Diphascon_Cl5b	0.19	0.18	0.20	0.20	0.20	0.20	0.20																																													
Ta5	9	T5_EF632535_Diphascon_Cl5c	0.20	0.19	0.20	0.20	0.20	0.20	0.20	0.03																																												
Ta6	10	T6_Diphascon_CO1_07_25_Cl5da	0.20	0.20	0.21	0.21	0.21	0.21	0.21	0.11	0.12																																											
Ta6	11	T7_Diphascon_CO1_07_64_Cl5db	0.20	0.21	0.20	0.20	0.20	0.20	0.20	0.12	0.13	0.02																																										
Ta7	12	T8_Diphascon_CO1_07_61_M13_Cl5e	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.11	0.12	0.07	0.08																																									
Ta7	13	T8_Diphascon_CO1_07_29_Cl5e	0.20	0.20	0.19	0.19	0.20	0.20	0.19	0.11	0.11	0.07	0.07	0.01																																								
Ta7	14	T8_Diphascon_CO1_07_28_M13_Cl5e	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.10	0.11	0.06	0.07	0.00	0.01																																							
Ta8	15	T11b_MSTAR728_12_PIA_C11_Tar01	0.23	0.22	0.21	0.21	0.21	0.21	0.21	0.22	0.22	0.23	0.23	0.22	0.22	0.22																																						
Ta8	16	T11b_MSTAR758_12_Pint_C12_Tar03	0.22	0.22	0.21	0.21	0.21	0.21	0.21	0.21	0.22	0.23	0.23	0.22	0.22	0.22	0.00																																					
Ta9	17	T11c_MSTAR748_12_Pint_S51_Tar01	0.20	0.21	0.21	0.21	0.21	0.21	0.21	0.22	0.22	0.23	0.23	0.22	0.22	0.22	0.19	0.19																																				
Ta10	18	T9_Diphascon_Cl9b_GardenSpur_61T2	0.25	0.22	0.23	0.23	0.23	0.23	0.23	0.21	0.22	0.22	0.23	0.21	0.21	0.21	0.22	0.21	0.24																																			
Ta11	19	T10_MSTAR028_AVC514_Tar07	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.19	0.19	0.19	0.20	0.19	0.18	0.19	0.21	0.20	0.22	0.16																																		
Ta11	20	T10_MSTAR029_AVC514_Tar08	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.19	0.19	0.19	0.20	0.19	0.19	0.19	0.20	0.20	0.21	0.16	0.01																																	
Ta11	21	T10_MSTAR026_AVC514_Tar05	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.19	0.19	0.19	0.20	0.19	0.18	0.19	0.21	0.20	0.22	0.16	0.00	0.00																																
Ta12	22	T11_Eutard_Sg4_AP_MSTAR493_AP025	0.22	0.21	0.22	0.22	0.22	0.23	0.22	0.23	0.24	0.22	0.22	0.22	0.22	0.22	0.24	0.23	0.21	0.23	0.21	0.22	0.21																															
Ta13	23	T12_Diphascon_06_315 *D. puneceum*	0.24	0.23	0.26	0.26	0.26	0.26	0.26	0.24	0.25	0.26	0.26	0.24	0.25	0.24	0.22	0.22	0.24	0.22	0.21	0.21	0.21	0.21																														
Ta13	24	T12_Diphascon_06_273 D. *puneceum*	0.24	0.23	0.26	0.26	0.26	0.26	0.26	0.24	0.25	0.26	0.26	0.24	0.24	0.24	0.22	0.22	0.24	0.22	0.22	0.21	0.22	0.20	0.00																													
Ta13	25	T12_Diphascon_06_271 *D. puneceum*	0.24	0.23	0.26	0.26	0.26	0.26	0.26	0.24	0.25	0.26	0.26	0.24	0.24	0.24	0.22	0.22	0.24	0.22	0.22	0.21	0.22	0.20	0.00	0.00																												
Ta13	26	T12_Diphascon_06_314 *D. puneceum*	0.24	0.23	0.26	0.26	0.26	0.26	0.26	0.24	0.25	0.26	0.26	0.24	0.24	0.24	0.22	0.22	0.24	0.22	0.22	0.21	0.22	0.20	0.00	0.00	0.00																											
Ta13	27	T13_Diphascon_06_287 *D. puneceum*	0.24	0.23	0.26	0.26	0.26	0.26	0.26	0.23	0.24	0.26	0.26	0.23	0.24	0.24	0.22	0.22	0.24	0.22	0.21	0.21	0.21	0.21	0.01	0.01	0.01	0.01																										
Ta14	28	T14_Sg8_Tanngarden_MSTAR410_Cdh42	0.25	0.25	0.24	0.24	0.24	0.24	0.24	0.26	0.27	0.26	0.27	0.27	0.27	0.27	0.26	0.25	0.26	0.24	0.24	0.24	0.24	0.25	0.23	0.22	0.23	0.23	0.22																									
Ta15	29	T15_MSTAR273_AVC06_Tar04	0.23	0.22	0.24	0.24	0.24	0.24	0.24	0.22	0.22	0.22	0.22	0.22	0.21	0.21	0.19	0.18	0.21	0.21	0.20	0.21	0.21	0.21	0.19	0.20	0.20	0.20	0.20	0.25																								
Ta15	30	T15_MSTAR147_AVC296_Tar08	0.23	0.22	0.24	0.24	0.24	0.24	0.24	0.22	0.22	0.22	0.22	0.22	0.21	0.21	0.19	0.18	0.21	0.21	0.20	0.21	0.21	0.21	0.19	0.20	0.20	0.20	0.20	0.25	0.00																							
Ta15	31	T15_MSTAR276_11_AVC06_Tar07	0.23	0.22	0.24	0.24	0.24	0.24	0.24	0.22	0.22	0.22	0.22	0.22	0.21	0.21	0.19	0.18	0.21	0.21	0.20	0.21	0.21	0.21	0.19	0.20	0.20	0.20	0.20	0.25	0.00	0.00																						
Ta16	32	T16_Hebesuncus_Sg18_MSTAR426_DML	0.23	0.21	0.24	0.24	0.24	0.24	0.24	0.23	0.22	0.24	0.24	0.23	0.24	0.24	0.23	0.23	0.24	0.23	0.22	0.22	0.22	0.23	0.24	0.24	0.24	0.24	0.24	0.26	0.21	0.21	0.21																					
Ta17	33	T17_EU251379_R_oberhaeuseri_Italy	0.23	0.23	0.25	0.25	0.26	0.25	0.26	0.24	0.24	0.26	0.26	0.25	0.26	0.25	0.24	0.24	0.25	0.27	0.24	0.24	0.24	0.24	0.25	0.25	0.25	0.25	0.26	0.29	0.20	0.20	0.20	0.16																				
Ta18	34	T18_FJ435799_R_oberhaeuseri_Spain	0.24	0.24	0.23	0.23	0.23	0.23	0.23	0.22	0.22	0.25	0.25	0.24	0.24	0.24	0.24	0.23	0.26	0.23	0.22	0.23	0.23	0.25	0.25	0.25	0.25	0.25	0.26	0.25	0.22	0.22	0.22	0.19	0.20																			
Ta19	35	T19b_MSTAR745_12_Pint_S42_Tar01	0.23	0.25	0.26	0.26	0.26	0.26	0.26	0.25	0.25	0.24	0.24	0.24	0.24	0.24	0.25	0.25	0.23	0.25	0.24	0.24	0.24	0.24	0.23	0.24	0.24	0.24	0.24	0.27	0.24	0.24	0.24	0.20	0.22	0.20																		
Ta20	36	T19_FJ435801_Murrayon_dianeae_Spain	0.24	0.24	0.23	0.23	0.23	0.22	0.23	0.23	0.23	0.24	0.24	0.24	0.24	0.25	0.23	0.23	0.23	0.26	0.22	0.22	0.22	0.23	0.26	0.26	0.26	0.26	0.26	0.28	0.24	0.24	0.24	0.19	0.22	0.21	0.18																	
Ta21	37	T18b_MSTAR756_12_Pint_C12_Tar01	0.24	0.23	0.23	0.23	0.23	0.23	0.23	0.22	0.22	0.24	0.23	0.23	0.22	0.23	0.25	0.25	0.23	0.23	0.22	0.22	0.22	0.23	0.24	0.25	0.25	0.25	0.25	0.28	0.22	0.22	0.22	0.23	0.21	0.21	0.22	0.21																
Ta22	38	T21e_MSTAR730_12_PIA_C13_Tar01	0.22	0.22	0.20	0.20	0.20	0.20	0.20	0.21	0.22	0.22	0.22	0.23	0.22	0.23	0.23	0.23	0.23	0.22	0.22	0.22	0.22	0.26	0.25	0.26	0.26	0.26	0.26	0.27	0.21	0.21	0.21	0.19	0.21	0.19	0.23	0.19	0.21															
Ta22	39	T21e_MSTAR733_12_PIA_C13_Tar04	0.23	0.22	0.20	0.20	0.20	0.20	0.20	0.21	0.22	0.23	0.22	0.23	0.23	0.23	0.23	0.23	0.24	0.22	0.23	0.23	0.22	0.26	0.26	0.26	0.26	0.26	0.26	0.27	0.21	0.21	0.21	0.19	0.21	0.20	0.23	0.19	0.21	0.00														
Ta22	40	T21e_MSTAR735_12_PIA_C23_Tar02	0.24	0.23	0.20	0.20	0.20	0.20	0.20	0.22	0.22	0.22	0.21	0.23	0.23	0.23	0.24	0.24	0.24	0.22	0.23	0.23	0.23	0.26	0.26	0.27	0.26	0.26	0.27	0.28	0.22	0.22	0.22	0.19	0.22	0.20	0.23	0.19	0.21	0.00	0.00													
Ta24	41	T21d_MSTAR727_12_PIA_S53_Tar02	0.24	0.22	0.23	0.23	0.23	0.23	0.23	0.25	0.23	0.24	0.23	0.24	0.24	0.24	0.22	0.22	0.24	0.26	0.24	0.24	0.24	0.22	0.24	0.24	0.24	0.24	0.24	0.25	0.22	0.22	0.22	0.23	0.22	0.24	0.20	0.22	0.24	0.18	0.18	0.18												
Ta23	42	T21d_MSTAR734_12_PIA_C23_Tar01	0.24	0.24	0.22	0.22	0.22	0.22	0.22	0.21	0.22	0.23	0.22	0.23	0.23	0.22	0.24	0.24	0.25	0.22	0.23	0.23	0.23	0.26	0.26	0.26	0.26	0.26	0.26	0.27	0.21	0.21	0.21	0.20	0.21	0.22	0.25	0.20	0.22	0.03	0.03	0.03	0.19											
Ta25	43	T20_EF632518_Hypsibius_KingGIsl	0.26	0.27	0.24	0.24	0.24	0.25	0.25	0.24	0.25	0.25	0.24	0.25	0.25	0.25	0.25	0.25	0.27	0.26	0.24	0.24	0.24	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.26	0.25	0.25	0.22	0.25	0.20	0.26	0.21	0.21	0.21	0.23	0.22										
Ta26	44	T21_FJ184601_Borealibius_zetlandicus_Ita	0.24	0.22	0.23	0.23	0.23	0.22	0.23	0.21	0.21	0.22	0.22	0.21	0.20	0.21	0.23	0.22	0.19	0.24	0.19	0.19	0.19	0.23	0.24	0.24	0.24	0.24	0.24	0.26	0.20	0.20	0.20	0.20	0.21	0.21	0.18	0.20	0.21	0.18	0.18	0.18	0.19	0.20	0.22									
Ta27	45	T21c_MSTAR750_12_Pint_S51_Tar03	0.23	0.26	0.23	0.23	0.24	0.23	0.24	0.24	0.24	0.26	0.25	0.26	0.26	0.26	0.23	0.23	0.24	0.25	0.25	0.25	0.25	0.25	0.26	0.26	0.26	0.26	0.26	0.25	0.22	0.22	0.22	0.22	0.22	0.21	0.20	0.21	0.23	0.20	0.20	0.20	0.20	0.22	0.22	0.18								
Ta27	46	T21b_MSTAR744_12_Pint_S41_Tar01	0.22	0.25	0.23	0.23	0.24	0.23	0.24	0.24	0.24	0.25	0.25	0.26	0.26	0.26	0.22	0.22	0.23	0.25	0.25	0.25	0.25	0.25	0.26	0.26	0.26	0.26	0.26	0.25	0.21	0.21	0.21	0.22	0.22	0.21	0.20	0.20	0.24	0.20	0.20	0.20	0.21	0.22	0.22	0.18	0.01							
Ta28	47	T22_MSTAR109_MBS019_02	0.24	0.25	0.25	0.25	0.25	0.24	0.25	0.26	0.26	0.25	0.26	0.24	0.25	0.25	0.25	0.25	0.24	0.27	0.26	0.25	0.26	0.24	0.28	0.28	0.28	0.28	0.29	0.31	0.21	0.21	0.21	0.23	0.24	0.24	0.27	0.22	0.23	0.22	0.22	0.22	0.22	0.22	0.27	0.21	0.23	0.22						
Ta28	48	T22_MSTAR228_AVC481_10	0.25	0.25	0.24	0.24	0.24	0.24	0.24	0.26	0.26	0.25	0.25	0.24	0.25	0.24	0.25	0.25	0.24	0.27	0.25	0.25	0.25	0.24	0.27	0.28	0.28	0.28	0.28	0.30	0.21	0.21	0.21	0.24	0.24	0.24	0.27	0.22	0.24	0.22	0.22	0.22	0.23	0.22	0.26	0.20	0.23	0.22	0.01					
Ta28	49	T22_MSTAR378_MBS011_02	0.24	0.25	0.25	0.25	0.25	0.24	0.25	0.26	0.25	0.25	0.25	0.24	0.25	0.24	0.25	0.25	0.24	0.27	0.26	0.25	0.26	0.24	0.28	0.28	0.28	0.28	0.29	0.31	0.21	0.21	0.21	0.23	0.24	0.24	0.27	0.22	0.23	0.22	0.22	0.22	0.22	0.23	0.26	0.21	0.23	0.22	0.00	0.01				
Ta28	50	T22_MSTAR310_AVC243_Tar04	0.24	0.25	0.25	0.25	0.25	0.24	0.25	0.26	0.26	0.25	0.26	0.24	0.25	0.25	0.25	0.25	0.24	0.27	0.26	0.25	0.26	0.24	0.28	0.28	0.28	0.28	0.29	0.31	0.21	0.21	0.21	0.23	0.24	0.24	0.27	0.22	0.23	0.22	0.22	0.22	0.22	0.22	0.27	0.21	0.23	0.22	0.00	0.01	0.00			
Ta28	51	T22_MSTAR169_AVC41_01	0.24	0.25	0.25	0.25	0.25	0.24	0.25	0.26	0.26	0.25	0.26	0.24	0.25	0.25	0.25	0.25	0.24	0.27	0.26	0.25	0.26	0.24	0.28	0.28	0.28	0.28	0.29	0.31	0.21	0.21	0.21	0.23	0.24	0.24	0.27	0.22	0.23	0.22	0.22	0.22	0.22	0.22	0.27	0.21	0.23	0.22	0.00	0.01	0.00	0.00		
Ta28	52	T22_MSTAR315_AVC243_09_wMstar720	0.24	0.25	0.25	0.25	0.25	0.24	0.25	0.26	0.26	0.25	0.26	0.24	0.25	0.25	0.25	0.25	0.24	0.27	0.26	0.25	0.26	0.24	0.28	0.28	0.28	0.28	0.29	0.31	0.21	0.21	0.21	0.23	0.24	0.24	0.27	0.22	0.23	0.22	0.22	0.22	0.22	0.22	0.27	0.21	0.23	0.22	0.00	0.01	0.00	0.00	0.00	
Ta28	53	T22_MSTAR048_AVC517_08_wMstar649	0.24	0.25	0.25	0.25	0.25	0.24	0.25	0.26	0.26	0.25	0.26	0.24	0.25	0.25	0.25	0.25	0.24	0.27	0.26	0.25	0.26	0.24	0.28	0.28	0.28	0.28	0.29	0.31	0.21	0.21	0.21	0.23	0.24	0.24	0.27	0.22	0.23	0.22	0.22	0.22	0.22	0.22	0.27	0.21	0.23	0.22	0.00	0.01	0.00	0.00	0.00	0.00	
Ta28	54	T22_JX865305_Acut_antarct_06_181	0.24	0.25	0.24	0.24	0.24	0.24	0.24	0.26	0.25	0.25	0.25	0.24	0.25	0.24	0.25	0.25	0.24	0.27	0.26	0.25	0.25	0.24	0.28	0.28	0.28	0.28	0.29	0.30	0.20	0.20	0.20	0.23	0.24	0.23	0.26	0.21	0.23	0.22	0.22	0.22	0.22	0.23	0.26	0.21	0.22	0.22	0.00	0.01	0.01	0.00	0.00	0.00	0.00
Ta28	55	T22_MSTAR420_Cdh51_04	0.24	0.26	0.24	0.24	0.24	0.24	0.24	0.26	0.25	0.25	0.25	0.24	0.25	0.24	0.25	0.25	0.24	0.27	0.26	0.25	0.25	0.23	0.28	0.28	0.28	0.28	0.29	0.30	0.20	0.20	0.20	0.23	0.23	0.23	0.27	0.22	0.23	0.22	0.22	0.22	0.22	0.23	0.26	0.20	0.22	0.22	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Ta28	56	T22_MSTAR119_AVC251_Tar04	0.24	0.25	0.25	0.25	0.25	0.24	0.25	0.26	0.26	0.25	0.26	0.24	0.25	0.25	0.25	0.25	0.24	0.27	0.26	0.25	0.26	0.24	0.28	0.28	0.28	0.28	0.29	0.31	0.21	0.21	0.21	0.23	0.24	0.24	0.27	0.22	0.23	0.22	0.22	0.22	0.22	0.22	0.27	0.21	0.23	0.22	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Ta28	57	T22_JX486022_Acutuncus_antarct_BH	0.24	0.25	0.25	0.25	0.25	0.24	0.25	0.26	0.26	0.25	0.26	0.24	0.25	0.25	0.25	0.25	0.24	0.27	0.26	0.25	0.26	0.24	0.28	0.28	0.28	0.28	0.29	0.31	0.21	0.21	0.21	0.23	0.24	0.24	0.27	0.22	0.23	0.22	0.22	0.22	0.22	0.22	0.27	0.21	0.23	0.22	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Ta28	58	T22_JX486021_Acutuncus_antarct_BH	0.24	0.25	0.25	0.25	0.25	0.24	0.25	0.26	0.26	0.25	0.26	0.24	0.25	0.25	0.25	0.25	0.24	0.27	0.26	0.25	0.26	0.24	0.28	0.28	0.28	0.28	0.29	0.31	0.21	0.21	0.21	0.23	0.24	0.24	0.27	0.22	0.23	0.22	0.22	0.22	0.22	0.22	0.27	0.21	0.23	0.22	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Ta28	59	T22_MSTAR166_11_AVC16_Tar05	0.24	0.25	0.25	0.25	0.25	0.24	0.25	0.26	0.26	0.25	0.26	0.24	0.25	0.25	0.25	0.25	0.24	0.27	0.26	0.25	0.26	0.24	0.28	0.28	0.28	0.28	0.29	0.31	0.21	0.21	0.21	0.23	0.24	0.24	0.27	0.22	0.23	0.22	0.22	0.22	0.22	0.22	0.27	0.21	0.23	0.22	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Ta28	60	T22_Hidden_Valley_9201B_15T7_COI2	0.24	0.25	0.24	0.24	0.24	0.24	0.24	0.26	0.26	0.25	0.26	0.24	0.25	0.25	0.25	0.25	0.24	0.27	0.26	0.25	0.25	0.23	0.27	0.28	0.28	0.28	0.28	0.30	0.21	0.21	0.21	0.23	0.24	0.24	0.27	0.22	0.23	0.22	0.22	0.22	0.22	0.22	0.27	0.21	0.22	0.22	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Ta28	61	T22_MSTAR058_10_AVC477_Tar06	0.24	0.25	0.24	0.24	0.24	0.24	0.24	0.26	0.26	0.25	0.25	0.24	0.25	0.24	0.25	0.25	0.24	0.27	0.26	0.25	0.25	0.24	0.28	0.28	0.28	0.28	0.29	0.30	0.20	0.20	0.20	0.23	0.24	0.23	0.27	0.22	0.23	0.22	0.22	0.22	0.22	0.22	0.26	0.21	0.22	0.22	0.01	0.00	0.01	0.01	0.01	0.01	0.01
Ta28	62	T22_MSTAR163_11_AVC16_Tar02	0.25	0.25	0.24	0.24	0.24	0.24	0.24	0.26	0.26	0.25	0.25	0.24	0.25	0.24	0.25	0.25	0.24	0.27	0.26	0.25	0.25	0.23	0.28	0.28	0.28	0.28	0.29	0.30	0.20	0.20	0.20	0.23	0.23	0.23	0.27	0.22	0.23	0.22	0.22	0.22	0.23	0.22	0.26	0.20	0.22	0.22	0.01	0.00	0.01	0.01	0.01	0.01	0.01
Ta28	63	T22_MSTAR189_11_AVC22_17	0.25	0.25	0.24	0.24	0.24	0.24	0.24	0.26	0.25	0.25	0.26	0.24	0.25	0.25	0.25	0.25	0.24	0.27	0.26	0.25	0.26	0.24	0.27	0.28	0.28	0.28	0.28	0.30	0.21	0.21	0.21	0.23	0.23	0.24	0.27	0.22	0.24	0.22	0.22	0.22	0.22	0.22	0.27	0.21	0.23	0.22	0.01	0.00	0.01	0.01	0.01	0.01	0.01
Ta28	64	T22_MSTAR171_11_AVC41_Tar03	0.24	0.25	0.24	0.24	0.24	0.24	0.24	0.26	0.25	0.25	0.25	0.24	0.25	0.25	0.25	0.25	0.24	0.27	0.25	0.25	0.25	0.24	0.28	0.28	0.28	0.28	0.29	0.30	0.21	0.21	0.21	0.24	0.23	0.23	0.26	0.22	0.23	0.21	0.22	0.21	0.22	0.23	0.26	0.20	0.22	0.22	0.01	0.00	0.01	0.01	0.01	0.01	0.01
Ta28	65	T22_w_MSTAR646_12_AVC162_Tar04	0.24	0.25	0.25	0.25	0.25	0.25	0.25	0.26	0.26	0.25	0.25	0.25	0.25	0.25	0.26	0.26	0.24	0.28	0.26	0.26	0.26	0.23	0.28	0.28	0.28	0.28	0.29	0.30	0.21	0.21	0.21	0.23	0.23	0.24	0.27	0.22	0.24	0.22	0.22	0.22	0.23	0.23	0.27	0.21	0.23	0.23	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Ta28	66	T22_w_MSTAR600_12_AVC70_Tar02	0.24	0.26	0.25	0.25	0.25	0.25	0.25	0.26	0.26	0.26	0.26	0.25	0.26	0.25	0.26	0.26	0.25	0.28	0.26	0.26	0.26	0.24	0.28	0.28	0.28	0.28	0.29	0.31	0.21	0.21	0.21	0.24	0.24	0.24	0.27	0.22	0.24	0.22	0.22	0.22	0.23	0.22	0.27	0.21	0.23	0.23	0.00	0.01	0.01	0.00	0.00	0.00	0.00
Ta28	67	T22_w_MSTAR680_12_AVC327_Tar01	0.24	0.25	0.25	0.25	0.25	0.25	0.25	0.26	0.26	0.26	0.26	0.25	0.25	0.25	0.25	0.25	0.24	0.28	0.26	0.26	0.26	0.24	0.28	0.28	0.28	0.28	0.29	0.31	0.21	0.21	0.21	0.23	0.23	0.24	0.27	0.22	0.24	0.22	0.22	0.22	0.23	0.23	0.27	0.21	0.22	0.22	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Ta28	68	T22_w_MSTAR642_12_AVC346_Tar08	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.26	0.26	0.25	0.26	0.24	0.25	0.25	0.25	0.25	0.24	0.27	0.26	0.25	0.26	0.23	0.28	0.28	0.28	0.28	0.29	0.30	0.21	0.21	0.21	0.23	0.23	0.24	0.27	0.22	0.23	0.22	0.22	0.22	0.23	0.22	0.26	0.21	0.22	0.22	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Ta28	69	T22_w_MSTAR640_12_AVC346_Tar06	0.25	0.26	0.25	0.25	0.25	0.25	0.25	0.26	0.26	0.26	0.26	0.25	0.25	0.25	0.26	0.26	0.24	0.28	0.26	0.26	0.26	0.24	0.28	0.28	0.28	0.28	0.29	0.31	0.21	0.21	0.21	0.23	0.24	0.24	0.27	0.22	0.24	0.22	0.22	0.22	0.23	0.22	0.27	0.21	0.23	0.22	0.00	0.01	0.01	0.00	0.00	0.00	0.00
Ta28	70	T22_w_MSTAR625_12_AVC205_Tar01	0.24	0.25	0.25	0.25	0.25	0.24	0.25	0.26	0.26	0.25	0.26	0.24	0.25	0.25	0.25	0.25	0.24	0.27	0.26	0.25	0.26	0.24	0.28	0.28	0.28	0.28	0.29	0.31	0.21	0.21	0.21	0.23	0.24	0.24	0.27	0.22	0.23	0.22	0.22	0.22	0.22	0.22	0.27	0.21	0.23	0.22	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Ta28	71	T22_w_MSTAR651_12_AVC236_Tar04	0.24	0.25	0.25	0.25	0.25	0.24	0.25	0.26	0.26	0.25	0.26	0.24	0.25	0.25	0.25	0.25	0.24	0.27	0.26	0.25	0.26	0.24	0.28	0.28	0.28	0.28	0.29	0.30	0.21	0.21	0.21	0.23	0.24	0.24	0.27	0.22	0.23	0.22	0.22	0.22	0.22	0.22	0.27	0.21	0.23	0.22	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Ta28	72	T23_Harcourt_Spur_7_80T6_COI2	0.24	0.25	0.24	0.24	0.24	0.24	0.24	0.26	0.26	0.26	0.26	0.24	0.25	0.24	0.25	0.25	0.24	0.27	0.25	0.25	0.25	0.24	0.28	0.28	0.28	0.28	0.29	0.30	0.21	0.21	0.21	0.23	0.24	0.24	0.26	0.22	0.23	0.22	0.22	0.22	0.22	0.22	0.27	0.21	0.22	0.22	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Ta28	73	T23_MSTAR268_11_AVC15_Tar09	0.24	0.25	0.24	0.24	0.24	0.24	0.24	0.26	0.26	0.26	0.26	0.24	0.25	0.24	0.25	0.25	0.24	0.27	0.25	0.25	0.25	0.24	0.28	0.28	0.28	0.28	0.29	0.30	0.21	0.21	0.21	0.23	0.24	0.24	0.26	0.22	0.23	0.22	0.22	0.22	0.22	0.22	0.27	0.21	0.22	0.22	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Ta29	74	T24_Gateway_Ridge_GR10_70T1_Cl2b	0.25	0.25	0.25	0.25	0.25	0.26	0.26	0.24	0.24	0.24	0.24	0.25	0.25	0.25	0.23	0.23	0.22	0.26	0.25	0.24	0.25	0.22	0.26	0.26	0.26	0.26	0.27	0.30	0.22	0.22	0.22	0.22	0.23	0.24	0.24	0.21	0.22	0.21	0.22	0.22	0.24	0.22	0.23	0.19	0.23	0.23	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Ta29	75	T25_MSTAR428_12_Cdh25_Tar07	0.25	0.26	0.26	0.26	0.26	0.26	0.26	0.25	0.25	0.24	0.24	0.25	0.25	0.25	0.24	0.24	0.22	0.26	0.25	0.25	0.25	0.23	0.26	0.27	0.27	0.27	0.27	0.30	0.22	0.22	0.22	0.23	0.24	0.24	0.24	0.21	0.22	0.21	0.22	0.22	0.24	0.22	0.24	0.20	0.24	0.23	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Ta29	76	T25_MSTAR387_Cdh38_07_jx296216	0.25	0.25	0.26	0.26	0.26	0.26	0.26	0.24	0.24	0.24	0.24	0.25	0.25	0.25	0.24	0.24	0.22	0.26	0.25	0.25	0.25	0.23	0.27	0.27	0.27	0.27	0.27	0.30	0.22	0.22	0.22	0.23	0.24	0.24	0.24	0.21	0.22	0.22	0.22	0.22	0.24	0.22	0.24	0.20	0.24	0.23	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Ta29	77	T25_JX296188_Acutuncus_Tanngarden	0.25	0.26	0.25	0.25	0.25	0.26	0.26	0.24	0.24	0.24	0.24	0.25	0.25	0.25	0.24	0.24	0.22	0.26	0.25	0.25	0.25	0.23	0.26	0.26	0.26	0.26	0.27	0.30	0.22	0.22	0.22	0.22	0.24	0.24	0.24	0.21	0.22	0.21	0.22	0.22	0.24	0.22	0.24	0.20	0.23	0.23	0.20	0.20	0.19	0.20	0.20	0.20	0.20
Ta30	78	T26_JX865308_06_282 *M.acro. furciger*	0.29	0.27	0.27	0.27	0.27	0.27	0.27	0.29	0.29	0.27	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.23	0.23	0.24	0.27	0.28	0.28	0.28	0.28	0.27	0.26	0.27	0.27	0.27	0.26	0.26	0.25	0.26	0.24	0.26	0.27	0.28	0.28	0.29	0.28	0.27	0.25	0.26	0.26	0.27	0.26	0.27	0.27	0.27	0.27	0.27
Ta31	79	T27_JX865306_Macrobiotus_05_148	0.26	0.25	0.25	0.25	0.26	0.26	0.25	0.26	0.24	0.27	0.26	0.27	0.25	0.27	0.27	0.26	0.26	0.27	0.27	0.27	0.27	0.26	0.28	0.28	0.28	0.28	0.28	0.28	0.24	0.24	0.24	0.24	0.25	0.26	0.22	0.25	0.25	0.25	0.25	0.26	0.23	0.26	0.28	0.25	0.25	0.25	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Ta32	80	T27b_MSTAR726_12_PIA_S53_Tar01	0.27	0.27	0.25	0.25	0.25	0.25	0.25	0.27	0.27	0.26	0.25	0.26	0.25	0.26	0.25	0.25	0.25	0.27	0.24	0.24	0.24	0.26	0.27	0.27	0.27	0.27	0.27	0.29	0.26	0.26	0.26	0.24	0.26	0.25	0.23	0.24	0.26	0.25	0.25	0.25	0.26	0.27	0.27	0.25	0.27	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Ta33	81	T28_Macrobiotus_Sg11_FM	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.31	0.29	0.31	0.30	0.30	0.30	0.30	0.25	0.25	0.26	0.30	0.27	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.28	0.31	0.25	0.25	0.25	0.24	0.26	0.27	0.26	0.26	0.27	0.30	0.30	0.29	0.26	0.31	0.30	0.29	0.26	0.25	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Ta34	82	T29_JX865314_Macrobiotus_06_161	0.25	0.25	0.28	0.28	0.28	0.27	0.28	0.28	0.27	0.28	0.28	0.27	0.27	0.27	0.26	0.26	0.25	0.29	0.23	0.23	0.23	0.26	0.27	0.27	0.27	0.27	0.28	0.28	0.24	0.24	0.24	0.24	0.24	0.24	0.22	0.24	0.28	0.29	0.29	0.29	0.27	0.30	0.29	0.25	0.27	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Ta35	83	T31_Garden_Spur_12_54T4_COI2_Cl11bf	0.28	0.28	0.26	0.26	0.26	0.26	0.26	0.28	0.27	0.28	0.28	0.28	0.28	0.29	0.26	0.26	0.28	0.29	0.26	0.26	0.26	0.27	0.28	0.28	0.28	0.28	0.27	0.31	0.25	0.25	0.25	0.27	0.28	0.27	0.26	0.25	0.27	0.28	0.28	0.28	0.27	0.29	0.31	0.27	0.26	0.26	0.28	0.28	0.27	0.28	0.28	0.28	0.28
Ta35	84	T31_Masan_Glacier_2_55T2_COI2	0.28	0.28	0.26	0.26	0.26	0.26	0.26	0.28	0.27	0.28	0.28	0.28	0.27	0.28	0.26	0.26	0.28	0.29	0.26	0.26	0.26	0.27	0.28	0.28	0.28	0.28	0.27	0.31	0.25	0.25	0.25	0.27	0.28	0.27	0.26	0.24	0.27	0.28	0.28	0.28	0.27	0.29	0.31	0.27	0.26	0.26	0.28	0.28	0.27	0.28	0.28	0.28	0.28
Ta36	85	T32_304_2_Biocomplexity_509_1_Cl11bb	0.28	0.27	0.26	0.26	0.26	0.25	0.26	0.27	0.27	0.27	0.27	0.27	0.26	0.27	0.27	0.27	0.28	0.29	0.25	0.25	0.25	0.26	0.27	0.27	0.27	0.27	0.27	0.30	0.26	0.26	0.26	0.28	0.28	0.27	0.26	0.24	0.26	0.29	0.29	0.29	0.28	0.30	0.31	0.27	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Ta36	86	T32_Hidden_Valley_9503A_19T10_13_COI2	0.28	0.28	0.26	0.26	0.26	0.26	0.26	0.28	0.27	0.28	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.29	0.25	0.25	0.26	0.26	0.27	0.28	0.28	0.28	0.27	0.30	0.26	0.26	0.26	0.28	0.27	0.27	0.26	0.24	0.26	0.29	0.30	0.29	0.27	0.30	0.31	0.27	0.25	0.25	0.27	0.27	0.26	0.27	0.27	0.27	0.27
Ta36	87	T32_Hidden_Valley_9503A_19T6_COI2	0.28	0.28	0.26	0.26	0.26	0.26	0.26	0.28	0.27	0.28	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.29	0.25	0.25	0.26	0.26	0.27	0.28	0.28	0.28	0.27	0.30	0.26	0.26	0.26	0.28	0.27	0.27	0.26	0.24	0.26	0.29	0.30	0.29	0.27	0.30	0.31	0.27	0.25	0.25	0.27	0.27	0.26	0.27	0.27	0.27	0.27
Ta37	88	T33_Hidden_Valley_9086A_17T6_7_Cl11be	0.28	0.28	0.26	0.26	0.26	0.26	0.26	0.28	0.27	0.28	0.28	0.27	0.27	0.28	0.28	0.28	0.28	0.29	0.25	0.25	0.25	0.26	0.27	0.28	0.28	0.28	0.27	0.30	0.26	0.26	0.26	0.27	0.27	0.27	0.26	0.25	0.27	0.29	0.29	0.29	0.27	0.30	0.32	0.27	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Ta38	89	T34_Harcourt_Spur_2_74T1_2_3_Cl11bc	0.28	0.28	0.26	0.26	0.26	0.26	0.26	0.28	0.27	0.28	0.28	0.28	0.27	0.28	0.27	0.27	0.28	0.31	0.26	0.26	0.26	0.27	0.27	0.28	0.28	0.28	0.27	0.31	0.26	0.26	0.26	0.27	0.27	0.27	0.26	0.25	0.27	0.29	0.29	0.29	0.28	0.30	0.31	0.27	0.27	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Ta39	90	T35_Harcourt_Spur_3_88T1_2_Cl11bd	0.27	0.27	0.26	0.26	0.26	0.26	0.26	0.28	0.26	0.28	0.27	0.28	0.27	0.28	0.28	0.28	0.28	0.30	0.26	0.25	0.25	0.27	0.28	0.28	0.28	0.28	0.28	0.30	0.26	0.26	0.26	0.27	0.28	0.26	0.26	0.24	0.27	0.30	0.30	0.30	0.28	0.30	0.31	0.27	0.26	0.26	0.27	0.27	0.26	0.27	0.27	0.27	0.27
Ta40	91	T30_Hidden_Valley_9060B_33T3_4_11	0.27	0.27	0.26	0.26	0.26	0.26	0.26	0.26	0.25	0.27	0.27	0.26	0.26	0.27	0.27	0.27	0.26	0.28	0.25	0.25	0.25	0.26	0.26	0.27	0.27	0.27	0.26	0.32	0.26	0.26	0.26	0.27	0.26	0.27	0.26	0.23	0.26	0.28	0.28	0.28	0.28	0.28	0.30	0.27	0.27	0.27	0.27	0.27	0.26	0.27	0.27	0.27	0.27
Ta40	92	T30_304_2_Biocomplexity_507_1_Cl11ba	0.26	0.27	0.25	0.25	0.26	0.26	0.25	0.25	0.24	0.27	0.27	0.26	0.26	0.27	0.27	0.27	0.26	0.28	0.25	0.25	0.25	0.25	0.26	0.26	0.26	0.26	0.26	0.31	0.25	0.25	0.25	0.27	0.26	0.27	0.26	0.23	0.26	0.28	0.28	0.28	0.28	0.28	0.30	0.27	0.27	0.27	0.27	0.27	0.26	0.27	0.27	0.27	0.27
Ta41	93	T36_JX865310_Macrobiotus_07_037	0.28	0.29	0.28	0.28	0.28	0.28	0.28	0.27	0.27	0.26	0.25	0.24	0.24	0.24	0.29	0.29	0.27	0.29	0.23	0.24	0.23	0.29	0.28	0.28	0.28	0.28	0.28	0.30	0.26	0.26	0.26	0.25	0.26	0.29	0.26	0.27	0.25	0.27	0.28	0.27	0.27	0.28	0.31	0.25	0.28	0.28	0.29	0.29	0.30	0.29	0.29	0.29	0.29
Ta42	94	T37_MSTAR393_Cdh57_Tar04	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.28	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.24	0.24	0.25	0.27	0.26	0.27	0.27	0.27	0.27	0.30	0.24	0.24	0.24	0.26	0.25	0.26	0.24	0.25	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Ta42	95	T37_MSTAR399_Cdh39_Tar02	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.28	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.24	0.24	0.25	0.27	0.26	0.27	0.27	0.27	0.27	0.30	0.24	0.24	0.24	0.26	0.25	0.26	0.24	0.25	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Ta42	96	T37_MSTAR391_Cdh57_Tar02	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.28	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.24	0.24	0.25	0.27	0.26	0.27	0.27	0.27	0.27	0.30	0.24	0.24	0.24	0.26	0.25	0.26	0.24	0.25	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Ta42	97	T37_JX296225_Macrobiot_Tanngarden	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.27	0.27	0.27	0.26	0.24	0.24	0.24	0.28	0.26	0.27	0.27	0.27	0.27	0.30	0.23	0.23	0.23	0.26	0.25	0.26	0.24	0.25	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Ta43	98	T39_JX296253_Macrobiot_DML_Cl12c	0.27	0.27	0.28	0.28	0.28	0.28	0.28	0.27	0.28	0.29	0.29	0.28	0.28	0.28	0.27	0.27	0.27	0.26	0.25	0.25	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.29	0.24	0.24	0.24	0.27	0.25	0.26	0.24	0.25	0.26	0.27	0.28	0.27	0.27	0.27	0.28	0.28	0.26	0.26	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Ta43	99	T40_JX888911_jx296254_258_261	0.28	0.28	0.28	0.28	0.29	0.29	0.29	0.28	0.29	0.28	0.28	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.25	0.25	0.26	0.27	0.26	0.26	0.26	0.26	0.26	0.30	0.23	0.23	0.23	0.27	0.25	0.27	0.24	0.26	0.27	0.27	0.28	0.28	0.26	0.27	0.28	0.28	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Ta43	100	T41_JX296260_Tanng_Cdh38_Cl12e	0.27	0.27	0.29	0.29	0.29	0.28	0.29	0.28	0.29	0.29	0.29	0.27	0.27	0.27	0.28	0.28	0.27	0.25	0.25	0.25	0.26	0.27	0.27	0.27	0.27	0.27	0.26	0.29	0.24	0.24	0.24	0.26	0.26	0.27	0.24	0.26	0.27	0.28	0.28	0.28	0.26	0.28	0.29	0.28	0.26	0.26	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Ta44	101	T38_JX888912_Macrobiot_Tanngarden	0.27	0.27	0.28	0.28	0.28	0.28	0.28	0.28	0.29	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.25	0.25	0.25	0.26	0.27	0.26	0.26	0.26	0.26	0.26	0.29	0.24	0.24	0.24	0.27	0.26	0.27	0.24	0.26	0.26	0.28	0.28	0.28	0.27	0.28	0.29	0.29	0.27	0.26	0.28	0.29	0.28	0.28	0.28	0.28	0.28
Ta44	102	T38_JX296237	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.28	0.28	0.28	0.28	0.28	0.27	0.28	0.27	0.27	0.25	0.25	0.25	0.25	0.27	0.26	0.26	0.26	0.26	0.26	0.29	0.24	0.24	0.24	0.26	0.25	0.27	0.24	0.26	0.25	0.27	0.28	0.28	0.26	0.27	0.28	0.28	0.26	0.26	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Ta44	103	T38_MSTAR404_Cdh39_07_jx296241	0.27	0.27	0.27	0.27	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.25	0.25	0.25	0.25	0.27	0.26	0.26	0.26	0.26	0.26	0.29	0.24	0.24	0.24	0.27	0.26	0.27	0.24	0.26	0.25	0.27	0.28	0.27	0.27	0.27	0.28	0.28	0.27	0.26	0.28	0.29	0.28	0.28	0.28	0.28	0.28
Ta44	104	T38_MSTAR398_Cdh39_01_jx296232	0.27	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.28	0.28	0.28	0.28	0.28	0.27	0.28	0.27	0.27	0.25	0.25	0.25	0.25	0.27	0.26	0.26	0.26	0.26	0.26	0.29	0.24	0.24	0.24	0.27	0.26	0.27	0.24	0.26	0.25	0.27	0.27	0.27	0.27	0.27	0.28	0.28	0.27	0.26	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Ta44	105	T38_MSTAR386_Cdh38_Tar06_jx296228	0.27	0.27	0.28	0.28	0.28	0.28	0.28	0.28	0.29	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.25	0.25	0.25	0.26	0.27	0.26	0.26	0.26	0.26	0.26	0.29	0.24	0.24	0.24	0.27	0.26	0.27	0.24	0.26	0.26	0.27	0.28	0.28	0.27	0.27	0.29	0.29	0.27	0.26	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Ta44	106	T38_JX296231	0.27	0.26	0.27	0.27	0.27	0.27	0.27	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.28	0.28	0.27	0.25	0.25	0.25	0.25	0.27	0.26	0.26	0.26	0.26	0.26	0.29	0.24	0.24	0.24	0.27	0.26	0.27	0.24	0.27	0.25	0.27	0.28	0.27	0.26	0.27	0.29	0.28	0.27	0.27	0.28	0.29	0.28	0.28	0.28	0.28	0.28
Ta44	107	T38_MSTAR406_Cdh42_Tar01	0.27	0.27	0.28	0.28	0.28	0.28	0.28	0.28	0.29	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.25	0.25	0.25	0.25	0.27	0.26	0.26	0.26	0.26	0.26	0.29	0.24	0.24	0.24	0.27	0.26	0.27	0.24	0.27	0.26	0.27	0.28	0.28	0.27	0.27	0.29	0.29	0.27	0.27	0.28	0.29	0.28	0.28	0.28	0.28	0.28
Ta45	108	T54_Milnesium_06_124_MarionIs_Cl14a	0.33	0.35	0.34	0.34	0.34	0.33	0.34	0.30	0.31	0.31	0.31	0.30	0.30	0.30	0.31	0.31	0.32	0.35	0.31	0.30	0.31	0.33	0.33	0.33	0.33	0.33	0.33	0.35	0.31	0.31	0.31	0.34	0.32	0.32	0.30	0.31	0.32	0.32	0.32	0.32	0.33	0.33	0.33	0.32	0.32	0.32	0.36	0.35	0.36	0.36	0.36	0.36	0.36
Ta45	109	T54_Milnesium_06_123 Marion Is	0.32	0.34	0.33	0.33	0.34	0.33	0.33	0.30	0.30	0.31	0.30	0.30	0.30	0.30	0.31	0.31	0.32	0.34	0.30	0.30	0.30	0.33	0.32	0.32	0.32	0.32	0.32	0.35	0.31	0.31	0.31	0.33	0.32	0.31	0.30	0.30	0.32	0.31	0.32	0.32	0.33	0.32	0.33	0.31	0.32	0.31	0.36	0.35	0.36	0.36	0.36	0.36	0.36
Ta46	110	T55_Milnesium_MSRAT235_SP_Cl15a	0.37	0.36	0.36	0.36	0.36	0.36	0.36	0.32	0.32	0.33	0.33	0.33	0.33	0.33	0.32	0.33	0.34	0.34	0.36	0.35	0.35	0.36	0.33	0.33	0.33	0.33	0.33	0.38	0.33	0.33	0.33	0.34	0.34	0.34	0.34	0.35	0.37	0.35	0.36	0.35	0.35	0.35	0.37	0.34	0.36	0.36	0.37	0.38	0.37	0.37	0.37	0.37	0.37
Ta47	111	T56_Milnesium_Mt_Kyffin_Cl16	0.34	0.34	0.33	0.33	0.33	0.33	0.33	0.30	0.30	0.32	0.32	0.32	0.32	0.32	0.34	0.34	0.33	0.35	0.33	0.32	0.32	0.36	0.35	0.35	0.35	0.35	0.35	0.36	0.32	0.32	0.32	0.35	0.32	0.32	0.34	0.33	0.34	0.32	0.32	0.33	0.32	0.32	0.33	0.32	0.35	0.35	0.37	0.36	0.36	0.37	0.37	0.37	0.37
Ta48	112	T57_EU244603_*Milnes*_Cl15b_eu244604	0.33	0.33	0.32	0.32	0.33	0.32	0.32	0.30	0.31	0.32	0.32	0.33	0.32	0.32	0.31	0.31	0.33	0.35	0.32	0.31	0.31	0.35	0.33	0.34	0.34	0.34	0.34	0.35	0.30	0.30	0.30	0.32	0.31	0.33	0.33	0.31	0.34	0.32	0.32	0.33	0.33	0.33	0.33	0.30	0.30	0.29	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Ta49	113	T58_JN664950_Cl14b_*Miln_tardigradum*	0.35	0.34	0.34	0.34	0.35	0.34	0.34	0.30	0.29	0.33	0.33	0.32	0.32	0.32	0.33	0.33	0.31	0.35	0.32	0.32	0.32	0.34	0.35	0.35	0.35	0.35	0.34	0.37	0.33	0.33	0.33	0.30	0.31	0.32	0.32	0.32	0.34	0.32	0.33	0.33	0.34	0.33	0.35	0.30	0.34	0.33	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Ta50	114	T59_Milnesium_Cl17_EF632553_AP.	0.34	0.33	0.34	0.34	0.34	0.34	0.34	0.31	0.30	0.33	0.33	0.33	0.32	0.32	0.32	0.32	0.33	0.32	0.30	0.30	0.30	0.34	0.32	0.32	0.32	0.32	0.33	0.35	0.31	0.31	0.31	0.29	0.29	0.31	0.32	0.31	0.32	0.30	0.30	0.31	0.31	0.30	0.33	0.31	0.31	0.30	0.36	0.36	0.35	0.36	0.36	0.36	0.36
Ta51	115	T60_Milnesium_06_224_ElsworthMt_Cl14c	0.33	0.34	0.35	0.35	0.35	0.35	0.35	0.32	0.31	0.34	0.35	0.33	0.33	0.33	0.33	0.33	0.34	0.35	0.32	0.32	0.32	0.35	0.35	0.35	0.35	0.35	0.35	0.37	0.32	0.32	0.32	0.30	0.28	0.32	0.32	0.32	0.32	0.32	0.33	0.33	0.31	0.33	0.34	0.32	0.31	0.31	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Ta52	116	T42_JF437735_Greenl_Cl13c_*Echiniscoi*	0.32	0.32	0.33	0.33	0.33	0.32	0.33	0.31	0.32	0.31	0.31	0.31	0.32	0.31	0.29	0.29	0.29	0.32	0.30	0.30	0.30	0.30	0.32	0.32	0.32	0.32	0.32	0.34	0.31	0.31	0.31	0.30	0.31	0.30	0.31	0.32	0.31	0.33	0.33	0.33	0.28	0.33	0.32	0.29	0.31	0.30	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Ta53	117	T43_Echiniscus_CO1_07_172	0.29	0.29	0.31	0.31	0.32	0.31	0.32	0.32	0.31	0.31	0.32	0.31	0.31	0.31	0.29	0.29	0.31	0.32	0.31	0.31	0.31	0.32	0.29	0.29	0.29	0.29	0.30	0.33	0.30	0.30	0.30	0.30	0.31	0.33	0.32	0.31	0.32	0.33	0.33	0.33	0.30	0.33	0.32	0.31	0.32	0.32	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Ta53	118	T43_MSTAR239_AVC210_01_Heterotar	0.29	0.30	0.32	0.32	0.32	0.31	0.32	0.32	0.31	0.31	0.32	0.31	0.31	0.31	0.30	0.29	0.32	0.32	0.32	0.31	0.31	0.32	0.29	0.29	0.30	0.30	0.30	0.33	0.30	0.30	0.30	0.31	0.31	0.33	0.32	0.31	0.32	0.33	0.33	0.33	0.31	0.33	0.32	0.32	0.32	0.32	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Ta53	119	T43_MSTAR261_AVC15_02_Heterotar	0.29	0.30	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.31	0.32	0.31	0.30	0.30	0.32	0.32	0.32	0.31	0.31	0.33	0.30	0.30	0.30	0.30	0.30	0.33	0.31	0.31	0.31	0.31	0.31	0.33	0.32	0.31	0.32	0.33	0.34	0.34	0.31	0.33	0.32	0.32	0.33	0.32	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Ta53	120	T43_MSTAR373_AVC17_06_Heterotar	0.29	0.29	0.31	0.31	0.32	0.31	0.32	0.32	0.32	0.32	0.32	0.31	0.32	0.31	0.29	0.29	0.31	0.32	0.32	0.31	0.31	0.33	0.29	0.29	0.30	0.30	0.30	0.33	0.30	0.30	0.30	0.31	0.31	0.33	0.32	0.31	0.32	0.33	0.33	0.33	0.31	0.33	0.32	0.32	0.33	0.32	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Ta54	121	T44_Echiniscus_Cl13d_MSTAR173_VH	0.31	0.32	0.33	0.33	0.33	0.32	0.33	0.31	0.30	0.32	0.32	0.34	0.33	0.33	0.32	0.32	0.32	0.33	0.31	0.31	0.32	0.32	0.33	0.33	0.33	0.33	0.33	0.31	0.32	0.32	0.32	0.30	0.31	0.32	0.33	0.32	0.32	0.33	0.34	0.34	0.31	0.33	0.33	0.29	0.31	0.31	0.33	0.34	0.33	0.33	0.33	0.33	0.33
Ta55	122	T47_EU046195_Cl13n *Echiniscus blumi*	0.32	0.30	0.32	0.32	0.32	0.32	0.32	0.29	0.29	0.30	0.30	0.29	0.29	0.29	0.31	0.30	0.33	0.31	0.32	0.32	0.32	0.32	0.30	0.30	0.30	0.30	0.30	0.33	0.28	0.28	0.28	0.31	0.33	0.30	0.31	0.33	0.32	0.31	0.32	0.31	0.30	0.31	0.36	0.31	0.33	0.33	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Ta56	123	T48_FJ435813_Cl13f_ef632541 *E. meroken*	0.35	0.31	0.35	0.35	0.35	0.34	0.35	0.32	0.32	0.34	0.34	0.32	0.32	0.32	0.32	0.31	0.35	0.32	0.32	0.32	0.32	0.34	0.31	0.31	0.31	0.31	0.31	0.36	0.31	0.31	0.31	0.32	0.33	0.30	0.31	0.32	0.34	0.33	0.33	0.33	0.32	0.33	0.36	0.32	0.32	0.31	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Ta57	124	T49_EF632542_Cl13h *Echiniscus testudo*	0.33	0.32	0.32	0.32	0.32	0.32	0.32	0.31	0.30	0.31	0.31	0.31	0.32	0.31	0.29	0.28	0.33	0.32	0.32	0.32	0.32	0.33	0.32	0.32	0.32	0.32	0.32	0.34	0.29	0.29	0.29	0.27	0.29	0.29	0.32	0.31	0.33	0.30	0.30	0.30	0.30	0.30	0.34	0.31	0.33	0.32	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Ta58	125	T45_Echiniscus_06_235_Cl13g	0.31	0.30	0.32	0.32	0.32	0.32	0.32	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.32	0.33	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.37	0.31	0.31	0.31	0.31	0.33	0.30	0.33	0.32	0.31	0.35	0.36	0.35	0.35	0.35	0.34	0.33	0.36	0.35	0.35	0.34	0.35	0.35	0.35	0.35	0.35
Ta59	126	T46_06_113_Cl13l *Echiniscus_jenningsi*	0.33	0.32	0.32	0.32	0.33	0.33	0.32	0.33	0.34	0.34	0.33	0.34	0.34	0.34	0.31	0.31	0.33	0.32	0.35	0.34	0.34	0.32	0.30	0.30	0.30	0.30	0.30	0.34	0.30	0.30	0.30	0.32	0.33	0.32	0.32	0.31	0.33	0.33	0.34	0.34	0.30	0.34	0.35	0.34	0.33	0.33	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Ta60	127	T50_Echiniscus_CO1_07_93_Cl13k	0.34	0.32	0.32	0.32	0.32	0.32	0.32	0.31	0.31	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.35	0.32	0.32	0.32	0.32	0.32	0.31	0.31	0.31	0.31	0.31	0.34	0.30	0.30	0.30	0.30	0.30	0.29	0.31	0.32	0.35	0.31	0.32	0.31	0.31	0.31	0.32	0.32	0.33	0.33	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Ta61	128	T52_Echiniscus_CO1_07_19_Cl13j	0.35	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.30	0.30	0.31	0.29	0.30	0.30	0.31	0.31	0.35	0.30	0.31	0.32	0.31	0.33	0.30	0.30	0.30	0.30	0.31	0.35	0.30	0.30	0.30	0.31	0.30	0.30	0.33	0.33	0.33	0.31	0.31	0.31	0.32	0.31	0.34	0.32	0.34	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
Ta62	129	T51_Echiniscus_CO1_07_47_Cl13p	0.35	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.30	0.31	0.31	0.31	0.31	0.31	0.32	0.31	0.36	0.31	0.32	0.32	0.32	0.33	0.30	0.30	0.30	0.30	0.31	0.35	0.29	0.29	0.29	0.32	0.31	0.30	0.33	0.32	0.33	0.31	0.32	0.32	0.33	0.32	0.33	0.33	0.35	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Ta63	130	T53_Echiniscus_CO1_07_53_Cl13m	0.35	0.32	0.32	0.32	0.32	0.32	0.32	0.30	0.30	0.32	0.33	0.32	0.33	0.32	0.32	0.32	0.35	0.32	0.32	0.32	0.32	0.36	0.32	0.33	0.33	0.33	0.33	0.35	0.32	0.32	0.32	0.31	0.33	0.30	0.33	0.32	0.35	0.32	0.32	0.32	0.34	0.32	0.33	0.33	0.34	0.33	0.35	0.35	0.35	0.35	0.35	0.35	0.35

Table S1 (continued)

Lineage		Haplotype	54	55	56	57	58	59	60	61	62	63	64	65	66
Ta28	**55**	T22_MSTAR420_Cdh51_04	0.00												
Ta28	**56**	T22_MSTAR119_AVC251_Tar04	0.00	0.01											
Ta28	**57**	T22_JX486022_Acutuncus_antarct_BH	0.00	0.01	0.00										
Ta28	**58**	T22_JX486021_Acutuncus_antarct_BH	0.00	0.01	0.00	0.00									
Ta28	**59**	T22_MSTAR166_11_AVC16_Tar05	0.00	0.01	0.00	0.00	0.00								
Ta28	**60**	T22_Hidden_Valley_9201B_15T7_COI2	0.01	0.01	0.00	0.00	0.00	0.00							
Ta28	**61**	T22_MSTAR058_10_AVC477_Tar06	0.01	0.01	0.01	0.01	0.01	0.01	0.00						
Ta28	**62**	T22_MSTAR163_11_AVC16_Tar02	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00					
Ta28	**63**	T22_MSTAR189_11_AVC22_17	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00				
Ta28	**64**	T22_MSTAR171_11_AVC41_Tar03	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00			
Ta28	**65**	T22_w_MSTAR646_12_AVC162_Tar04	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01		
Ta28	**66**	T22_w_MSTAR600_12_AVC70_Tar02	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	
Ta28	**67**	T22_w_MSTAR680_12_AVC327_Tar01	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.01
Ta28	**68**	T22_w_MSTAR642_12_AVC346_Tar08	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.01
Ta28	**69**	T22_w_MSTAR640_12_AVC346_Tar06	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Ta28	**70**	T22_w_MSTAR625_12_AVC205_Tar01	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.01
Ta28	**71**	T22_w_MSTAR651_12_AVC236_Tar04	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.01
Ta28	**72**	T23_Harcourt_Spur_7_80T6_COI2	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Ta28	**73**	T23_MSTAR268_11_AVC15_Tar09	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Ta29	**74**	T24_Gateway_Ridge_GR10_70T1_Cl2b	0.19	0.18	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Ta29	**75**	T25_MSTAR428_12_Cdh25_Tar07	0.19	0.19	0.20	0.20	0.20	0.20	0.20	0.20	0.19	0.20	0.19	0.20	0.20
Ta29	**76**	T25_MSTAR387_Cdh38_07_jx296216	0.19	0.19	0.20	0.20	0.20	0.20	0.20	0.20	0.19	0.20	0.19	0.20	0.20
Ta29	**77**	T25_JX296188_Acutuncus_Tanngarden	0.19	0.19	0.20	0.20	0.20	0.20	0.20	0.19	0.19	0.20	0.19	0.19	0.20
Ta30	**78**	T26_JX865308_06_282 *M.acro. furciger*	0.26	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.26	0.26	0.27	0.27
Ta31	**79**	T27_JX865306_Macrobiotus_05_148	0.29	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.29	0.30	0.30
Ta32	**80**	T27b_MSTAR726_12_PIA_S53_Tar01	0.25	0.26	0.26	0.26	0.26	0.26	0.26	0.25	0.26	0.26	0.25	0.26	0.26
Ta33	**81**	T28_Macrobiotus_Sg11_FM	0.27	0.27	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Ta34	**82**	T29_JX865314_Macrobiotus_06_161	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.26	0.27	0.27
Ta35	**83**	T31_Garden_Spur_12_54T4_COI2_Cl11bf	0.27	0.27	0.28	0.28	0.28	0.28	0.28	0.27	0.27	0.27	0.27	0.28	0.28
Ta35	**84**	T31_Masan_Glacier_2_55T2_COI2	0.27	0.27	0.28	0.28	0.28	0.28	0.28	0.27	0.27	0.27	0.27	0.28	0.28
Ta36	**85**	T32_304_2_Biocomplexity_509_1_Cl11bb	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.27	0.27
Ta36	**86**	T32_Hidden_Valley_9503A_19T10_13_COI2	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.26	0.27	0.27
Ta36	**87**	T32_Hidden_Valley_9503A_19T6_COI2	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.26	0.27	0.27
Ta37	**88**	T33_Hidden_Valley_9086A_17T6_7_Cl11be	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.27	0.27
Ta38	**89**	T34_Harcourt_Spur_2_74T1_2_3_Cl11bc	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.28	0.28
Ta39	**90**	T35_Harcourt_Spur_3_88T1_2_Cl11bd	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.26	0.27	0.27
Ta40	**91**	T30_Hidden_Valley_9060B_33T3_4_11	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.26	0.27	0.27
Ta40	**92**	T30_304_2_Biocomplexity_507_1_Cl11ba	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.26	0.27	0.27
Ta41	**93**	T36_JX865310_Macrobiotus_07_037	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.30	0.30
Ta42	**94**	T37_MSTAR393_Cdh57_Tar04	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Ta42	**95**	T37_MSTAR399_Cdh39_Tar02	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Ta42	**96**	T37_MSTAR391_Cdh57_Tar02	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Ta42	**97**	T37_JX296225_Macrobiot_Tanngarden	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Ta43	**98**	T39_JX296253_Macrobiot_DML_Cl12c	0.27	0.27	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Ta43	**99**	T40_JX888911_jx296254_258_261	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Ta43	**100**	T41_JX296260_Tanng_Cdh38_Cl12e	0.27	0.27	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.28	0.28
Ta44	**101**	T38_JX888912_Macrobiot_Tanngarden	0.28	0.28	0.28	0.28	0.28	0.28	0.29	0.29	0.29	0.29	0.29	0.29	0.29
Ta44	**102**	T38_JX296237	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Ta44	**103**	T38_MSTAR404_Cdh39_07_jx296241	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.29	0.28	0.28	0.28
Ta44	**104**	T38_MSTAR398_Cdh39_01_jx296232	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Ta44	**105**	T38_MSTAR386_Cdh38_Tar06_jx296228	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Ta44	**106**	T38_JX296231	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.29	0.28	0.28	0.28
Ta44	**107**	T38_MSTAR406_Cdh42_Tar01	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.29	0.29	0.29	0.28	0.28	0.28
Ta45	**108**	T54_Milnesium_06_124_MarionIs_Cl14a	0.35	0.36	0.36	0.36	0.36	0.36	0.36	0.35	0.36	0.36	0.35	0.36	0.36
Ta45	**109**	T54_Milnesium_06_123 Marion Is	0.35	0.36	0.36	0.36	0.36	0.36	0.36	0.35	0.36	0.36	0.35	0.36	0.36
Ta46	**110**	T55_Milnesium_MSRAT235_SP_Cl15a	0.37	0.37	0.37	0.37	0.37	0.37	0.38	0.37	0.38	0.37	0.38	0.38	0.38
Ta47	**111**	T56_Milnesium_Mt_Kyffin_Cl16	0.36	0.37	0.37	0.37	0.37	0.37	0.37	0.36	0.37	0.37	0.36	0.36	0.36
Ta48	**112**	T57_EU244603_*Milnes*_Cl15b_eu244604	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.36	0.36
Ta49	**113**	T58_JN664950_Cl14b_*Miln_tardigradum*	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Ta50	**114**	T59_Milnesium_Cl17_EF632553_AP.	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.35	0.36	0.36	0.36
Ta51	**115**	T60_Milnesium_06_224_ElsworthMt_Cl14c	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Ta52	**116**	T42_JF437735_Greenl_Cl13c_*Echiniscoi*	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Ta53	**117**	T43_Echiniscus_CO1_07_172	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.30	0.30	0.31	0.31
Ta53	**118**	T43_MSTAR239_AVC210_01_Heterotar	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Ta53	**119**	T43_MSTAR261_AVC15_02_Heterotar	0.31	0.32	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.32	0.31
Ta53	**120**	T43_MSTAR373_AVC17_06_Heterotar	0.31	0.32	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.32	0.31
Ta54	**121**	T44_Echiniscus_Cl13d_MSTAR173_VH	0.34	0.33	0.33	0.33	0.33	0.33	0.33	0.34	0.33	0.33	0.33	0.33	0.33
Ta55	**122**	T47_EU046195_Cl13n *Echiniscus blumi*	0.33	0.33	0.34	0.34	0.34	0.34	0.34	0.33	0.34	0.33	0.34	0.34	0.33
Ta56	**123**	T48_FJ435813_Cl13f_ef632541 *E. meroken*	0.34	0.35	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.35	0.34
Ta57	**124**	T49_EF632542_Cl13h *Echiniscus testudo*	0.34	0.34	0.35	0.35	0.35	0.35	0.35	0.34	0.34	0.34	0.34	0.35	0.35
Ta58	**125**	T45_Echiniscus_06_235_Cl13g	0.34	0.35	0.35	0.35	0.35	0.35	0.35	0.34	0.35	0.34	0.35	0.35	0.34
Ta59	**126**	T46_06_113_Cl13l *Echiniscus_jenningsi*	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Ta60	**127**	T50_Echiniscus_CO1_07_93_Cl13k	0.35	0.35	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Ta61	**128**	T52_Echiniscus_CO1_07_19_Cl13j	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.34	0.34
Ta62	**129**	T51_Echiniscus_CO1_07_47_Cl13p	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.33	0.34	0.34	0.34
Ta63	**130**	T53_Echiniscus_CO1_07_53_Cl13m	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35

	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88
68	0.00																					
69	0.01	0.01																				
70	0.00	0.00	0.01																			
71	0.00	0.00	0.01	0.00																		
72	0.01	0.01	0.01	0.01	0.01																	
73	0.01	0.01	0.01	0.01	0.01	0.00																
74	0.19	0.19	0.19	0.19	0.19	0.19	0.19															
75	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.01														
76	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.01	0.01													
77	0.19	0.19	0.19	0.20	0.19	0.20	0.20	0.01	0.00	0.01												
78	0.27	0.27	0.26	0.27	0.27	0.26	0.26	0.28	0.29	0.29	0.29											
79	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.28	0.29	0.28	0.28	0.22										
80	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.27	0.27	0.27	0.21	0.20									
81	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.30	0.31	0.30	0.31	0.26	0.21	0.22								
82	0.27	0.26	0.27	0.27	0.27	0.27	0.27	0.28	0.29	0.29	0.29	0.24	0.22	0.20	0.21							
83	0.28	0.28	0.28	0.28	0.28	0.27	0.27	0.28	0.28	0.28	0.28	0.27	0.21	0.21	0.21	0.20						
84	0.28	0.28	0.28	0.28	0.28	0.27	0.27	0.28	0.28	0.28	0.28	0.26	0.21	0.21	0.21	0.20	0.01					
85	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.28	0.28	0.28	0.28	0.26	0.23	0.20	0.23	0.20	0.05	0.06				
86	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.28	0.28	0.28	0.28	0.26	0.23	0.20	0.22	0.19	0.05	0.06	0.00			
87	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.28	0.28	0.28	0.28	0.26	0.23	0.20	0.22	0.19	0.05	0.06	0.00	0.00		
88	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.27	0.28	0.28	0.28	0.26	0.22	0.20	0.23	0.19	0.05	0.06	0.03	0.03	0.03	
89	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.28	0.28	0.28	0.28	0.27	0.22	0.20	0.23	0.19	0.05	0.06	0.04	0.03	0.03	0.04
90	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.28	0.28	0.28	0.28	0.26	0.21	0.21	0.22	0.20	0.05	0.06	0.03	0.03	0.03	0.04
91	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.27	0.27	0.26	0.26	0.25	0.22	0.19	0.22	0.20	0.06	0.07	0.07	0.07	0.07	0.06
92	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.27	0.27	0.26	0.26	0.25	0.22	0.19	0.22	0.20	0.07	0.07	0.07	0.07	0.07	0.07
93	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.28	0.28	0.28	0.24	0.23	0.22	0.24	0.20	0.21	0.21	0.21	0.22	0.22	0.21
94	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.27	0.27	0.27	0.22	0.23	0.21	0.24	0.21	0.20	0.20	0.21	0.21	0.21	0.19
95	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.27	0.27	0.27	0.22	0.23	0.21	0.24	0.21	0.20	0.20	0.21	0.21	0.21	0.19
96	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.27	0.27	0.27	0.22	0.23	0.21	0.24	0.21	0.20	0.20	0.21	0.21	0.21	0.19
97	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.27	0.27	0.27	0.23	0.23	0.21	0.23	0.21	0.19	0.19	0.21	0.20	0.20	0.19
98	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.28	0.27	0.27	0.23	0.23	0.21	0.24	0.21	0.20	0.20	0.20	0.20	0.20	0.19
99	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.23	0.23	0.21	0.24	0.20	0.20	0.20	0.21	0.20	0.20	0.19
100	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.28	0.27	0.27	0.23	0.23	0.22	0.24	0.20	0.21	0.21	0.21	0.20	0.20	0.20
101	0.28	0.28	0.29	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.28	0.25	0.24	0.22	0.25	0.21	0.22	0.22	0.22	0.22	0.22	0.20
102	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.27	0.27	0.27	0.24	0.24	0.21	0.24	0.21	0.21	0.21	0.22	0.21	0.21	0.20
103	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.28	0.27	0.27	0.24	0.24	0.21	0.24	0.21	0.21	0.21	0.22	0.22	0.22	0.20
104	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.27	0.27	0.27	0.24	0.24	0.21	0.24	0.22	0.21	0.21	0.22	0.22	0.22	0.20
105	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.28	0.27	0.27	0.24	0.24	0.22	0.24	0.21	0.21	0.21	0.22	0.22	0.22	0.20
106	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.27	0.27	0.27	0.24	0.23	0.21	0.24	0.22	0.21	0.21	0.22	0.22	0.22	0.20
107	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.28	0.24	0.24	0.22	0.25	0.22	0.21	0.22	0.22	0.22	0.22	0.21
108	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.34	0.34	0.34	0.34	0.38	0.32	0.35	0.36	0.32	0.34	0.34	0.33	0.33	0.33	0.34
109	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.33	0.33	0.34	0.33	0.38	0.32	0.34	0.36	0.32	0.33	0.33	0.33	0.33	0.33	0.33
110	0.38	0.38	0.37	0.37	0.37	0.38	0.38	0.35	0.35	0.35	0.35	0.36	0.34	0.34	0.37	0.35	0.36	0.35	0.36	0.36	0.36	0.36
111	0.37	0.37	0.37	0.36	0.37	0.37	0.37	0.35	0.35	0.36	0.36	0.39	0.35	0.35	0.39	0.35	0.36	0.36	0.36	0.35	0.35	0.37
112	0.36	0.36	0.35	0.36	0.35	0.35	0.35	0.36	0.36	0.36	0.36	0.35	0.32	0.35	0.36	0.34	0.36	0.35	0.36	0.36	0.36	0.37
113	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.34	0.32	0.34	0.35	0.33	0.35	0.35	0.35	0.35	0.35	0.35
114	0.36	0.36	0.36	0.35	0.36	0.36	0.36	0.35	0.34	0.35	0.34	0.33	0.30	0.30	0.34	0.32	0.33	0.33	0.34	0.34	0.34	0.33
115	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.32	0.34	0.34	0.32	0.34	0.34	0.35	0.34	0.34	0.34
116	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.31	0.30	0.31	0.30	0.33	0.33	0.33	0.33	0.33	0.34
117	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.34	0.34	0.34	0.34	0.34	0.30	0.32	0.30	0.31	0.32	0.31	0.32	0.32	0.32	0.32
118	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.34	0.34	0.34	0.34	0.34	0.30	0.32	0.31	0.31	0.32	0.31	0.32	0.32	0.32	0.33
119	0.32	0.32	0.31	0.31	0.32	0.32	0.32	0.34	0.35	0.34	0.34	0.34	0.30	0.32	0.31	0.31	0.32	0.32	0.32	0.33	0.33	0.33
120	0.32	0.32	0.31	0.31	0.32	0.32	0.32	0.34	0.35	0.34	0.34	0.34	0.30	0.32	0.30	0.31	0.32	0.32	0.32	0.33	0.33	0.33
121	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.31	0.32	0.32	0.32	0.32	0.30	0.32	0.32	0.33	0.35	0.35	0.34	0.34	0.34	0.34
122	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.35	0.35	0.35	0.35	0.34	0.29	0.33	0.32	0.30	0.33	0.33	0.33	0.32	0.32	0.33
123	0.34	0.35	0.35	0.34	0.34	0.34	0.34	0.36	0.35	0.36	0.36	0.34	0.30	0.32	0.32	0.29	0.33	0.33	0.32	0.32	0.32	0.32
124	0.35	0.34	0.35	0.35	0.35	0.35	0.35	0.32	0.32	0.32	0.32	0.34	0.30	0.30	0.32	0.30	0.30	0.31	0.31	0.31	0.31	0.31
125	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.34	0.34	0.34	0.34	0.35	0.32	0.30	0.31	0.31	0.33	0.34	0.33	0.32	0.32	0.33
126	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.35	0.35	0.35	0.34	0.33	0.32	0.31	0.33	0.32	0.32	0.32	0.32	0.32	0.32	0.32
127	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.35	0.35	0.36	0.35	0.31	0.31	0.31	0.32	0.31	0.32	0.32	0.32	0.32	0.32	0.31
128	0.34	0.34	0.34	0.33	0.33	0.34	0.34	0.35	0.36	0.36	0.35	0.31	0.30	0.30	0.33	0.29	0.31	0.31	0.32	0.32	0.32	0.31
129	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.35	0.35	0.35	0.35	0.32	0.30	0.29	0.33	0.30	0.32	0.32	0.32	0.32	0.32	0.31
130	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.36	0.36	0.36	0.36	0.31	0.32	0.29	0.33	0.30	0.32	0.32	0.33	0.33	0.33	0.32

	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105
90	0.03																
91	0.08	0.08															
92	0.08	0.08	0.00														
93	0.22	0.22	0.20	0.20													
94	0.21	0.21	0.18	0.18	0.17												
95	0.21	0.21	0.18	0.18	0.17	0.00											
96	0.21	0.21	0.18	0.18	0.17	0.00	0.00										
97	0.20	0.21	0.18	0.18	0.17	0.01	0.01	0.01									
98	0.21	0.21	0.18	0.18	0.18	0.03	0.03	0.03	0.03								
99	0.20	0.21	0.18	0.18	0.17	0.03	0.03	0.03	0.03	0.01							
100	0.21	0.21	0.18	0.18	0.18	0.04	0.04	0.04	0.04	0.02	0.01						
101	0.22	0.22	0.19	0.19	0.19	0.04	0.04	0.04	0.05	0.03	0.03	0.03					
102	0.22	0.22	0.18	0.18	0.18	0.04	0.04	0.04	0.04	0.02	0.02	0.03	0.01				
103	0.22	0.22	0.19	0.19	0.19	0.04	0.04	0.04	0.04	0.02	0.02	0.03	0.01	0.00			
104	0.22	0.22	0.19	0.19	0.19	0.03	0.03	0.03	0.04	0.02	0.02	0.03	0.01	0.01	0.00		
105	0.22	0.22	0.19	0.19	0.19	0.04	0.04	0.04	0.04	0.02	0.02	0.03	0.01	0.00	0.00	0.01	
106	0.22	0.22	0.19	0.19	0.18	0.04	0.04	0.04	0.04	0.02	0.02	0.03	0.01	0.01	0.00	0.00	0.01
107	0.22	0.22	0.19	0.19	0.19	0.04	0.04	0.04	0.04	0.02	0.02	0.03	0.01	0.01	0.00	0.00	0.01
108	0.33	0.34	0.33	0.33	0.35	0.33	0.33	0.33	0.33	0.34	0.33	0.33	0.33	0.33	0.33	0.33	0.33
109	0.33	0.33	0.32	0.32	0.35	0.33	0.33	0.33	0.32	0.33	0.33	0.33	0.33	0.32	0.32	0.32	0.32
110	0.36	0.36	0.34	0.34	0.37	0.36	0.36	0.36	0.36	0.36	0.37	0.37	0.38	0.37	0.37	0.37	0.38
111	0.36	0.36	0.35	0.35	0.37	0.36	0.36	0.36	0.36	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
112	0.36	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.34	0.35	0.34	0.34	0.35	0.35	0.35	0.35	0.35
113	0.34	0.34	0.35	0.34	0.37	0.33	0.33	0.33	0.34	0.35	0.34	0.34	0.35	0.35	0.34	0.34	0.35
114	0.32	0.33	0.32	0.32	0.35	0.33	0.33	0.33	0.33	0.34	0.34	0.35	0.35	0.34	0.34	0.34	0.35
115	0.33	0.32	0.33	0.33	0.35	0.33	0.33	0.33	0.33	0.35	0.35	0.35	0.36	0.35	0.35	0.35	0.35
116	0.33	0.33	0.32	0.32	0.31	0.32	0.32	0.32	0.33	0.33	0.32	0.32	0.32	0.32	0.32	0.32	0.32
117	0.32	0.32	0.32	0.31	0.32	0.33	0.33	0.33	0.33	0.34	0.33	0.34	0.33	0.32	0.32	0.32	0.32
118	0.33	0.32	0.32	0.32	0.32	0.33	0.33	0.33	0.33	0.34	0.34	0.34	0.33	0.33	0.33	0.33	0.33
119	0.33	0.32	0.32	0.32	0.33	0.33	0.33	0.33	0.33	0.34	0.34	0.34	0.33	0.33	0.33	0.33	0.33
120	0.33	0.32	0.32	0.32	0.33	0.33	0.33	0.33	0.33	0.34	0.34	0.34	0.33	0.33	0.33	0.33	0.33
121	0.34	0.33	0.33	0.32	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.32	0.33	0.33	0.33
122	0.32	0.32	0.33	0.32	0.33	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
123	0.32	0.33	0.32	0.32	0.35	0.32	0.32	0.32	0.32	0.33	0.32	0.33	0.33	0.33	0.32	0.32	0.33
124	0.30	0.30	0.30	0.30	0.31	0.32	0.32	0.32	0.32	0.33	0.32	0.32	0.33	0.32	0.32	0.32	0.33
125	0.33	0.33	0.33	0.33	0.33	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.34	0.34	0.34	0.34
126	0.32	0.32	0.32	0.31	0.34	0.34	0.34	0.34	0.34	0.33	0.33	0.32	0.32	0.32	0.32	0.32	0.32
127	0.31	0.31	0.32	0.32	0.33	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.32	0.32	0.31	0.31	0.32
128	0.30	0.31	0.31	0.30	0.32	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.33	0.34	0.34	0.34
129	0.30	0.32	0.31	0.31	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.33	0.34	0.34	0.34
130	0.31	0.32	0.32	0.31	0.33	0.35	0.35	0.35	0.35	0.35	0.35	0.34	0.34	0.34	0.34	0.34	0.34

Lineage		Haplotype	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129
Ta44	**107**	T38_MSTAR406_Cdh42_Tar01	0.00																							
Ta45	**108**	T54_Milnesium_06_124_MarionIs_Cl14a	0.33	0.33																						
Ta45	**109**	T54_Milnesium_06_123 Marion Is	0.32	0.32	0.00																					
Ta46	**110**	T55_Milnesium_MSRAT235_SP_Cl15a	0.38	0.38	0.23	0.22																				
Ta47	**111**	T56_Milnesium_Mt_Kyffin_Cl16	0.37	0.37	0.24	0.23	0.19																			
Ta48	**112**	T57_EU244603_*Milnes*_Cl15b_eu244604	0.35	0.35	0.25	0.25	0.24	0.24																		
Ta49	**113**	T58_JN664950_Cl14b_*Miln_tardigradum*	0.35	0.35	0.22	0.22	0.22	0.23	0.20																	
Ta50	**114**	T59_Milnesium_Cl17_EF632553_AP.	0.35	0.35	0.23	0.23	0.21	0.20	0.19	0.18																
Ta51	**115**	T60_Milnesium_06_224_ElsworthMt_Cl14c	0.35	0.35	0.23	0.24	0.23	0.23	0.21	0.20	0.08															
Ta52	**116**	T42_JF437735_Greenl_Cl13c_*Echiniscoi*	0.32	0.32	0.35	0.35	0.35	0.34	0.34	0.32	0.33	0.34														
Ta53	**117**	T43_Echiniscus_CO1_07_172	0.32	0.33	0.35	0.35	0.35	0.35	0.35	0.35	0.34	0.34	0.30													
Ta53	**118**	T43_MSTAR239_AVC210_01_Heterotar	0.33	0.33	0.35	0.35	0.35	0.35	0.35	0.35	0.34	0.34	0.31	0.00												
Ta53	**119**	T43_MSTAR261_AVC15_02_Heterotar	0.33	0.33	0.35	0.35	0.35	0.35	0.35	0.35	0.34	0.34	0.31	0.00	0.00											
Ta53	**120**	T43_MSTAR373_AVC17_06_Heterotar	0.33	0.33	0.35	0.35	0.35	0.35	0.35	0.35	0.34	0.34	0.31	0.00	0.00	0.00										
Ta54	**121**	T44_Echiniscus_Cl13d_MSTAR173_VH	0.32	0.33	0.37	0.37	0.36	0.36	0.36	0.35	0.34	0.36	0.31	0.21	0.22	0.22	0.22									
Ta55	**122**	T47_EU046195_Cl13n *Echiniscus blumi*	0.32	0.32	0.30	0.30	0.30	0.33	0.30	0.32	0.30	0.32	0.30	0.28	0.28	0.28	0.28	0.31								
Ta56	**123**	T48_FJ435813_Cl13f_ef632541 *E. meroken*	0.33	0.33	0.32	0.31	0.31	0.33	0.32	0.31	0.30	0.32	0.28	0.27	0.27	0.28	0.27	0.30	0.16							
Ta57	**124**	T49_EF632542_Cl13h *Echiniscus testudo*	0.32	0.32	0.32	0.32	0.31	0.33	0.32	0.32	0.31	0.32	0.26	0.27	0.28	0.28	0.28	0.28	0.16	0.18						
Ta58	**125**	T45_Echiniscus_06_235_Cl13g	0.34	0.34	0.35	0.34	0.34	0.36	0.34	0.36	0.33	0.35	0.31	0.26	0.27	0.27	0.26	0.29	0.20	0.21	0.21					
Ta59	**126**	T46_06_113_Cl13l *Echiniscus_jenningsi*	0.32	0.32	0.37	0.36	0.35	0.38	0.35	0.37	0.34	0.36	0.32	0.28	0.28	0.29	0.29	0.30	0.20	0.24	0.21	0.21				
Ta60	**127**	T50_Echiniscus_CO1_07_93_Cl13k	0.32	0.32	0.35	0.34	0.32	0.34	0.34	0.32	0.32	0.34	0.28	0.28	0.29	0.29	0.29	0.29	0.16	0.19	0.17	0.21	0.19			
Ta61	**128**	T52_Echiniscus_CO1_07_19_Cl13j	0.34	0.34	0.35	0.36	0.31	0.35	0.35	0.34	0.30	0.32	0.29	0.28	0.28	0.29	0.28	0.30	0.17	0.19	0.17	0.19	0.19	0.15		
Ta62	**129**	T51_Echiniscus_CO1_07_47_Cl13p	0.34	0.34	0.36	0.36	0.31	0.36	0.36	0.35	0.30	0.33	0.31	0.30	0.30	0.30	0.30	0.30	0.17	0.21	0.18	0.19	0.19	0.16	0.03	
Ta63	**130**	T53_Echiniscus_CO1_07_53_Cl13m	0.35	0.35	0.35	0.35	0.33	0.36	0.36	0.34	0.32	0.33	0.30	0.29	0.29	0.29	0.29	0.30	0.19	0.21	0.18	0.20	0.20	0.17	0.06	0.06

Table S2. Pair-wise distance comparison matrix among COI haplotypes from 135 bdelloid rotifers and two monogononts (Outgroup), showing all 47 established mitochondrial lineages and the two Outgroups used in the main manuscript and in Fig. 3

Lineage		Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Bd1	**1**	Bdelloidea_Cl21b_EA														
Bd1	**2**	MSROT630_AVC508_Rot02	0.00													
Bd1	**3**	MSROT362_AVC304_Rot11	0.00	0.00												
Bd1	**4**	MSROT597_AVC11_Rot05	0.00	0.00	0.00											
Bd1	**5**	MSROT364_AVC98_Rot02	0.01	0.01	0.00	0.00										
Bd1	**6**	MSROT474_AVC208_Rot11	0.01	0.01	0.01	0.01	0.01									
Bd1	**7**	MSROT587_AVC243_Rot08	0.01	0.01	0.01	0.01	0.01	0.00								
Bd1	**8**	Bdelloidea_Cl21a_EA	0.01	0.01	0.01	0.01	0.01	0.00	0.00							
Bd1	**9**	MSROT037_AVC245_Rot01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00						
Bd1	**10**	MSROT029_AVC514_Rot07	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01					
Bd1	**11**	MSROT327_AVC481_Rot13	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01				
Bd2	**12**	MSROT666_MBS018_Rot01	0.11	0.11	0.11	0.11	0.12	0.11	0.11	0.11	0.11	0.11	0.11			
Bd2	**13**	Bdelloidea_Cl22b_MS_VH	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.01		
Bd2	**14**	MSROT375_AVC36_Rot05	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.10	0.01	0.02	
Bd2	**15**	Bdelloidea_Cl22a_BP	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.10	0.01	0.02	0.00
Bd2	**16**	w_MSROT1089_AVC104_Bdell03	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.10	0.02	0.02	0.00
Bd2	**17**	w_MSROT1101_AVC226_Bdell01	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.02	0.02	0.00
Bd2	**18**	w_MSROT1087_AVC104_Bdell01	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.02	0.02	0.00
Bd2	**19**	w_MSROT922_AVC61_Bdell01	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.02	0.02	0.00
Bd3	**20**	Bdell_MSROT642_AVC505_06	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14
Bd4	**21**	Bdelloidea_Cl23_SP	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14
Bd4	**22**	MSROT614_AVC242_Rot05	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14
Bd5	**23**	Bdelloidea_Sg31_AP	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.16	0.15	0.15
Bd6	**24**	Bdelloidea_Sg32_SP	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.13
Bd7	**25**	MSROT006_AVC467_Rot06	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15
Bd7	**26**	MSROT229_AVC73_Rot02	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15
Bd7	**27**	Bdelloidea_Cl33_DS	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15
Bd7	**28**	MSROT724_AVC445_Rot11	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15
Bd8	**29**	Adineta_Cl16_CS_DS	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.13
Bd8	**30**	MSROT445_AVC41_Rot07	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.13
Bd8	**31**	Adineta-EF173193	0.13	0.13	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.15	0.15	0.14
Bd8	**32**	Adineta-EF173190	0.13	0.13	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.15	0.15	0.14
Bd8	**33**	EF173191-Adineta_sp	0.13	0.13	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.15	0.14	0.14
Bd8	**34**	MSROT115_AVC350_Rot05	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.13
Bd9	**35**	MSROT910_Cdh25_Rot7	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Bd10	**36**	Bdelloidea_Cl15b_Tanng	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.16	0.16	0.15
Bd10	**37**	MSROT914_Cdh25_Rot11	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.16	0.15	0.15
Bd10	**38**	Bdelloidea_Sg15a_MP	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.16	0.15	0.15
Bd11	**39**	Bdelloidea_Cl17_DS_MS_SI	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.13	0.13
Bd11	**40**	MSROT509_MBS008_Rot07	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.13	0.13
Bd11	**41**	MSROT090_AVC482_Rot10	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.13	0.13
Bd11	**42**	MSROT411_MBS015_Rot04	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.13
Bd12	**43**	Bdelloidea_Cl18_DS_MS	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14
Bd12	**44**	MSROT439_AVC41_Rot01	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Bd12	**45**	MSROT394_AVC239_Rot07	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Bd13	**46**	Bdelloidea_Cl20_BP_VH	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.14	0.14	0.14
Bd14	**47**	MSROT131_AVC449_Rot08	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Bd14	**48**	Bdelloidea_Cl19_CS_DS_SI	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.13
Bd14	**49**	MSROT466_AVC208_Rot03	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.13
Bd15	**50**	Bdelloidea_Cl14_EA	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.16	0.16	0.16
Bd15	**51**	Bdelloidea_Sg14b_CS	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16
Bd16	**52**	Bdelloidea_Cl14a_SP_MP_FM	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.16	0.16	0.16
Bd16	**53**	Bdelloidea_Cl14c_CS	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.16	0.16	0.16
Bd17	**54**	Bdel_MSROT019_AVC483_04	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Bd18	**55**	MSROT1129_PIA_S53_Bdell04	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.16	0.16	0.15
Bd19	**56**	MSROT1283_Pint_S42_Bdell02	0.16	0.16	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15
Bd20	**57**	w_MSROT1056_AVC236_Bdell04	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.17	0.16	0.16	0.16
Bd20	**58**	w_MSROT1049_AVC162_Bdell01	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
Bd21	**59**	Bdel_MSROT034_AVC492_04	0.19	0.19	0.18	0.18	0.18	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Bd22	**60**	EF173192-Adineta_sp	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.18	0.17	0.17
Bd23	**61**	Adineta_Cl12a_CS	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Bd23	**62**	Adineta_MSROT023_AVC514_01	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16
Bd23	**63**	MSROT586_AVC243_Rot07	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16
Bd24	**64**	Adineta_Cl29a_HI_MP	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.16	0.16	0.15
Bd24	**65**	Adineta_Sg29b_SP	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.15	0.15	0.14
Bd25	**66**	Bdelloidea_Cl30_BP	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.15	0.15	0.14
Bd25	**67**	MSROT253_AVC42_Rot08	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.15	0.15	0.14
Bd26	**68**	MSROT1137_PIA_C12_Bdell03	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.13	0.12	0.12
Bd27	**69**	w_MSROT1086_AVC461_Bdell04	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15
Bd27	**70**	w_MSROT1065_AVC82_Bdell02	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.15
Bd27	**71**	w_MSROT921_AVC66_Bdell06	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.15
Bd27	**72**	MSROT695_AVC475_Rot08	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.17	0.17	0.16	0.16
Bd27	**73**	Bdelloidea_Cl4_EA	0.17	0.17	0.18	0.18	0.17	0.18	0.18	0.18	0.18	0.17	0.17	0.17	0.16	0.16
Bd27	**74**	MSROT174_AVC68_Rot09	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16
Bd28	**75**	Bdelloidea_Cl10_FM	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15
Bd28	**76**	MSROT641_AVC505_Rot05	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.16	0.16	0.15
Bd29	**77**	MSROT1326_Pint_C33_Bdell02	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.18	0.17	0.17
Bd30	**78**	MSROT1319_Pint_C31_Bdell01	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.12
Bd31	**79**	MSROT158_AVC173_Rot03	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.13
Bd31	**80**	MSROT713_AVC439_Rot05	0.14	0.14	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.13
Bd31	**81**	w_MSROT920_AVC66_Bdell05	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.13
Bd31	**82**	Bdelloidea_Cl1_EA	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.13
Bd32	**83**	MSROT1257_Pint_S13_Bdell02	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.13
Bd32	**84**	MSROT1302_Pint_C21_Bdell01	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.13	0.13	0.12
Bd33	**85**	MSROT343_AVC210_Rot05	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.17	0.16	0.16
Bd33	**86**	Bdelloidea_Cl2a_SP	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.16	0.16	0.16
Bd33	**87**	Bdelloidea_Cl2b_CS	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.17	0.17	0.17
Bd33	**88**	MSROT347_AVC157_Rot02	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.16	0.16	0.16
Bd33	**89**	Bdelloidea_Cl2c_EA	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.16	0.16	0.16
Bd33	**90**	Bdelloidea_Cl2_EA	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.16	0.16	0.16
Bd33	**91**	MSROT080_AVC477_Rot07	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.17	0.16	0.16
Bd33	**92**	MSROT658_AVC1_Rot06	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.16	0.16	0.16
Bd34	**93**	MSROT1322_Pint_C32_Bdell01	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16
Bd34	**94**	MSROT1320_Pint_C31_Bdell02	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16
Bd35	**95**	Bdel_MSROT913_Cdh25_10_DML	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.16	0.16	0.14	0.14	0.14
Bd36	**96**	MSROT1329_Pint_C41_Bdell03	0.24	0.24	0.24	0.24	0.24	0.24	0.23	0.23	0.23	0.23	0.23	0.24	0.23	0.23
Bd37	**97**	Bdelloidea_CL27_FM	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.18	0.17
Bd38	**98**	MSROT1269_Pint_S31_Bdell03	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.18	0.18	0.17
Bd39	**99**	MSROT918_AP025_Rot1	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.16
Bd40	**100**	MSROT385_AVC179_Rot05	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.17
Bd40	**101**	Bdelloidea_Cl28_SP	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.17
Bd41	**102**	MSROT1307_Pint_C22_Bdell01	0.15	0.15	0.16	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Bd42	**103**	MSROT057_AVC268_Rot09	0.17	0.17	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.18	0.18	0.17
Bd42	**104**	MSROT147_MBS019_Rot11	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.18	0.18	0.17
Bd42	**105**	Bdelloidea_Cl5_CS_LH_HI_VH	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.18	0.18	0.18
Bd42	**106**	MSROT139_MBS019_Rot03	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.18	0.18	0.17
Bd42	**107**	MSROT354_AVC304_Rot03	0.17	0.17	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.18	0.18	0.17
Bd42	**108**	MSROT073_AVC283_Rot05	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.18	0.18	0.17
Bd42	**109**	MSROT368_AVC98_Rot06	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.18	0.18	0.17
Bd43	**110**	Bdelloidea_Cl9_AP	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.15
Bd44	**111**	Bdelloidea_Sg3_FM	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14
Bd45	**112**	w_MSROT1096_AVC222_Bdell01	0.19	0.19	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.18	0.17
Bd45	**113**	w_MSROT947_AVC168_Bdell01	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.18	0.18	0.17	0.18	0.18
Bd45	**114**	w_MSROT1093_AVC95_Bdell02	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.18
Bd45	**115**	Bdelloidea_Cl7b_SP	0.19	0.19	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Bd45	**116**	MSROT619_AVC256_Rot02	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.18
Bd45	**117**	Bdelloidea_Sg7a_CS	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.18	0.17
Bd46	**118**	w_MSROT1112_AVC127_Bdell03	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15	0.15
Bd46	**119**	Philodina_Cl6_CS_VH_BP_SP_MS	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15
Bd46	**120**	MSROT459_AVC22_Rot03	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15
Bd46	**121**	MSROT625_AVC256_Rot08	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15
Bd47	**122**	Philodina_Cl8b_VH_SI_SP_BP_MS	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.15	0.15
Bd47	**123**	w_MSROT1104_AVC110_Bdell01	0.17	0.17	0.18	0.18	0.18	0.18	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.15
Bd47	**124**	w_MSROT1078_MBS014_Bdell01	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.17	0.16	0.16	0.15
Bd47	**125**	w_MSROT1079_MBS014_Bdell02	0.17	0.17	0.18	0.18	0.18	0.18	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.15
Bd47	**126**	MSROT553_MBS011_Rot03	0.17	0.17	0.17	0.17	0.17	0.18	0.17	0.17	0.17	0.17	0.17	0.16	0.15	0.14
Bd47	**127**	MSROT554_MBS011_Rot04	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.17	0.16	0.16	0.15
Bd47	**128**	MSROT501_MBS021_Rot01	0.17	0.17	0.18	0.18	0.18	0.18	0.17	0.17	0.17	0.17	0.17	0.16	0.15	0.14
Bd47	**129**	MSROT011_AVC495_Rot03	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.17	0.16	0.16	0.15
Bd47	**130**	Philodina_Cl8a_MS_BP	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.17	0.16	0.16	0.15
Bd47	**131**	Philodina_RS_13_Wood	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.15	0.15	0.14
Bd47	**132**	Philodina_RS_5_HOC	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.15	0.15	0.14
Bd47	**133**	Philodina_RS_6_Mero	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.16	0.15	0.14
Bd47	**134**	Philodina_RS_2_Fog	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.16	0.15	0.14
Bd47	**135**	Philodina_RS_4_HOC	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.16	0.16	0.15
Outgroup	**136**	Outgroup_Monogononta_JN936618	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Outgroup	**137**	Outgroup_Monogononta_JX216519	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34

Lineage		15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Bd2	**16**	0.00																		
Bd2	**17**	0.00	0.00																	
Bd2	**18**	0.00	0.00	0.00																
Bd2	**19**	0.00	0.00	0.00	0.00															
Bd3	**20**	0.14	0.15	0.15	0.14	0.15														
Bd4	**21**	0.14	0.14	0.14	0.13	0.14	0.04													
Bd4	**22**	0.14	0.14	0.14	0.13	0.14	0.04	0.00												
Bd5	**23**	0.15	0.15	0.15	0.15	0.15	0.16	0.15	0.15											
Bd6	**24**	0.13	0.13	0.14	0.13	0.14	0.15	0.14	0.14	0.07										
Bd7	**25**	0.15	0.15	0.16	0.16	0.16	0.16	0.16	0.16	0.13	0.13									
Bd7	**26**	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.16	0.13	0.13	0.00								
Bd7	**27**	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.16	0.13	0.13	0.01	0.01							
Bd7	**28**	0.15	0.15	0.16	0.16	0.16	0.16	0.16	0.16	0.13	0.13	0.00	0.00	0.00						
Bd8	**29**	0.13	0.13	0.14	0.14	0.14	0.16	0.15	0.15	0.15	0.13	0.16	0.15	0.16	0.16					
Bd8	**30**	0.13	0.13	0.14	0.14	0.14	0.16	0.15	0.15	0.15	0.13	0.16	0.15	0.16	0.16	0.00				
Bd8	**31**	0.14	0.13	0.14	0.14	0.14	0.16	0.15	0.15	0.15	0.13	0.16	0.16	0.16	0.16	0.01	0.01			
Bd8	**32**	0.14	0.14	0.14	0.14	0.14	0.17	0.15	0.16	0.15	0.13	0.16	0.16	0.16	0.16	0.01	0.01	0.01		
Bd8	**33**	0.14	0.13	0.14	0.14	0.14	0.16	0.15	0.15	0.14	0.13	0.16	0.16	0.16	0.16	0.01	0.01	0.00	0.00	
Bd8	**34**	0.13	0.13	0.13	0.13	0.13	0.16	0.15	0.15	0.14	0.13	0.15	0.15	0.15	0.15	0.01	0.01	0.01	0.01	0.01
Bd9	**35**	0.14	0.14	0.14	0.13	0.13	0.15	0.14	0.14	0.14	0.13	0.13	0.13	0.14	0.13	0.13	0.13	0.12	0.12	0.12
Bd10	**36**	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.12	0.13	0.17	0.16	0.17	0.17	0.14	0.14	0.14	0.14	0.14
Bd10	**37**	0.15	0.15	0.15	0.15	0.15	0.17	0.16	0.16	0.12	0.13	0.16	0.16	0.16	0.16	0.14	0.14	0.14	0.14	0.14
Bd10	**38**	0.15	0.15	0.15	0.15	0.15	0.17	0.16	0.16	0.13	0.13	0.17	0.16	0.17	0.17	0.13	0.13	0.14	0.14	0.14
Bd11	**39**	0.13	0.13	0.13	0.13	0.13	0.17	0.15	0.15	0.14	0.13	0.15	0.15	0.15	0.15	0.11	0.11	0.11	0.11	0.11
Bd11	**40**	0.13	0.13	0.13	0.13	0.13	0.17	0.15	0.15	0.14	0.13	0.15	0.15	0.15	0.15	0.11	0.11	0.11	0.11	0.11
Bd11	**41**	0.13	0.13	0.13	0.13	0.13	0.17	0.15	0.15	0.14	0.13	0.15	0.15	0.15	0.15	0.11	0.11	0.11	0.11	0.11
Bd11	**42**	0.13	0.13	0.13	0.13	0.14	0.17	0.16	0.16	0.14	0.13	0.15	0.15	0.15	0.15	0.11	0.11	0.11	0.11	0.11
Bd12	**43**	0.14	0.14	0.14	0.14	0.14	0.18	0.17	0.17	0.15	0.13	0.16	0.15	0.16	0.16	0.14	0.13	0.14	0.14	0.14
Bd12	**44**	0.14	0.14	0.14	0.14	0.14	0.18	0.17	0.17	0.15	0.13	0.16	0.16	0.16	0.16	0.13	0.13	0.14	0.14	0.14
Bd12	**45**	0.14	0.14	0.14	0.14	0.14	0.18	0.17	0.17	0.15	0.13	0.16	0.15	0.16	0.16	0.13	0.13	0.14	0.14	0.14
Bd13	**46**	0.14	0.14	0.14	0.15	0.14	0.18	0.17	0.17	0.15	0.14	0.16	0.16	0.16	0.16	0.13	0.12	0.13	0.13	0.13
Bd14	**47**	0.14	0.14	0.14	0.14	0.14	0.18	0.16	0.16	0.15	0.13	0.15	0.15	0.16	0.15	0.13	0.13	0.13	0.13	0.13
Bd14	**48**	0.13	0.13	0.14	0.14	0.14	0.18	0.16	0.16	0.15	0.13	0.15	0.15	0.15	0.15	0.13	0.12	0.13	0.13	0.13
Bd14	**49**	0.13	0.13	0.13	0.14	0.14	0.18	0.16	0.17	0.15	0.13	0.15	0.15	0.15	0.15	0.13	0.12	0.13	0.13	0.13
Bd15	**50**	0.16	0.16	0.16	0.17	0.16	0.18	0.17	0.17	0.14	0.14	0.15	0.15	0.15	0.15	0.14	0.14	0.15	0.15	0.14
Bd15	**51**	0.16	0.16	0.16	0.17	0.16	0.17	0.17	0.17	0.13	0.13	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.15
Bd16	**52**	0.16	0.16	0.16	0.16	0.16	0.18	0.18	0.18	0.15	0.14	0.16	0.16	0.16	0.16	0.14	0.14	0.15	0.15	0.15
Bd16	**53**	0.16	0.16	0.16	0.17	0.16	0.17	0.17	0.17	0.15	0.14	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.16	0.15
Bd17	**54**	0.14	0.14	0.14	0.14	0.14	0.16	0.15	0.15	0.14	0.13	0.15	0.15	0.15	0.15	0.13	0.13	0.13	0.13	0.13
Bd18	**55**	0.15	0.15	0.15	0.15	0.15	0.16	0.17	0.17	0.15	0.15	0.18	0.18	0.18	0.18	0.14	0.14	0.13	0.14	0.13
Bd19	**56**	0.15	0.15	0.15	0.15	0.15	0.19	0.18	0.18	0.15	0.15	0.18	0.18	0.18	0.18	0.15	0.16	0.15	0.15	0.15
Bd20	**57**	0.16	0.15	0.15	0.15	0.16	0.17	0.17	0.17	0.16	0.15	0.16	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16
Bd20	**58**	0.16	0.15	0.16	0.15	0.16	0.17	0.17	0.17	0.16	0.15	0.16	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16
Bd21	**59**	0.19	0.19	0.19	0.18	0.19	0.19	0.20	0.20	0.18	0.18	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Bd22	**60**	0.17	0.17	0.17	0.16	0.17	0.18	0.18	0.18	0.14	0.14	0.16	0.16	0.17	0.17	0.15	0.15	0.15	0.15	0.15
Bd23	**61**	0.15	0.15	0.15	0.14	0.15	0.16	0.15	0.15	0.14	0.13	0.15	0.15	0.15	0.15	0.14	0.14	0.15	0.15	0.14
Bd23	**62**	0.16	0.16	0.16	0.15	0.16	0.17	0.17	0.17	0.15	0.14	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15	0.15
Bd23	**63**	0.16	0.16	0.16	0.15	0.16	0.17	0.16	0.16	0.14	0.14	0.15	0.15	0.16	0.16	0.14	0.14	0.15	0.15	0.15
Bd24	**64**	0.15	0.15	0.15	0.16	0.16	0.16	0.15	0.15	0.15	0.13	0.15	0.15	0.15	0.15	0.13	0.13	0.13	0.13	0.13
Bd24	**65**	0.14	0.15	0.14	0.15	0.15	0.15	0.14	0.14	0.14	0.13	0.16	0.15	0.15	0.15	0.13	0.12	0.13	0.13	0.13
Bd25	**66**	0.14	0.14	0.14	0.14	0.14	0.16	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.15
Bd25	**67**	0.14	0.14	0.14	0.14	0.14	0.16	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.15
Bd26	**68**	0.12	0.12	0.12	0.12	0.12	0.15	0.15	0.15	0.13	0.12	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14
Bd27	**69**	0.15	0.14	0.15	0.15	0.15	0.16	0.16	0.16	0.14	0.14	0.18	0.18	0.18	0.18	0.14	0.14	0.15	0.15	0.14
Bd27	**70**	0.15	0.14	0.15	0.15	0.15	0.16	0.16	0.16	0.14	0.14	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.14
Bd27	**71**	0.15	0.14	0.15	0.15	0.15	0.16	0.16	0.16	0.14	0.14	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.14
Bd27	**72**	0.16	0.15	0.15	0.16	0.16	0.17	0.16	0.17	0.15	0.15	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15
Bd27	**73**	0.16	0.15	0.16	0.16	0.16	0.17	0.16	0.17	0.15	0.15	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15
Bd27	**74**	0.16	0.15	0.15	0.16	0.16	0.17	0.16	0.17	0.15	0.15	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15
Bd28	**75**	0.15	0.15	0.15	0.15	0.16	0.18	0.15	0.16	0.13	0.13	0.17	0.17	0.17	0.17	0.14	0.14	0.15	0.15	0.14
Bd28	**76**	0.15	0.15	0.16	0.16	0.16	0.18	0.15	0.16	0.14	0.13	0.17	0.17	0.17	0.17	0.14	0.14	0.15	0.15	0.14
Bd29	**77**	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.15	0.14	0.18	0.18	0.18	0.18	0.14	0.14	0.13	0.13	0.13
Bd30	**78**	0.12	0.11	0.12	0.12	0.12	0.18	0.16	0.16	0.15	0.14	0.16	0.16	0.16	0.16	0.12	0.12	0.12	0.12	0.12
Bd31	**79**	0.13	0.13	0.13	0.13	0.13	0.15	0.14	0.14	0.13	0.11	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.13
Bd31	**80**	0.13	0.13	0.13	0.13	0.13	0.15	0.14	0.14	0.13	0.11	0.13	0.13	0.14	0.13	0.12	0.12	0.13	0.13	0.13
Bd31	**81**	0.13	0.13	0.13	0.13	0.13	0.15	0.14	0.14	0.13	0.11	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.13
Bd31	**82**	0.13	0.13	0.13	0.13	0.13	0.15	0.14	0.14	0.13	0.11	0.13	0.13	0.14	0.13	0.13	0.13	0.13	0.13	0.13
Bd32	**83**	0.13	0.12	0.13	0.13	0.13	0.15	0.15	0.15	0.15	0.12	0.15	0.15	0.16	0.15	0.13	0.13	0.13	0.13	0.13
Bd32	**84**	0.12	0.12	0.12	0.12	0.12	0.15	0.15	0.15	0.15	0.12	0.14	0.14	0.15	0.14	0.13	0.13	0.13	0.13	0.13
Bd33	**85**	0.16	0.16	0.16	0.17	0.17	0.18	0.16	0.16	0.15	0.14	0.16	0.16	0.17	0.17	0.13	0.13	0.14	0.14	0.14
Bd33	**86**	0.16	0.16	0.16	0.16	0.16	0.17	0.16	0.16	0.13	0.13	0.14	0.14	0.15	0.15	0.14	0.14	0.14	0.14	0.14
Bd33	**87**	0.17	0.17	0.17	0.16	0.17	0.17	0.16	0.16	0.16	0.15	0.17	0.17	0.17	0.17	0.15	0.15	0.15	0.15	0.15
Bd33	**88**	0.16	0.16	0.16	0.16	0.16	0.18	0.15	0.15	0.14	0.14	0.16	0.16	0.16	0.16	0.13	0.13	0.13	0.14	0.13
Bd33	**89**	0.16	0.16	0.16	0.16	0.16	0.18	0.15	0.15	0.14	0.14	0.16	0.16	0.16	0.16	0.13	0.13	0.13	0.14	0.13
Bd33	**90**	0.16	0.16	0.16	0.17	0.16	0.18	0.16	0.16	0.15	0.14	0.16	0.16	0.16	0.16	0.13	0.13	0.14	0.14	0.13
Bd33	**91**	0.16	0.16	0.16	0.17	0.17	0.18	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.13	0.13	0.14	0.14	0.14
Bd33	**92**	0.16	0.16	0.16	0.17	0.16	0.18	0.15	0.15	0.15	0.14	0.16	0.16	0.16	0.16	0.13	0.13	0.14	0.14	0.13
Bd34	**93**	0.16	0.15	0.16	0.16	0.16	0.18	0.18	0.18	0.15	0.14	0.17	0.17	0.17	0.17	0.15	0.15	0.15	0.15	0.15
Bd34	**94**	0.16	0.15	0.15	0.16	0.16	0.18	0.18	0.18	0.15	0.14	0.17	0.17	0.17	0.17	0.15	0.15	0.15	0.15	0.15
Bd35	**95**	0.14	0.14	0.14	0.14	0.14	0.17	0.16	0.17	0.13	0.13	0.16	0.16	0.16	0.16	0.13	0.13	0.14	0.14	0.13
Bd36	**96**	0.23	0.23	0.23	0.22	0.23	0.23	0.23	0.23	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.21	0.21	0.21
Bd37	**97**	0.17	0.17	0.17	0.17	0.17	0.18	0.18	0.18	0.18	0.16	0.17	0.17	0.17	0.17	0.16	0.16	0.15	0.16	0.15
Bd38	**98**	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.15	0.16	0.17	0.17	0.17	0.17	0.15	0.15	0.15	0.15	0.15
Bd39	**99**	0.16	0.16	0.16	0.17	0.16	0.17	0.16	0.16	0.14	0.14	0.16	0.16	0.16	0.16	0.13	0.13	0.13	0.13	0.13
Bd40	**100**	0.17	0.17	0.18	0.17	0.17	0.18	0.17	0.17	0.15	0.13	0.16	0.16	0.16	0.16	0.14	0.14	0.15	0.15	0.15
Bd40	**101**	0.17	0.17	0.18	0.17	0.17	0.18	0.17	0.17	0.15	0.13	0.16	0.16	0.16	0.16	0.14	0.14	0.15	0.15	0.15
Bd41	**102**	0.15	0.14	0.14	0.14	0.14	0.16	0.16	0.16	0.15	0.14	0.20	0.20	0.20	0.20	0.15	0.15	0.15	0.15	0.15
Bd42	**103**	0.17	0.17	0.18	0.18	0.18	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.17	0.16
Bd42	**104**	0.17	0.17	0.18	0.18	0.18	0.17	0.16	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Bd42	**105**	0.18	0.18	0.18	0.18	0.18	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.16
Bd42	**106**	0.17	0.17	0.18	0.17	0.18	0.17	0.16	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Bd42	**107**	0.17	0.17	0.17	0.17	0.18	0.17	0.16	0.16	0.17	0.15	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Bd42	**108**	0.17	0.17	0.17	0.18	0.18	0.17	0.16	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Bd42	**109**	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Bd43	**110**	0.15	0.15	0.15	0.15	0.15	0.18	0.18	0.18	0.14	0.15	0.15	0.15	0.16	0.16	0.14	0.14	0.14	0.14	0.14
Bd44	**111**	0.14	0.14	0.14	0.13	0.15	0.16	0.16	0.16	0.15	0.13	0.17	0.17	0.17	0.17	0.14	0.14	0.15	0.15	0.14
Bd45	**112**	0.17	0.17	0.17	0.17	0.17	0.18	0.18	0.18	0.18	0.17	0.18	0.18	0.18	0.18	0.16	0.16	0.16	0.16	0.16
Bd45	**113**	0.18	0.17	0.17	0.17	0.18	0.18	0.18	0.18	0.18	0.17	0.18	0.18	0.18	0.18	0.16	0.16	0.16	0.16	0.16
Bd45	**114**	0.18	0.17	0.17	0.17	0.18	0.18	0.18	0.18	0.18	0.17	0.18	0.18	0.18	0.18	0.16	0.16	0.16	0.16	0.16
Bd45	**115**	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.19	0.17	0.19	0.19	0.19	0.19	0.16	0.16	0.16	0.16	0.16
Bd45	**116**	0.18	0.18	0.18	0.18	0.19	0.18	0.18	0.18	0.19	0.17	0.19	0.19	0.19	0.19	0.16	0.16	0.16	0.16	0.16
Bd45	**117**	0.17	0.17	0.17	0.17	0.17	0.18	0.18	0.18	0.18	0.16	0.18	0.17	0.17	0.17	0.15	0.15	0.15	0.16	0.15
Bd46	**118**	0.15	0.14	0.14	0.14	0.15	0.16	0.15	0.15	0.14	0.14	0.18	0.17	0.18	0.17	0.14	0.14	0.14	0.14	0.13
Bd46	**119**	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.15	0.14	0.18	0.18	0.18	0.18	0.14	0.14	0.14	0.14	0.14
Bd46	**120**	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.15	0.14	0.18	0.18	0.18	0.18	0.14	0.14	0.14	0.14	0.14
Bd46	**121**	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.15	0.14	0.18	0.18	0.18	0.18	0.14	0.14	0.14	0.14	0.14
Bd47	**122**	0.15	0.15	0.15	0.14	0.15	0.19	0.17	0.17	0.16	0.15	0.18	0.17	0.18	0.18	0.15	0.15	0.15	0.15	0.15
Bd47	**123**	0.15	0.15	0.15	0.14	0.15	0.19	0.18	0.18	0.16	0.15	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15
Bd47	**124**	0.15	0.14	0.15	0.13	0.15	0.19	0.18	0.18	0.16	0.16	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15
Bd47	**125**	0.15	0.14	0.14	0.13	0.15	0.19	0.18	0.18	0.16	0.15	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15
Bd47	**126**	0.14	0.14	0.15	0.14	0.15	0.19	0.18	0.18	0.16	0.15	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15
Bd47	**127**	0.15	0.15	0.15	0.14	0.15	0.19	0.18	0.18	0.16	0.16	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15
Bd47	**128**	0.14	0.14	0.15	0.14	0.15	0.19	0.18	0.18	0.16	0.15	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15
Bd47	**129**	0.15	0.15	0.15	0.14	0.15	0.19	0.18	0.18	0.16	0.16	0.18	0.18	0.18	0.18	0.16	0.16	0.16	0.16	0.16
Bd47	**130**	0.15	0.15	0.15	0.14	0.15	0.19	0.18	0.18	0.16	0.16	0.18	0.18	0.18	0.18	0.16	0.16	0.16	0.16	0.16
Bd47	**131**	0.14	0.14	0.14	0.13	0.14	0.19	0.18	0.18	0.16	0.16	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15
Bd47	**132**	0.14	0.14	0.14	0.13	0.14	0.19	0.18	0.18	0.16	0.16	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15
Bd47	**133**	0.14	0.14	0.14	0.13	0.15	0.19	0.18	0.18	0.16	0.16	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15
Bd47	**134**	0.14	0.14	0.14	0.13	0.15	0.19	0.18	0.18	0.16	0.16	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15
Bd47	**135**	0.15	0.14	0.14	0.14	0.15	0.19	0.18	0.18	0.17	0.16	0.18	0.18	0.18	0.18	0.16	0.16	0.15	0.15	0.15
Outgroup	**136**	0.30	0.30	0.30	0.30	0.30	0.32	0.31	0.31	0.31	0.30	0.33	0.33	0.33	0.33	0.31	0.31	0.31	0.31	0.31
Outgroup	**137**	0.34	0.34	0.34	0.35	0.35	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33

Lineage		34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
Bd9	**35**	0.12																			
Bd10	**36**	0.13	0.13																		
Bd10	**37**	0.13	0.12	0.00																	
Bd10	**38**	0.13	0.13	0.01	0.01																
Bd11	**39**	0.10	0.11	0.12	0.12	0.12															
Bd11	**40**	0.10	0.11	0.12	0.12	0.12	0.00														
Bd11	**41**	0.10	0.11	0.12	0.12	0.12	0.00	0.00													
Bd11	**42**	0.10	0.11	0.12	0.12	0.12	0.00	0.00	0.00												
Bd12	**43**	0.13	0.13	0.12	0.11	0.12	0.10	0.10	0.10	0.10											
Bd12	**44**	0.13	0.13	0.11	0.11	0.11	0.10	0.10	0.10	0.10	0.00										
Bd12	**45**	0.13	0.13	0.11	0.11	0.11	0.10	0.10	0.10	0.10	0.00	0.00									
Bd13	**46**	0.12	0.14	0.13	0.13	0.12	0.10	0.10	0.10	0.10	0.05	0.05	0.05								
Bd14	**47**	0.12	0.13	0.12	0.12	0.12	0.09	0.09	0.09	0.09	0.04	0.04	0.04	0.04							
Bd14	**48**	0.12	0.13	0.12	0.12	0.11	0.09	0.09	0.09	0.09	0.05	0.04	0.04	0.04	0.01						
Bd14	**49**	0.12	0.13	0.12	0.12	0.11	0.09	0.09	0.09	0.09	0.05	0.04	0.04	0.04	0.01	0.00					
Bd15	**50**	0.14	0.15	0.13	0.13	0.13	0.12	0.12	0.12	0.12	0.14	0.14	0.14	0.15	0.15	0.14	0.14				
Bd15	**51**	0.14	0.15	0.14	0.14	0.14	0.12	0.12	0.12	0.12	0.15	0.14	0.14	0.15	0.15	0.14	0.14	0.01			
Bd16	**52**	0.14	0.16	0.15	0.15	0.15	0.12	0.12	0.12	0.12	0.15	0.15	0.14	0.16	0.15	0.15	0.15	0.05	0.05		
Bd16	**53**	0.14	0.16	0.15	0.15	0.15	0.12	0.12	0.12	0.11	0.15	0.15	0.14	0.16	0.15	0.15	0.15	0.02	0.02	0.03	
Bd17	**54**	0.12	0.14	0.14	0.13	0.13	0.11	0.11	0.11	0.11	0.13	0.13	0.13	0.14	0.13	0.13	0.13	0.11	0.11	0.10	0.11
Bd18	**55**	0.13	0.14	0.15	0.15	0.16	0.13	0.13	0.13	0.14	0.15	0.15	0.14	0.17	0.15	0.14	0.14	0.13	0.13	0.13	0.13
Bd19	**56**	0.15	0.17	0.17	0.17	0.17	0.12	0.12	0.12	0.12	0.15	0.15	0.15	0.15	0.14	0.13	0.13	0.16	0.16	0.15	0.16
Bd20	**57**	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.14	0.14	0.14	0.14	0.15	0.16	0.15	0.15
Bd20	**58**	0.16	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.14	0.14	0.14	0.14	0.16	0.16	0.15	0.16
Bd21	**59**	0.19	0.17	0.16	0.16	0.17	0.18	0.18	0.18	0.18	0.17	0.17	0.17	0.18	0.17	0.17	0.17	0.18	0.18	0.17	0.18
Bd22	**60**	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.14	0.14	0.13	0.13	0.15	0.15	0.15	0.15
Bd23	**61**	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.13	0.12	0.13	0.12	0.13	0.12	0.12	0.12	0.14	0.14	0.14	0.14
Bd23	**62**	0.14	0.15	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.13	0.13	0.13	0.15	0.15	0.14	0.15
Bd23	**63**	0.14	0.15	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.15	0.15	0.15	0.15
Bd24	**64**	0.12	0.14	0.15	0.15	0.15	0.13	0.13	0.13	0.13	0.13	0.13	0.12	0.14	0.13	0.13	0.13	0.14	0.14	0.14	0.15
Bd24	**65**	0.12	0.14	0.15	0.15	0.15	0.13	0.13	0.13	0.13	0.13	0.12	0.12	0.14	0.13	0.13	0.13	0.14	0.14	0.15	0.14
Bd25	**66**	0.14	0.15	0.14	0.14	0.14	0.13	0.13	0.13	0.14	0.13	0.13	0.13	0.14	0.14	0.14	0.13	0.14	0.13	0.14	0.15
Bd25	**67**	0.14	0.15	0.14	0.14	0.14	0.13	0.13	0.13	0.14	0.13	0.13	0.13	0.14	0.14	0.14	0.13	0.14	0.13	0.14	0.15
Bd26	**68**	0.14	0.13	0.14	0.14	0.15	0.14	0.14	0.14	0.14	0.14	0.15	0.14	0.15	0.14	0.14	0.14	0.15	0.15	0.16	0.15
Bd27	**69**	0.14	0.16	0.15	0.15	0.15	0.13	0.13	0.13	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.14	0.14	0.16	0.15
Bd27	**70**	0.14	0.16	0.15	0.15	0.15	0.13	0.13	0.13	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.14	0.14	0.16	0.15
Bd27	**71**	0.14	0.16	0.15	0.15	0.15	0.13	0.13	0.13	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.14	0.14	0.16	0.15
Bd27	**72**	0.14	0.16	0.16	0.15	0.16	0.14	0.14	0.14	0.14	0.15	0.14	0.14	0.15	0.14	0.14	0.13	0.14	0.14	0.16	0.15
Bd27	**73**	0.14	0.16	0.16	0.16	0.16	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.16	0.15
Bd27	**74**	0.14	0.16	0.16	0.16	0.16	0.14	0.14	0.14	0.14	0.15	0.15	0.14	0.15	0.14	0.14	0.14	0.14	0.14	0.16	0.15
Bd28	**75**	0.14	0.15	0.14	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.14	0.13	0.14	0.13	0.13	0.15	0.14	0.14	0.15
Bd28	**76**	0.14	0.15	0.14	0.14	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.15	0.14	0.15	0.15
Bd29	**77**	0.13	0.15	0.13	0.14	0.14	0.14	0.14	0.14	0.13	0.15	0.15	0.15	0.16	0.15	0.15	0.14	0.15	0.15	0.16	0.16
Bd30	**78**	0.12	0.13	0.13	0.12	0.12	0.11	0.11	0.11	0.11	0.11	0.11	0.10	0.11	0.11	0.11	0.11	0.13	0.14	0.14	0.14
Bd31	**79**	0.12	0.13	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.13	0.12	0.11	0.11	0.13	0.13	0.13	0.13
Bd31	**80**	0.12	0.13	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.13	0.11	0.11	0.11	0.12	0.13	0.13	0.13
Bd31	**81**	0.12	0.13	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.13	0.12	0.11	0.11	0.13	0.13	0.13	0.13
Bd31	**82**	0.12	0.13	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.13	0.11	0.11	0.11	0.13	0.13	0.13	0.13
Bd32	**83**	0.12	0.14	0.15	0.16	0.16	0.11	0.11	0.11	0.12	0.14	0.14	0.14	0.13	0.12	0.12	0.12	0.15	0.15	0.15	0.15
Bd32	**84**	0.12	0.13	0.15	0.15	0.16	0.11	0.11	0.11	0.11	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.15	0.15	0.15	0.15
Bd33	**85**	0.13	0.15	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.13	0.13	0.13	0.15	0.16	0.16	0.16
Bd33	**86**	0.13	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.14	0.14	0.13	0.14	0.13	0.13	0.13	0.15	0.14	0.16	0.16
Bd33	**87**	0.14	0.16	0.16	0.16	0.15	0.13	0.13	0.13	0.14	0.15	0.14	0.14	0.15	0.14	0.14	0.14	0.16	0.16	0.16	0.16
Bd33	**88**	0.12	0.15	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.14	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.15	0.15	0.16
Bd33	**89**	0.12	0.15	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.14	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.15	0.15	0.16
Bd33	**90**	0.13	0.15	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.14	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.15	0.15	0.16
Bd33	**91**	0.13	0.15	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.14	0.14	0.13	0.14	0.13	0.13	0.13	0.14	0.15	0.16	0.15
Bd33	**92**	0.13	0.15	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.14	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.15	0.15	0.16
Bd34	**93**	0.14	0.16	0.15	0.16	0.16	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.14	0.13	0.13	0.13	0.14	0.14	0.16	0.14
Bd34	**94**	0.14	0.16	0.15	0.16	0.16	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.14	0.13	0.13	0.13	0.14	0.14	0.15	0.14
Bd35	**95**	0.13	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.15	0.14	0.15
Bd36	**96**	0.21	0.21	0.22	0.22	0.22	0.20	0.20	0.20	0.21	0.23	0.23	0.23	0.21	0.22	0.22	0.22	0.22	0.21	0.22	0.21
Bd37	**97**	0.15	0.16	0.16	0.16	0.16	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.16	0.15	0.15	0.15	0.18	0.17	0.18	0.18
Bd38	**98**	0.14	0.16	0.14	0.14	0.14	0.13	0.13	0.13	0.14	0.14	0.13	0.13	0.15	0.14	0.14	0.13	0.14	0.14	0.15	0.14
Bd39	**99**	0.13	0.15	0.14	0.13	0.14	0.14	0.14	0.14	0.15	0.14	0.14	0.14	0.15	0.14	0.13	0.13	0.16	0.15	0.16	0.16
Bd40	**100**	0.14	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.15	0.15	0.14	0.15
Bd40	**101**	0.14	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.15	0.15	0.14	0.15
Bd41	**102**	0.14	0.15	0.15	0.14	0.14	0.13	0.13	0.13	0.13	0.13	0.13	0.12	0.14	0.12	0.12	0.12	0.15	0.15	0.16	0.15
Bd42	**103**	0.16	0.16	0.15	0.15	0.15	0.13	0.13	0.13	0.13	0.14	0.14	0.14	0.13	0.14	0.14	0.14	0.14	0.13	0.14	0.14
Bd42	**104**	0.15	0.16	0.15	0.15	0.15	0.12	0.12	0.12	0.13	0.14	0.14	0.13	0.13	0.14	0.14	0.14	0.14	0.13	0.14	0.14
Bd42	**105**	0.16	0.16	0.15	0.15	0.15	0.13	0.13	0.13	0.13	0.14	0.14	0.14	0.13	0.14	0.14	0.14	0.14	0.13	0.14	0.14
Bd42	**106**	0.15	0.16	0.15	0.15	0.15	0.12	0.12	0.12	0.13	0.14	0.14	0.13	0.13	0.14	0.14	0.14	0.14	0.13	0.14	0.14
Bd42	**107**	0.15	0.16	0.15	0.15	0.15	0.12	0.12	0.12	0.13	0.14	0.13	0.13	0.13	0.14	0.14	0.13	0.14	0.13	0.14	0.14
Bd42	**108**	0.15	0.16	0.15	0.15	0.15	0.13	0.13	0.13	0.13	0.14	0.14	0.13	0.13	0.14	0.14	0.14	0.14	0.13	0.14	0.14
Bd42	**109**	0.15	0.16	0.15	0.15	0.15	0.12	0.12	0.12	0.13	0.14	0.14	0.13	0.13	0.14	0.14	0.14	0.14	0.13	0.14	0.14
Bd43	**110**	0.14	0.15	0.16	0.16	0.16	0.14	0.14	0.14	0.14	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.14	0.13	0.14	0.14
Bd44	**111**	0.14	0.15	0.13	0.13	0.13	0.12	0.12	0.12	0.12	0.13	0.14	0.13	0.14	0.13	0.13	0.12	0.15	0.16	0.16	0.16
Bd45	**112**	0.16	0.16	0.16	0.16	0.17	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.17	0.16	0.16	0.16	0.18	0.17	0.19	0.18
Bd45	**113**	0.16	0.16	0.17	0.17	0.17	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.17	0.16	0.16	0.16	0.18	0.18	0.19	0.18
Bd45	**114**	0.16	0.16	0.16	0.16	0.16	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.17	0.16	0.16	0.16	0.17	0.17	0.18	0.17
Bd45	**115**	0.17	0.17	0.17	0.17	0.17	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.17	0.16	0.16	0.16	0.18	0.18	0.19	0.18
Bd45	**116**	0.17	0.17	0.17	0.17	0.17	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.17	0.17	0.16	0.16	0.18	0.18	0.19	0.18
Bd45	**117**	0.15	0.16	0.16	0.16	0.16	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.16	0.15	0.16	0.15	0.17	0.17	0.17	0.17
Bd46	**118**	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.14	0.13	0.14	0.13	0.13	0.13	0.16	0.15	0.16	0.16
Bd46	**119**	0.13	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.15	0.13	0.14	0.14	0.16	0.15	0.16	0.16
Bd46	**120**	0.13	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.15	0.13	0.14	0.14	0.16	0.15	0.16	0.16
Bd46	**121**	0.13	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.15	0.13	0.14	0.14	0.16	0.15	0.16	0.16
Bd47	**122**	0.14	0.16	0.16	0.17	0.17	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.18	0.17	0.20	0.19
Bd47	**123**	0.14	0.16	0.16	0.17	0.17	0.15	0.15	0.15	0.16	0.15	0.15	0.15	0.16	0.15	0.16	0.15	0.18	0.18	0.20	0.19
Bd47	**124**	0.14	0.16	0.16	0.17	0.17	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.18	0.18	0.20	0.19
Bd47	**125**	0.14	0.16	0.16	0.17	0.17	0.15	0.15	0.15	0.16	0.15	0.15	0.15	0.16	0.15	0.16	0.15	0.18	0.18	0.20	0.19
Bd47	**126**	0.14	0.16	0.16	0.17	0.17	0.15	0.15	0.15	0.16	0.15	0.15	0.15	0.16	0.15	0.15	0.15	0.18	0.18	0.20	0.19
Bd47	**127**	0.14	0.16	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.18	0.18	0.20	0.19
Bd47	**128**	0.14	0.16	0.16	0.17	0.17	0.15	0.15	0.15	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.18	0.18	0.20	0.19
Bd47	**129**	0.15	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.15	0.16	0.15	0.16	0.16	0.16	0.16	0.19	0.18	0.20	0.20
Bd47	**130**	0.15	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.15	0.16	0.15	0.16	0.16	0.16	0.16	0.19	0.18	0.20	0.20
Bd47	**131**	0.14	0.16	0.16	0.17	0.17	0.15	0.15	0.15	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.19	0.18	0.20	0.19
Bd47	**132**	0.14	0.16	0.16	0.17	0.17	0.15	0.15	0.15	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.19	0.18	0.20	0.19
Bd47	**133**	0.15	0.16	0.16	0.17	0.17	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.16	0.15	0.16	0.15	0.19	0.18	0.20	0.20
Bd47	**134**	0.15	0.16	0.16	0.17	0.17	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.16	0.15	0.16	0.15	0.19	0.18	0.20	0.20
Bd47	**135**	0.15	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.19	0.18	0.20	0.20
Outgroup	**136**	0.31	0.30	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.30	0.30	0.31	0.30	0.30	0.30	0.33	0.32	0.32	0.33
Outgroup	**137**	0.33	0.34	0.33	0.33	0.33	0.33	0.33	0.33	0.34	0.34	0.34	0.34	0.34	0.33	0.32	0.32	0.34	0.34	0.35	0.35

Table S2 (continued)

Lineage		Haplotype	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105
Bd18	**55**	MSROT1129_PIA_S53_Bdell04	0.11
Bd19	**56**	MSROT1283_Pint_S42_Bdell02	0.15	0.15
Bd20	**57**	w_MSROT1056_AVC236_Bdell04	0.12	0.13	0.13
Bd20	**58**	w_MSROT1049_AVC162_Bdell01	0.13	0.14	0.13	0.00
Bd21	**59**	Bdel_MSROT034_AVC492_04	0.16	0.17	0.18	0.11	0.11
Bd22	**60**	EF173192-Adineta_sp	0.13	0.13	0.13	0.10	0.10	0.13
Bd23	**61**	Adineta_Cl12a_CS	0.12	0.13	0.12	0.09	0.09	0.12	0.05
Bd23	**62**	Adineta_MSROT023_AVC514_01	0.12	0.14	0.13	0.10	0.10	0.13	0.05	0.02
Bd23	**63**	MSROT586_AVC243_Rot07	0.12	0.14	0.13	0.10	0.10	0.12	0.05	0.02	0.00
Bd24	**64**	Adineta_Cl29a_HI_MP	0.13	0.13	0.16	0.15	0.15	0.19	0.15	0.14	0.16	0.16
Bd24	**65**	Adineta_Sg29b_SP	0.13	0.13	0.16	0.15	0.16	0.19	0.15	0.14	0.15	0.15	0.03
Bd25	**66**	Bdelloidea_Cl30_BP	0.14	0.15	0.17	0.16	0.16	0.19	0.16	0.16	0.16	0.16	0.11	0.13
Bd25	**67**	MSROT253_AVC42_Rot08	0.14	0.15	0.17	0.16	0.16	0.19	0.16	0.16	0.16	0.16	0.11	0.13	0.00
Bd26	**68**	MSROT1137_PIA_C12_Bdell03	0.14	0.15	0.15	0.15	0.16	0.18	0.16	0.15	0.16	0.16	0.14	0.14	0.13	0.13
Bd27	**69**	w_MSROT1086_AVC461_Bdell04	0.14	0.13	0.14	0.16	0.16	0.20	0.17	0.15	0.17	0.16	0.14	0.14	0.14	0.14	0.13
Bd27	**70**	w_MSROT1065_AVC82_Bdell02	0.14	0.13	0.14	0.16	0.16	0.20	0.17	0.15	0.17	0.16	0.14	0.14	0.14	0.14	0.13	0.00
Bd27	**71**	w_MSROT921_AVC66_Bdell06	0.14	0.13	0.14	0.16	0.16	0.20	0.17	0.15	0.17	0.16	0.14	0.14	0.14	0.14	0.13	0.00	0.00
Bd27	**72**	MSROT695_AVC475_Rot08	0.15	0.14	0.15	0.16	0.17	0.20	0.17	0.16	0.17	0.17	0.14	0.14	0.14	0.14	0.14	0.01	0.01	0.01
Bd27	**73**	Bdelloidea_Cl4_EA	0.15	0.14	0.15	0.16	0.17	0.20	0.17	0.16	0.17	0.17	0.15	0.14	0.14	0.14	0.14	0.01	0.01	0.01	0.00
Bd27	**74**	MSROT174_AVC68_Rot09	0.15	0.14	0.15	0.16	0.17	0.20	0.17	0.16	0.17	0.17	0.15	0.14	0.14	0.14	0.14	0.01	0.01	0.01	0.00	0.00
Bd28	**75**	Bdelloidea_Cl10_FM	0.13	0.14	0.15	0.15	0.15	0.18	0.16	0.15	0.14	0.15	0.15	0.15	0.13	0.13	0.14	0.16	0.16	0.16	0.16	0.17	0.16
Bd28	**76**	MSROT641_AVC505_Rot05	0.13	0.14	0.16	0.15	0.16	0.18	0.16	0.15	0.15	0.15	0.15	0.15	0.13	0.13	0.14	0.16	0.16	0.16	0.17	0.17	0.17	0.00
Bd29	**77**	MSROT1326_Pint_C33_Bdell02	0.15	0.15	0.15	0.16	0.16	0.19	0.15	0.15	0.16	0.16	0.12	0.14	0.14	0.14	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.13
Bd30	**78**	MSROT1319_Pint_C31_Bdell01	0.13	0.11	0.13	0.14	0.14	0.17	0.15	0.13	0.14	0.13	0.13	0.12	0.13	0.13	0.13	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.12
Bd31	**79**	MSROT158_AVC173_Rot03	0.10	0.15	0.15	0.14	0.14	0.18	0.14	0.13	0.14	0.14	0.13	0.13	0.13	0.13	0.11	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.11
Bd31	**80**	MSROT713_AVC439_Rot05	0.10	0.14	0.15	0.14	0.14	0.18	0.14	0.13	0.14	0.14	0.13	0.13	0.13	0.13	0.11	0.12	0.12	0.12	0.13	0.13	0.13	0.12	0.13	0.13	0.11	0.00
Bd31	**81**	w_MSROT920_AVC66_Bdell05	0.10	0.14	0.14	0.14	0.14	0.18	0.14	0.13	0.14	0.14	0.13	0.13	0.13	0.13	0.11	0.12	0.12	0.12	0.13	0.13	0.13	0.13	0.13	0.13	0.11	0.00	0.00
Bd31	**82**	Bdelloidea_Cl1_EA	0.10	0.14	0.15	0.14	0.14	0.17	0.14	0.13	0.14	0.14	0.13	0.13	0.13	0.13	0.11	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.11	0.00	0.00	0.00
Bd32	**83**	MSROT1257_Pint_S13_Bdell02	0.13	0.15	0.13	0.15	0.15	0.18	0.15	0.13	0.15	0.14	0.14	0.15	0.14	0.14	0.13	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.15	0.14	0.11	0.06	0.06	0.06	0.06
Bd32	**84**	MSROT1302_Pint_C21_Bdell01	0.13	0.15	0.13	0.14	0.15	0.17	0.15	0.13	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.14	0.14	0.14	0.15	0.15	0.15	0.14	0.14	0.14	0.11	0.06	0.06	0.05	0.06	0.01
Bd33	**85**	MSROT343_AVC210_Rot05	0.13	0.16	0.15	0.15	0.16	0.19	0.14	0.14	0.14	0.14	0.15	0.15	0.17	0.17	0.15	0.15	0.15	0.15	0.15	0.16	0.15	0.15	0.15	0.14	0.13	0.11	0.11	0.11	0.11	0.13	0.13
Bd33	**86**	Bdelloidea_Cl2a_SP	0.13	0.16	0.15	0.15	0.15	0.19	0.14	0.14	0.13	0.13	0.14	0.14	0.16	0.16	0.15	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.13	0.13	0.13	0.12	0.13	0.13	0.02
Bd33	**87**	Bdelloidea_Cl2b_CS	0.13	0.17	0.15	0.15	0.15	0.18	0.12	0.11	0.11	0.11	0.16	0.15	0.18	0.18	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.14	0.13	0.14	0.14	0.13	0.13	0.03	0.03
Bd33	**88**	MSROT347_AVC157_Rot02	0.13	0.16	0.15	0.14	0.15	0.19	0.14	0.13	0.13	0.13	0.14	0.14	0.17	0.17	0.15	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.15	0.14	0.13	0.12	0.12	0.12	0.12	0.13	0.13	0.02	0.01	0.03
Bd33	**89**	Bdelloidea_Cl2c_EA	0.13	0.16	0.15	0.14	0.15	0.19	0.14	0.13	0.13	0.13	0.14	0.14	0.17	0.17	0.15	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.15	0.14	0.13	0.12	0.12	0.12	0.12	0.13	0.13	0.02	0.01	0.03	0.00
Bd33	**90**	Bdelloidea_Cl2_EA	0.13	0.16	0.15	0.15	0.15	0.19	0.14	0.14	0.14	0.13	0.15	0.14	0.17	0.17	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.13	0.12	0.12	0.12	0.12	0.13	0.13	0.02	0.02	0.03	0.01	0.01
Bd33	**91**	MSROT080_AVC477_Rot07	0.13	0.17	0.15	0.14	0.15	0.19	0.14	0.14	0.14	0.13	0.15	0.14	0.17	0.17	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.15	0.13	0.13	0.12	0.13	0.13	0.13	0.13	0.02	0.02	0.03	0.00	0.00	0.01
Bd33	**92**	MSROT658_AVC1_Rot06	0.13	0.16	0.15	0.14	0.15	0.19	0.14	0.13	0.13	0.13	0.14	0.14	0.17	0.17	0.15	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.15	0.14	0.13	0.12	0.12	0.12	0.12	0.13	0.13	0.02	0.01	0.03	0.00	0.00	0.00	0.00
Bd34	**93**	MSROT1322_Pint_C32_Bdell01	0.14	0.15	0.15	0.15	0.15	0.18	0.15	0.14	0.15	0.15	0.15	0.16	0.17	0.17	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.15	0.16	0.14	0.12	0.14	0.13	0.13	0.14	0.14	0.13	0.14	0.14	0.15	0.14	0.14	0.14	0.14	0.14
Bd34	**94**	MSROT1320_Pint_C31_Bdell02	0.14	0.15	0.15	0.15	0.15	0.18	0.15	0.14	0.15	0.15	0.15	0.16	0.17	0.17	0.15	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.16	0.14	0.12	0.14	0.13	0.13	0.14	0.14	0.13	0.14	0.14	0.15	0.14	0.14	0.14	0.14	0.14	0.00
Bd35	**95**	Bdel_MSROT913_Cdh25_10_DML	0.14	0.17	0.17	0.16	0.16	0.18	0.16	0.16	0.16	0.16	0.15	0.14	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.14	0.14	0.13	0.13	0.14	0.15	0.15	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.14	0.14
Bd36	**96**	MSROT1329_Pint_C41_Bdell03	0.20	0.22	0.22	0.24	0.25	0.27	0.23	0.23	0.23	0.23	0.21	0.21	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.22	0.22	0.24	0.22	0.23	0.23	0.23	0.23	0.21	0.21	0.21	0.20	0.21	0.21	0.21	0.21	0.21	0.21	0.25	0.25	0.22
Bd37	**97**	Bdelloidea_CL27_FM	0.17	0.17	0.16	0.18	0.18	0.21	0.19	0.17	0.18	0.18	0.17	0.17	0.18	0.18	0.18	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.17	0.15	0.17	0.16	0.16	0.16	0.16	0.15	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.15	0.15	0.17	0.21
Bd38	**98**	MSROT1269_Pint_S31_Bdell03	0.14	0.13	0.15	0.15	0.15	0.20	0.14	0.14	0.14	0.15	0.14	0.15	0.14	0.14	0.15	0.14	0.14	0.14	0.15	0.15	0.15	0.13	0.14	0.14	0.12	0.14	0.14	0.13	0.13	0.13	0.13	0.14	0.14	0.15	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.14	0.22	0.17
Bd39	**99**	MSROT918_AP025_Rot1	0.14	0.13	0.16	0.16	0.16	0.19	0.16	0.15	0.16	0.16	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.15	0.15	0.13	0.13	0.12	0.12	0.12	0.13	0.13	0.14	0.14	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.15	0.22	0.16	0.11
Bd40	**100**	MSROT385_AVC179_Rot05	0.14	0.14	0.13	0.16	0.17	0.20	0.15	0.15	0.16	0.16	0.15	0.14	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.14	0.14	0.16	0.15	0.14	0.14	0.14	0.13	0.14	0.14	0.15	0.15	0.16	0.15	0.15	0.16	0.15	0.15	0.16	0.16	0.14	0.23	0.15	0.13	0.13
Bd40	**101**	Bdelloidea_Cl28_SP	0.14	0.14	0.13	0.16	0.17	0.20	0.15	0.15	0.16	0.16	0.15	0.14	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.14	0.14	0.16	0.15	0.14	0.14	0.14	0.13	0.14	0.14	0.15	0.15	0.16	0.15	0.15	0.16	0.15	0.15	0.16	0.16	0.14	0.23	0.15	0.13	0.13	0.00
Bd41	**102**	MSROT1307_Pint_C22_Bdell01	0.14	0.15	0.15	0.15	0.16	0.18	0.16	0.15	0.16	0.16	0.14	0.14	0.15	0.15	0.14	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.13	0.11	0.13	0.12	0.12	0.12	0.13	0.13	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.13	0.13	0.15	0.23	0.17	0.12	0.14	0.16	0.16
Bd42	**103**	MSROT057_AVC268_Rot09	0.14	0.17	0.17	0.15	0.15	0.18	0.16	0.16	0.16	0.16	0.16	0.17	0.14	0.14	0.17	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.15	0.14	0.14	0.14	0.14	0.15	0.14	0.15	0.14	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.22	0.17	0.15	0.16	0.14	0.14	0.16
Bd42	**104**	MSROT147_MBS019_Rot11	0.14	0.17	0.17	0.14	0.15	0.18	0.17	0.16	0.16	0.16	0.16	0.16	0.14	0.14	0.17	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.15	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.16	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.22	0.17	0.15	0.16	0.14	0.14	0.16	0.00
Bd42	**105**	Bdelloidea_Cl5_CS_LH_HI_VH	0.14	0.17	0.17	0.15	0.15	0.18	0.17	0.16	0.16	0.16	0.16	0.17	0.14	0.14	0.17	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.15	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.16	0.15	0.15	0.15	0.15	0.15	0.16	0.15	0.16	0.22	0.17	0.15	0.16	0.14	0.14	0.16	0.01	0.01
Bd42	**106**	MSROT139_MBS019_Rot03	0.14	0.17	0.16	0.14	0.15	0.18	0.17	0.16	0.16	0.16	0.16	0.16	0.14	0.14	0.17	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.15	0.14	0.14	0.14	0.14	0.15	0.14	0.15	0.15	0.16	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.22	0.17	0.15	0.16	0.14	0.14	0.16	0.01	0.00	0.01
Bd42	**107**	MSROT354_AVC304_Rot03	0.14	0.17	0.16	0.14	0.15	0.18	0.16	0.16	0.16	0.16	0.15	0.16	0.14	0.14	0.17	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.16	0.15	0.14	0.14	0.14	0.13	0.15	0.14	0.15	0.14	0.16	0.15	0.15	0.15	0.15	0.15	0.16	0.15	0.16	0.22	0.17	0.15	0.16	0.14	0.14	0.16	0.01	0.00	0.01
Bd42	**108**	MSROT073_AVC283_Rot05	0.14	0.17	0.16	0.14	0.15	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.14	0.14	0.17	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.15	0.14	0.14	0.14	0.13	0.15	0.14	0.15	0.14	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.22	0.17	0.15	0.16	0.14	0.14	0.16	0.00	0.01	0.01
Bd42	**109**	MSROT368_AVC98_Rot06	0.14	0.17	0.16	0.14	0.15	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.14	0.14	0.17	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.15	0.14	0.14	0.14	0.14	0.15	0.14	0.15	0.14	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.22	0.17	0.15	0.16	0.14	0.14	0.16	0.00	0.00	0.01
Bd43	**110**	Bdelloidea_Cl9_AP	0.13	0.14	0.16	0.16	0.16	0.17	0.14	0.13	0.14	0.14	0.16	0.15	0.16	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.13	0.14	0.14	0.13	0.14	0.13	0.13	0.13	0.14	0.13	0.16	0.15	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.14	0.15	0.22	0.16	0.14	0.15	0.15	0.15	0.16	0.15	0.15	0.15
Bd44	**111**	Bdelloidea_Sg3_FM	0.13	0.14	0.13	0.14	0.15	0.18	0.16	0.14	0.15	0.15	0.14	0.14	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.12	0.12	0.12	0.12	0.12	0.13	0.13	0.15	0.15	0.16	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.23	0.17	0.15	0.15	0.17	0.17	0.13	0.15	0.14	0.15
Bd45	**112**	w_MSROT1096_AVC222_Bdell01	0.17	0.18	0.19	0.17	0.17	0.18	0.17	0.16	0.17	0.17	0.16	0.17	0.16	0.16	0.16	0.17	0.17	0.17	0.18	0.18	0.18	0.18	0.18	0.17	0.14	0.16	0.15	0.15	0.16	0.15	0.15	0.18	0.17	0.18	0.18	0.18	0.18	0.18	0.18	0.16	0.16	0.18	0.25	0.20	0.15	0.17	0.19	0.19	0.15	0.16	0.16	0.16
Bd45	**113**	w_MSROT947_AVC168_Bdell01	0.17	0.18	0.19	0.17	0.17	0.18	0.17	0.16	0.17	0.17	0.16	0.17	0.16	0.16	0.16	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.15	0.16	0.16	0.15	0.16	0.15	0.15	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.16	0.16	0.18	0.25	0.20	0.16	0.17	0.20	0.20	0.15	0.17	0.17	0.17
Bd45	**114**	w_MSROT1093_AVC95_Bdell02	0.17	0.18	0.18	0.17	0.17	0.18	0.17	0.16	0.17	0.17	0.16	0.17	0.16	0.16	0.17	0.17	0.17	0.17	0.18	0.18	0.18	0.18	0.18	0.17	0.14	0.16	0.15	0.15	0.16	0.15	0.15	0.18	0.18	0.19	0.18	0.18	0.18	0.18	0.18	0.17	0.17	0.18	0.25	0.20	0.16	0.16	0.19	0.19	0.15	0.17	0.17	0.17
Bd45	**115**	Bdelloidea_Cl7b_SP	0.17	0.18	0.19	0.18	0.18	0.19	0.18	0.16	0.17	0.17	0.17	0.17	0.16	0.16	0.17	0.18	0.18	0.18	0.19	0.19	0.19	0.18	0.18	0.18	0.15	0.16	0.16	0.16	0.16	0.15	0.15	0.18	0.18	0.19	0.18	0.18	0.18	0.18	0.18	0.16	0.16	0.19	0.26	0.20	0.16	0.17	0.20	0.20	0.16	0.17	0.17	0.17
Bd45	**116**	MSROT619_AVC256_Rot02	0.17	0.18	0.19	0.18	0.18	0.18	0.18	0.16	0.17	0.17	0.17	0.17	0.16	0.16	0.17	0.18	0.18	0.18	0.19	0.19	0.19	0.18	0.18	0.18	0.15	0.16	0.16	0.16	0.16	0.16	0.15	0.18	0.18	0.19	0.18	0.18	0.18	0.18	0.18	0.16	0.16	0.19	0.26	0.20	0.16	0.17	0.20	0.20	0.16	0.17	0.17	0.17
Bd45	**117**	Bdelloidea_Sg7a_CS	0.16	0.17	0.18	0.17	0.17	0.18	0.17	0.15	0.16	0.16	0.16	0.16	0.15	0.15	0.16	0.17	0.17	0.17	0.18	0.18	0.18	0.17	0.17	0.17	0.15	0.15	0.15	0.15	0.15	0.16	0.15	0.18	0.18	0.19	0.18	0.18	0.18	0.18	0.18	0.16	0.16	0.18	0.25	0.19	0.16	0.16	0.19	0.19	0.16	0.16	0.16	0.16
Bd46	**118**	w_MSROT1112_AVC127_Bdell03	0.13	0.14	0.16	0.15	0.15	0.17	0.16	0.13	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.15	0.14	0.15	0.15	0.14	0.12	0.13	0.13	0.13	0.13	0.13	0.13	0.15	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.16	0.23	0.19	0.16	0.15	0.16	0.16	0.13	0.15	0.14	0.15
Bd46	**119**	Philodina_Cl6_CS_VH_BP_SP_MS	0.14	0.15	0.17	0.15	0.16	0.17	0.16	0.14	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.16	0.16	0.15	0.13	0.13	0.13	0.13	0.13	0.14	0.13	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.16	0.23	0.18	0.16	0.15	0.16	0.16	0.13	0.15	0.14	0.15
Bd46	**120**	MSROT459_AVC22_Rot03	0.14	0.15	0.17	0.15	0.16	0.17	0.16	0.14	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.16	0.16	0.15	0.13	0.13	0.13	0.13	0.13	0.14	0.13	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.16	0.23	0.18	0.16	0.15	0.16	0.16	0.13	0.15	0.14	0.15
Bd46	**121**	MSROT625_AVC256_Rot08	0.14	0.15	0.17	0.15	0.16	0.17	0.16	0.14	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.16	0.16	0.15	0.13	0.13	0.13	0.13	0.13	0.14	0.13	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.16	0.23	0.18	0.16	0.15	0.16	0.16	0.13	0.15	0.14	0.15
Bd47	**122**	Philodina_Cl8b_VH_SI_SP_BP_MS	0.16	0.18	0.17	0.16	0.16	0.20	0.17	0.16	0.16	0.16	0.17	0.17	0.15	0.15	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.17	0.15	0.15	0.15	0.15	0.14	0.13	0.13	0.16	0.15	0.16	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.18	0.24	0.18	0.18	0.17	0.19	0.19	0.16	0.17	0.17	0.17
Bd47	**123**	w_MSROT1104_AVC110_Bdell01	0.17	0.18	0.16	0.16	0.16	0.20	0.16	0.16	0.17	0.17	0.18	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.18	0.18	0.17	0.15	0.15	0.15	0.15	0.15	0.13	0.13	0.16	0.15	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.18	0.24	0.19	0.18	0.17	0.19	0.19	0.16	0.17	0.18	0.17
Bd47	**124**	w_MSROT1078_MBS014_Bdell01	0.17	0.18	0.17	0.16	0.16	0.20	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15	0.13	0.13	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.19	0.24	0.19	0.18	0.18	0.19	0.19	0.16	0.18	0.18	0.18
Bd47	**125**	w_MSROT1079_MBS014_Bdell02	0.17	0.18	0.16	0.16	0.16	0.20	0.16	0.16	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15	0.13	0.13	0.16	0.15	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.18	0.24	0.19	0.18	0.17	0.19	0.19	0.16	0.17	0.18	0.17
Bd47	**126**	MSROT553_MBS011_Rot03	0.16	0.18	0.17	0.16	0.16	0.20	0.17	0.16	0.17	0.17	0.17	0.17	0.15	0.15	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.17	0.18	0.18	0.15	0.15	0.15	0.15	0.15	0.13	0.14	0.16	0.15	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.19	0.24	0.19	0.18	0.18	0.19	0.19	0.16	0.17	0.18	0.17
Bd47	**127**	MSROT554_MBS011_Rot04	0.17	0.18	0.17	0.17	0.17	0.20	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.18	0.17	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.18	0.18	0.19	0.24	0.19	0.18	0.18	0.19	0.19	0.16	0.18	0.18	0.18
Bd47	**128**	MSROT501_MBS021_Rot01	0.17	0.18	0.17	0.17	0.17	0.20	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.17	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.16	0.15	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.19	0.24	0.19	0.18	0.18	0.19	0.19	0.16	0.17	0.17	0.17
Bd47	**129**	MSROT011_AVC495_Rot03	0.17	0.18	0.17	0.17	0.17	0.19	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.18	0.17	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15	0.13	0.14	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.18	0.18	0.19	0.24	0.19	0.18	0.18	0.20	0.20	0.16	0.18	0.18	0.18
Bd47	**130**	Philodina_Cl8a_MS_BP	0.17	0.18	0.17	0.17	0.17	0.19	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.18	0.17	0.18	0.18	0.18	0.15	0.15	0.15	0.15	0.15	0.13	0.14	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.18	0.18	0.19	0.24	0.19	0.18	0.18	0.20	0.20	0.16	0.18	0.18	0.18
Bd47	**131**	Philodina_RS_13_Wood	0.17	0.18	0.17	0.16	0.17	0.20	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.18	0.18	0.17	0.14	0.15	0.15	0.15	0.15	0.13	0.13	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.18	0.24	0.19	0.18	0.17	0.19	0.19	0.16	0.18	0.18	0.18
Bd47	**132**	Philodina_RS_5_HOC	0.17	0.18	0.16	0.17	0.17	0.20	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.17	0.18	0.18	0.17	0.14	0.15	0.15	0.15	0.15	0.13	0.13	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.18	0.24	0.19	0.18	0.17	0.19	0.19	0.16	0.18	0.18	0.18
Bd47	**133**	Philodina_RS_6_Mero	0.17	0.18	0.17	0.17	0.17	0.20	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.17	0.18	0.18	0.17	0.14	0.15	0.15	0.15	0.15	0.13	0.13	0.16	0.16	0.17	0.16	0.16	0.16	0.17	0.16	0.17	0.17	0.19	0.24	0.19	0.18	0.18	0.20	0.20	0.16	0.18	0.18	0.18
Bd47	**134**	Philodina_RS_2_Fog	0.17	0.18	0.17	0.17	0.17	0.20	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.17	0.18	0.18	0.17	0.14	0.15	0.15	0.15	0.15	0.13	0.13	0.16	0.16	0.17	0.16	0.16	0.16	0.17	0.16	0.17	0.17	0.19	0.24	0.19	0.18	0.18	0.20	0.20	0.16	0.18	0.18	0.18
Bd47	**135**	Philodina_RS_4_HOC	0.17	0.18	0.17	0.16	0.17	0.20	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.18	0.17	0.18	0.18	0.17	0.14	0.16	0.15	0.15	0.15	0.14	0.14	0.16	0.16	0.18	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.19	0.24	0.19	0.18	0.18	0.20	0.20	0.16	0.18	0.18	0.18
Outgroup	**136**	Outgroup_Monogononta_JN936618	0.31	0.32	0.33	0.33	0.33	0.34	0.31	0.30	0.31	0.31	0.32	0.32	0.32	0.32	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.32	0.32	0.31	0.30	0.31	0.30	0.30	0.30	0.30	0.31	0.31	0.31	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.35	0.32	0.32	0.32	0.32	0.32	0.28	0.33	0.33	0.33
Outgroup	**137**	Outgroup_Monogononta_JX216519	0.33	0.35	0.33	0.34	0.34	0.35	0.34	0.34	0.34	0.34	0.34	0.35	0.33	0.33	0.33	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.34	0.33	0.33	0.34	0.34	0.33	0.34	0.34	0.34	0.34	0.34	0.33	0.34	0.34	0.34	0.33	0.35	0.35	0.35

Lineage		Haplotype	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136
Bd42	**107**	MSROT354_AVC304_Rot03	0.00
Bd42	**108**	MSROT073_AVC283_Rot05	0.01	0.00
Bd42	**109**	MSROT368_AVC98_Rot06	0.00	0.00	0.00
Bd43	**110**	Bdelloidea_Cl9_AP	0.15	0.15	0.15	0.14
Bd44	**111**	Bdelloidea_Sg3_FM	0.14	0.14	0.14	0.14	0.15
Bd45	**112**	w_MSROT1096_AVC222_Bdell01	0.16	0.16	0.16	0.16	0.16	0.16
Bd45	**113**	w_MSROT947_AVC168_Bdell01	0.17	0.16	0.16	0.16	0.16	0.16	0.00
Bd45	**114**	w_MSROT1093_AVC95_Bdell02	0.17	0.16	0.16	0.16	0.16	0.16	0.00	0.00
Bd45	**115**	Bdelloidea_Cl7b_SP	0.17	0.17	0.17	0.17	0.17	0.16	0.02	0.02	0.02
Bd45	**116**	MSROT619_AVC256_Rot02	0.17	0.17	0.17	0.16	0.17	0.16	0.01	0.01	0.01	0.01
Bd45	**117**	Bdelloidea_Sg7a_CS	0.16	0.16	0.16	0.16	0.16	0.16	0.03	0.03	0.03	0.03	0.03
Bd46	**118**	w_MSROT1112_AVC127_Bdell03	0.14	0.14	0.14	0.14	0.15	0.14	0.14	0.14	0.15	0.15	0.15	0.14
Bd46	**119**	Philodina_Cl6_CS_VH_BP_SP_MS	0.14	0.14	0.14	0.14	0.15	0.14	0.15	0.15	0.15	0.15	0.16	0.14	0.01
Bd46	**120**	MSROT459_AVC22_Rot03	0.14	0.14	0.14	0.14	0.15	0.14	0.15	0.15	0.15	0.15	0.16	0.14	0.01	0.00
Bd46	**121**	MSROT625_AVC256_Rot08	0.14	0.14	0.14	0.14	0.15	0.14	0.15	0.15	0.15	0.15	0.16	0.14	0.01	0.00	0.00
Bd47	**122**	Philodina_Cl8b_VH_SI_SP_BP_MS	0.17	0.17	0.17	0.17	0.18	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.13	0.14	0.14	0.14
Bd47	**123**	w_MSROT1104_AVC110_Bdell01	0.17	0.18	0.17	0.17	0.18	0.16	0.16	0.16	0.16	0.16	0.17	0.16	0.13	0.14	0.14	0.14	0.00
Bd47	**124**	w_MSROT1078_MBS014_Bdell01	0.18	0.18	0.17	0.18	0.18	0.16	0.16	0.16	0.16	0.16	0.17	0.16	0.14	0.14	0.14	0.14	0.01	0.00
Bd47	**125**	w_MSROT1079_MBS014_Bdell02	0.17	0.18	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.16	0.13	0.14	0.14	0.14	0.01	0.00	0.00
Bd47	**126**	MSROT553_MBS011_Rot03	0.17	0.17	0.17	0.17	0.18	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.14	0.14	0.14	0.14	0.01	0.01	0.00	0.01
Bd47	**127**	MSROT554_MBS011_Rot04	0.18	0.18	0.17	0.18	0.18	0.16	0.16	0.17	0.16	0.16	0.17	0.16	0.14	0.14	0.14	0.14	0.01	0.01	0.00	0.01	0.00
Bd47	**128**	MSROT501_MBS021_Rot01	0.17	0.17	0.17	0.17	0.18	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.14	0.15	0.15	0.15	0.01	0.01	0.01	0.01	0.01	0.01
Bd47	**129**	MSROT011_AVC495_Rot03	0.18	0.18	0.17	0.18	0.18	0.16	0.17	0.17	0.17	0.16	0.17	0.16	0.14	0.14	0.14	0.14	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Bd47	**130**	Philodina_Cl8a_MS_BP	0.18	0.18	0.17	0.18	0.18	0.16	0.17	0.17	0.17	0.16	0.17	0.16	0.14	0.14	0.14	0.14	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00
Bd47	**131**	Philodina_RS_13_Wood	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.17	0.16	0.17	0.17	0.16	0.13	0.14	0.14	0.14	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01
Bd47	**132**	Philodina_RS_5_HOC	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16	0.16	0.17	0.17	0.16	0.13	0.14	0.14	0.14	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.01	0.00
Bd47	**133**	Philodina_RS_6_Mero	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16	0.16	0.17	0.17	0.16	0.13	0.14	0.14	0.14	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.01	0.01	0.00	0.00
Bd47	**134**	Philodina_RS_2_Fog	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16	0.16	0.17	0.17	0.16	0.13	0.14	0.14	0.14	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.00	0.00	0.00
Bd47	**135**	Philodina_RS_4_HOC	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.17	0.16	0.17	0.17	0.17	0.14	0.14	0.14	0.14	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.00	0.00
Outgroup	**136**	Outgroup_Monogononta_JN936618	0.33	0.33	0.33	0.33	0.32	0.32	0.32	0.32	0.33	0.32	0.32	0.32	0.31	0.31	0.31	0.31	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Outgroup	**137**	Outgroup_Monogononta_JX216519	0.35	0.35	0.35	0.35	0.35	0.34	0.34	0.34	0.35	0.35	0.34	0.35	0.32	0.31	0.31	0.31	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.21

Table S3. Tardigrade accessions numbers from COI sequences retrieved and uploaded in GenBank

Lineage	Taxa	accession numbers
Ta1	*Diphascon*	**KJ856915**
Ta2	*Diphascon*	EF632530
Ta3	*Diphascon*	**KJ856916 - KJ856920**
Ta4	*Diphascon*	EF632531 EF632533
Ta5	*Diphascon*	EF632532 EF632534 - EF632536
Ta6	*Diphascon*	**KJ856921 KJ856922**
Ta7	*Diphascon*	**KJ856923 - KJ856925**
Ta8	Parachela	**KJ856926 KJ856927**
Ta9	Parachela	**KJ856928**
Ta10	*Diphascon*	**KJ856929**
Ta11	Parachela	**KJ856930 - KJ856932**
Ta12	Parachela	**KJ856933**
Ta13	*D. puneceum*	**KJ856934 - KJ856938**
Ta14	Parachela	**KJ856939**
Ta15	Parachela	**KJ856940 - KJ856942**
Ta16	Parachela	**KJ856943**
Ta17	*Ramazzottius*	EU251379 EU251380
Ta18	*Ramazzottius*	FJ435799
Ta19	Parachela	**KJ856944**
Ta20	*Murrayon?*	FJ435801
Ta21	Parachela	**KJ856945**
Ta22	Parachela	**KJ856946 - KJ856948**
Ta23	Parachela	**KJ856949**
Ta24	Parachela	**KJ856950**
Ta25	*Hypsibius*	EF632518 EF632519
Ta26	*Borealibius*	FJ184601 FJ184602
Ta27	Parachela	**KJ856951 KJ856952**
Ta28	*A. antarcticus*	**KJ856953 - KJ856976** JX486021 JX486022 JX486023 JX486024 JX486025 JX865305
Ta29	*A. antarcticus*	**KJ856977 - KJ856979** JX296185 JX296188 - JX296190 JX296198 JX296205 JX296213 JX296215
Ta30	*Ma. furciger*	JX865308
Ta31	*Macrobiotus*	JX865306
Ta32	Parachela	**KJ856980**
Ta33	*Macrobiotus*	**KJ856981**
Ta34	*Macrobiotus*	JX865314
Ta35	*Macrobiotus*	**KJ856982 KJ856983**
Ta36	*Macrobiotus*	**KJ856984 - KJ856986**
Ta37	*Macrobiotus*	**KJ856987**
Ta38	*Macrobiotus*	**KJ856988**
Ta39	*Macrobiotus*	**KJ856989**
Ta40	*Macrobiotus*	**KJ856990 - KJ856991**
Ta41	*Macrobiotus*	JX865310
Ta42	*Macrobiotus*	**KJ856992 - KJ856994** JX296219 JX296220 JX296222 - JX296225
Ta43	*Macrobiotus*	JX296253 JX296254 - JX296256 JX296358 JX296260 JX296261 JX296359 JX296361 JX888911
		KJ856995 - KJ856998 JX296226 - JX296229 JX296231 - JX296233 JX296235 JX296237 JX296238
Ta44	*Macrobiotus*	JX296240 - JX296243 JX296246 JX296247 JX296249 - JX296251 JX296278 JX296282 JX296295
		JX296306 JX296312 JX296313
Ta45	*Milnesium*	**KJ856999 KJ857000**
Ta46	*Milnesium*	**KJ857001**
Ta47	*Milnesium*	**KJ857002**
Ta48	*Mi.tardigradum*	EU244603 EU244604
Ta49	*Mi.tardigradum*	JN664950
Ta50	*Mi.tardigradum*	EF632553
Ta51	*Milnesium*	**KJ857003**
		JF437735 - JF437737 JF437738 - JF437742 JF437744 JF437747 JF437749 - JF437752 JF437755
Ta52	Echiniscoides	JF437757 JF437764 JF437765 JF437766 JF437771 JF437772 JF437775 JF437777 JF437784
		JF437779 - JF437782
Ta53	*Echiniscus*	**KJ857004 - KJ857007**
Ta54	*Echiniscus*	**KJ857008**
		EU046090 EU046091 EU046093 - EU046100 EU046102 - EU046107 EU046109 - EU046128 EU046130
Ta55	*Echiniscus*	EU046131 EU046133 - EU046140 EU046145 - EU046160 EU046162 - EU046165 EU046172 EU046175
		EU046176 FJ435815 EU046178 - EU046180 EU046183 - EU046196
Ta56	*E. merokensis*	EF632541 EF632539
Ta57	*E. testudo*	EF632542 EF632543 EF620377 EF620378 EU244601 EF630370 - EF630372 EF630374
Ta58	*Echiniscus*	**KJ857009**
Ta59	*E. jenningsi*	**KJ857010**
Ta60	*Echiniscus*	**KJ857011**
Ta61	*Echiniscus*	**KJ857012**
Ta62	*Echiniscus*	**KJ857013**
Ta63	*Echiniscus*	**KJ857014**

Accession numbers underlined refer to sequences gathered from GenBank. Accession numbers in bold correspond to haplotypes generated for this study and uploaded in GenBank

Table S4. Bdelloid accessions numbers from COI sequences generated during the study and uploaded in GenBank

Lineage	Taxa	accession numbers
Bd1	*Adineta*	KJ543570 - KJ543580
Bd2	*Adineta*	KJ543581 - KJ543588
Bd3	Bdelloidea	KJ543589
Bd4	*Adineta*	KJ543590 - KJ543591
Bd5	Bdelloidea	KJ543592
Bd6	Bdelloidea	KJ543593
Bd7	Bdelloidea	KJ543594 - KJ543597
Bd8	*Adineta gracilis*	KJ543598 - KJ543600
Bd9	Bdelloidea	KJ543601
Bd10	Bdelloidea	KJ543602 - KJ543604
Bd11	Bdelloidea	KJ543605 - KJ543608
Bd12	Bdelloidea	KJ543609 - KJ543611
Bd13	Bdelloidea	KJ543612
Bd14	Bdelloidea	KJ543613 - KJ543615
Bd15	Bdelloidea	KJ543616 - KJ543617
Bd16	Bdelloidea	KJ543618 - KJ543619
Bd17	Bdelloidea	KJ543620
Bd18	Bdelloidea	KJ543621
Bd19	Bdelloidea	KJ543622
Bd20	Bdelloidea	KJ543623 - KJ543624
Bd21	Bdelloidea	KJ543625
Bd23	*Adineta*	KJ543626 - KJ543628
Bd24	*Adineta*	KJ543629 - KJ543630
Bd25	Bdelloidea	KJ543631 - KJ543632
Bd26	Bdelloidea	KJ543633
Bd27	Bdelloidea	KJ543634 - KJ543639
Bd28	Bdelloidea	KJ543640 - KJ543641
Bd29	Bdelloidea	KJ543642
Bd30	Bdelloidea	KJ543643
Bd31	Bdelloidea	KJ543644 - KJ543647
Bd32	Bdelloidea	KJ543648 - KJ543649
Bd33	Bdelloidea	KJ543650 - KJ543657
Bd34	Bdelloidea	KJ543658 - KJ543659
Bd35	Bdelloidea	KJ543660
Bd36	Bdelloidea	KJ543661
Bd37	Bdelloidea	KJ543662
Bd38	Bdelloidea	KJ543663
Bd39	Bdelloidea	KJ543664
Bd40	Bdelloidea	KJ543665 - KJ543666
Bd41	Bdelloidea	KJ543667
Bd42	Bdelloidea	KJ543668 - KJ543674
Bd43	Bdelloidea	KJ543675
Bd44	Bdelloidea	KJ543676
Bd45	Bdelloidea	KJ543677 - KJ543682
Bd46	*Philodina* sp.	KJ543683 - KJ543686
Bd47	*P.* cf. *gregaria*	KJ543687 - KJ543700

Table S5. Percentage sequence identity among lineages and bdelloid genera for the COI gene. Lineages mentioned in the table were used in the main manuscript and in Fig. 3.

Lineage	Bradyscela	Philodina	Adineta	Habrotrocha	Pleuretra	Macrotrachela	Rotaria	Abrochtha
Bd1	-	86	88	-	86	87	86	-
Bd2	86	87	88	87	86	88	86	88
Bd3		84	86	84	84	85	84	84
Bd4	85	85	87	85	87	86	86	-
Bd5	-	89	89	88	88	89	88	-
Bd6	-	88	89	88	88	89	87	-
Bd7	-	86	88	85	86	85	85	-
Bd8	87	87	88 (**99***)	88	88	87	87	88
Bd9	-	88	89	88	88	88	89	-
Bd10	88	90	89	88	90	89	89	88
Bd11	89	89	91	92	89	91	90	91
Bd12	87	89	90	89	87	89	88	91
Bd13	86	88	89	89	-	88	88	88
Bd14	88	90	90	90	88	90	89	90
Bd15	87	89	88	88	88	87	87	-
Bd16	-	88	88	88	86	88	88	-
Bd17	87	88	89	87	-	89	87	87
Bd18	87	88	88	87	87	88	87	-
Bd19	-	87	88	87	87	88	89	-
Bd20	-	88	90	87	87	87	87	87
Bd21	-	85	87	83	83	83	83	-
Bd22	-	85	87	86	87	86	85	-
Bd23	-	88	88 (**95‡**)	88	88	88	89	88
Bd24	86	89	89	89	88	88	87	-
Bd25	-	89	88	87	88	87	87	89
Bd26	-	88	89	88	87	87	88	89
Bd27	-	87	88	87	87	83	88	-
Bd28	87	88	89	88	88	88	87	-
Bd29	-	89	88	87	-	89	88	-
Bd30	88	91	90	90	89	89	89	-
Bd31	-	89	89	89	88	88	88	91
Bd32	-	90	89	-	88	90	89	89
Bd33	86	89	88	88	88	88	87	87
Bd34	-	-	88	88	87	89	87	-
Bd35	-	88	88	85	85	87	86	-
Bd36	-	79	79	79	80	80	78	-
Bd37	-	86	86	86	86	85	84	-
Bd38	-	89	88	87	-	89	87	-
Bd39	-	88	88	88	89	87	87	-
Bd40	-	87	87	87	86	88	86	-
Bd41	-	90	90	87	-	90	87	89
Bd42	-	88	87	88	87	87	87	-
Bd43	-	87	88	86	87	88	87	-
Bd44	-	89	88	88	87	88	87	-
Bd45	-	87	85	86	86	85	85	84
Bd46	-	89	88	-	89	87	88	-
Bd47	84	87	86	86	-	86	85	87

Sequences from each lineage were Blastn search (and BOLD search) for the top 100 hits (maximum scores). Only sequence coverage above 90% was considered.

*** 99**% corresponds to the sequence similarity of Adineta lineage Bd8 from EA and GenBank records for *Adineta gracilis* from Signy Island (EF173190, EF173191, EF173193).

‡ 95% indicates the closest match for lineage Bd23 which corresponds to lineage Bd22. Lineage Bd22 was formed exclusively by a GenBank *Adineta* sp sequence from Signy Island (EF173192).

Table S6. Water samples from EA lakes showing presence of bdelloid and tardigrade lineages (as in Fig. 3 and Fig. 2) and values for electrical conductivity (EC) and pH.

Region	Sample	Bd2	Bd20	Bd27	Bd31	Bd45	Bd46	*Philodina* Bd47	*Acutuncus* Ta28	*Diphascon* Ta3	EC (dS/m)	pH
VH	VH-1	-	-	-	-	-	-	✓	✓	-	1.30	8.0
VH	VH-2	✓	-	✓	-	-	-	-	✓	-	2.36	8.3
VH	VH-3	-	-	-	-	-	-	-	✓	-	0.37	7.5
HI	HI-1	-	-	-	-	-	-	-	✓	-	1.71	6.4
HI	HI-2	-	-	-	-	-	-	-	✓	-	3.39	8.1
SP	SP-1	-	-	-	✓	✓	✓	-	-	-	0.24	7.8
SP	SP-2	-	-	-	-	✓	✓	-	✓	✓	0.11	7.0
SP	SP-3	✓	✓	-	-	-	-	-	✓	-	0.52	7.4
SP	SP-4	-	✓	-	-	-	-	-	✓	✓	0.52	7.5
SP	SP-5	-	-	-	-	-	-	-	✓	-	0.14	7.4
SP	SP-6	-	-	-	-	-	-	-	✓	✓	0.15	6.7
SP	SP-7	-	-	-	-	-	-	-	✓	✓	0.19	7.6
SP	SP-8	-	-	-	-	-	-	-	✓	-	0.19	7.3
SP	SP-9	-	-	-	-	-	-	-	✓	✓	0.23	7.0
SP	SP-10	-	-	-	-	-	-	-	✓	✓	0.70	7.2
BP	BP-1	-	-	-	-	-	-	✓	-	-	1.70	4.9
BP	BP-2	-	-	✓	✓	-	✓	-	-	-	0.33	6.8
BP	BP-3	-	-	-	-	-	✓	-	✓	-	0.34	7.3
BP	BP-4	-	-	✓	-	✓	-	-	✓	-	0.15	6.9
BP	BP-5	✓	-	-	✓	-	-	-	-	-	0.32	7.1
BP	BP-6	-	-	✓	-	-	-	-	✓	-	0.24	7.1
BP	BP-7	✓	-	-	-	-	-	-	✓	-	2.70	7.2
BP	BP-8	-	-	-	-	-	-	-	-	✓	0.15	7.0
BP	BP-9	-	-	-	-	-	-	-	✓	✓	0.26	7.8
BP	BP-10	-	-	-	-	-	-	-	-	✓	0.28	7.2
BP	BP-11	-	-	-	-	-	-	-	✓	-	0.38	6.7
BP	BP-12	-	-	-	-	-	-	-	✓	-	0.44	7.4
BP	BP-13	-	-	-	-	-	-	-	✓	-	0.94	7.2

Presence of lineage in sample is indicated by '✓' and absence by '-'.List of Region acronyms: Vestfold Hills (VH), Hop Island (HI), Stornes Peninsula (SP), and Broknes Peninsula (BP).