ANGLIA RUSKIN UNIVERSITY

FACULTY OF SCIENCE AND TECHNOLOGY
DEPARTMENT OF LIFE SCIENCES

DOCTOR OF PHILOSOPHY BY PUBLISHED WORK

TAXONOMY, BIODIVERSITY AND BIOGEOGRAPHY: TARDIGRADA AND ANTARCTIC MEIOFAUNA

COPIES OF THE PAPERS ON WHICH THIS SUBMISSION IS BASED

Sandra J McInnes

A Thesis in partial fulfilment of the requirements of Anglia Ruskin University for the degree of Doctor of Philosophy by Published Work

NOVEMBER 2010

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ABSTRACT

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TAXONOMY, BIODIVERSITY AND BIOGEOGRAPHY: TARDIGRADA AND
ANTARCTIC MEIOFAUNA

BY SANDRA J MCINNES

NOVEMBER 2010

The main subject of my papers has been the phylum Tardigrada, with particular emphasis on the Antarctic fauna. While this is one of the 'lesser known phyla' the group forms an important element of Antarctic non-marine ecosystems. My work has centred on four interrelated themes: i) taxonomy, predominantly Tardigrada and with an Antarctic bias though including wider global species and high taxon groupings (I have also worked on other taxa such as Fungi-imperfecti and freshwater invertebrates); ii) biodiversity of tardigrades, particularly Antarctic habitats; iii) data-based studies based on the terrestrial Tardigrada and Antarctic freshwater crustaceans; and iv) biogeographic analysis of these databases.

The thesis presented consists of papers published in major, peer-reviewed journals, along with book chapters published and in press. Several of my papers have been cited 10-30 times according to the ISI Web of Science citation system.

Highlights of my work include being the first to publish a paper on the global terrestrial biogeography of a phylum and showing the extant limno-terrestrial tardigrade fauna reflect the early tectonic break-up of the Laurasia, West and East Gondwana super continents between 65 and 135 million years ago.

CRITICAL APPRAISAL

Introduction

Personal

I am broadly speaking a biologist who has spent many years working in limnology. My main area of research revolved around four interrelated themes: i) taxonomy, predominantly Tardigrada and with an Antarctic bias though including other regional/ global faunas and high taxon groupings (I have also worked on other taxa such as Fungi-imperfecti, Pycnogonida, freshwater crustaceans, rotifers, nematodes and gastrotrichs); ii) biodiversity of tardigrades, particularly Antarctic habitats; iii) data-based studies, again predominantly based on the terrestrial Tardigrada but diverging into marine tardigrades (not presented herein), Antarctic freshwater crustaceans, and Pycnogonida (not presented herein); and iv) biogeographic analysis of these databases.

I joined The British Antarctic Survey (BAS) in 1980 working in what was the 'Terrestrial and Freshwater Division' of the Biological Department. At that time I was a research assistant within the Freshwater Section (and the Birds & Seals Group) and over time was moved to a more general 'BioSciences Division' support role. My initial remit within the Freshwater Section was to compile databases and roughly analyse the raw data supplied by the Freshwater Assistants at Signy Island in the South Orkney Archipelago. This entailed regularly incorporating physico-chemical data for sixteen lakes and analysing data to increase the resolution of lake bathymetry. I was also tasked with the analysis of frozen and preserved benthic lake sample and floral/faunal trawls, which were then consolidated into a central data source

(some of this was presented at the Second International Congress of Limnology (Noon, McInnes and Pugh 1999)). This work highlighted the presence, indeed ubiquity, of tardigrades in many of the benthic samples. The BAS library held work by Jennings, an earlier BAS employee, on tardigrades at designated terrestrial study sites on Signy Island, but limited (especially freshwater environments) additional data (Jennings, 1975; 1976a; 1979). A previous personal interest in tardigrades prompted me to request permission to explore the tardigrades of the Signy Island lakes which was granted. In the further exploration of Antarctic material and, having digressed to examine tardigrades from Europe (Pyrenees – McInnes, 1991), I found I needed to verify earlier references and therefore began assembling a database of tardigrade literature. I became interested in the biogeography of the tardigrades, a trend developing in other groups at the time and, as the information was centrally unavailable, added such information to the database. These early studies of Signy Island lake tardigrades and the initial development of the database resulted in a successfully defended MPhil (McInnes, 1996), four peer reviewed papers (McInnes, 1994; 1995; McInnes & Pugh, 1998; 1999 - not presented herein) and five presentations at international conferences.

Introduction to the Tardigrada

The phylum Tardigrada, often referred to as one of the minor or lesser known phyla, comprises a group of animals with an almost ubiquitous worldwide distribution. The Tardigrada, colloquially referred to as tardigrades, water bears and moss piglets, are found in a variety of diverse habitats from tropical rain forest to arid polar deserts, and from mountain tops to the abyssal depths of the oceans. Where there is free water available and a food source there is a good possibility of finding terrestrial or, more correctly, limno-terrestrial tardigrades.
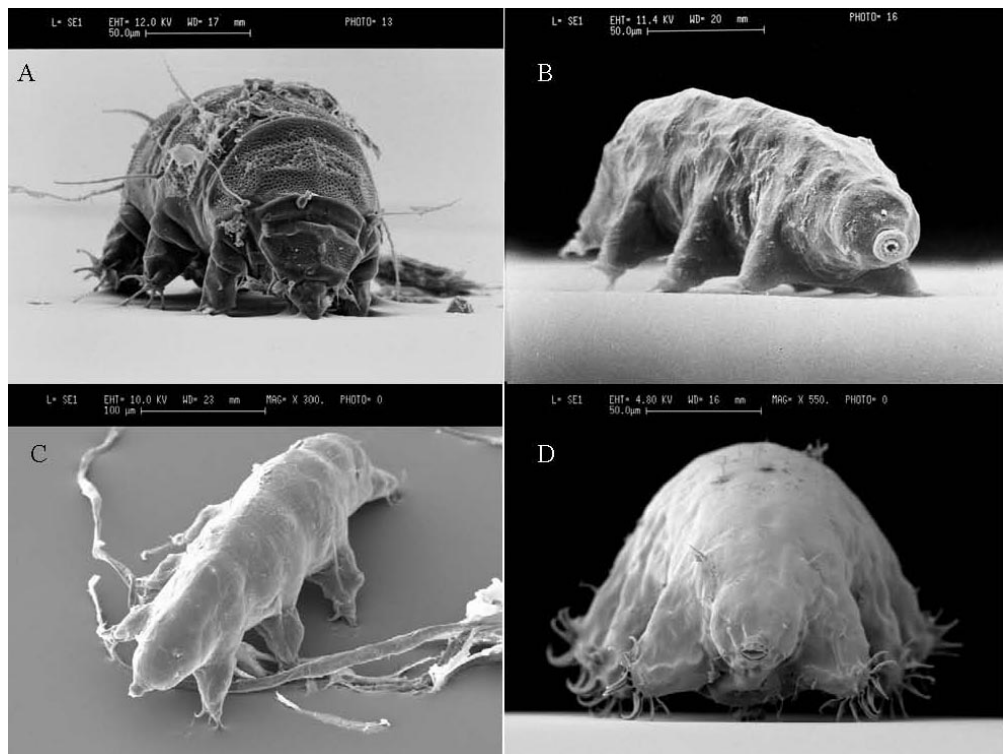
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Figure 1. Scanning electron microscope (SEM) images: A. *Echiniscus punctus*, terrestrial Heterotardigrada; B. *Macrobiotus furciger*, terrestrial Eutardigrada; C. *Ramazzottius* sp, terrestrial Eutardigrada; D. *Echiniscoides sigismundi*, marine (intertidal) Heterotardigrada.   Images: Original.
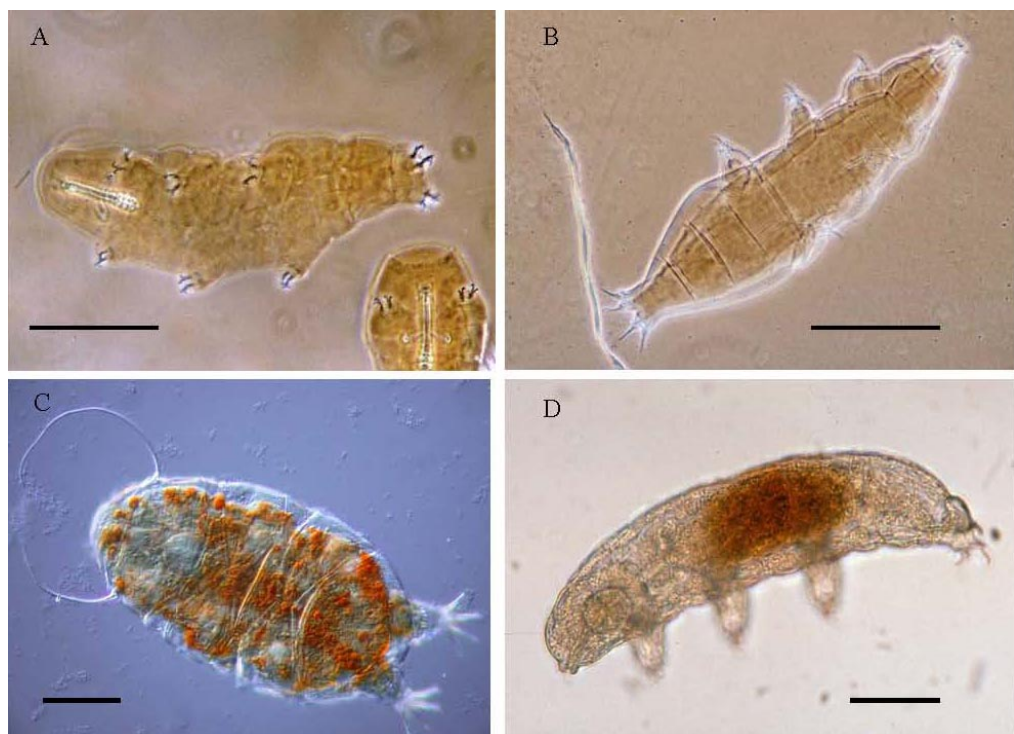

Figure 2. A. *Macrobiotus furciger*, terrestrial, Eutardigrada (Phase contrast – scale = 100µm); B. *Milnesium tardigradum*, terrestrial, Eutardigrada (Phase contrast – scale = 150µm); C. *Echiniscus* sp., terrestrial, Heterotardigrada (Differential interference contrast – scale = 50µm); D. *Acutuncus antarcticus*, terrestrial, Eutardigrada (Köhler illumination – scale = 100µm).   Images: Original.

The micrometazoan, Tardigrada, average 250-500 µm as mature adults with juveniles *ca.* 50 µm and a few larger taxa reaching 1200 µm. Tardigrades are bilaterally symmetrical with five distinct 'pseudosegments': four body segments, each bearing a pair of lobopod limbs, and a head (Figures 1 & 2). In most terrestrial tardigrades the limbs terminate in claws, which are absent in a few taxa. The body cavity is a fluid filled haemocoel, with circulation and respiration functions. There is a complete digestive system, a nervous system comprising dorsal lobed brain and ventral nerve cord with paired, fused ganglia, reproductive and excretory systems (Figures 3 & 4, and see: Nelson, 2002; Kinchin, 1994). Reproductive methods include sexual reproduction and parthenogenesis, both of which are present in Antarctic taxa. Like other micrometazoans tardigrades have the ability to survive unfavourable conditions by cryptobiosis (see: Wright 2001). This combination of attributes makes tardigrades efficient colonists of newly exposed terrain and well suited to extreme habitats such as those occurring in the Antarctic.

Tardigrades feed on plant and animal cells by piercing the cell with their stylets and then sucking out the contents via the musculature of the pharyngeal bulb. Some graze on the bacteria that cover the surfaces of their habitat, while others are able to ingest whole animals such as rotifers, nematodes and indeed other tardigrades.

Morphology (attributed with modifications to Marcus 1929; 1936), divides the phylum Tardigrada into two classes: Heterotardigrada Marcus, 1927 (marine and "armoured" terrestrial tardigrades) and Eutardigrada Marcus, 1927 ("unarmoured" terrestrial and freshwater taxa). A third class, Mesotardigrada, had included a single species (Rahm, 1937) from a hot spring in Japan, but neither the type locality nor a type specimen survive and, based on the limited literature description of the specimen, this species is now considered either dubious (see Nelson 2002; Nelson and McInnes 2002), or possibly related to Carphaniidae Binda & Kristensen, 1986 (Heterotardigrada; Echiniscoidea) (Binda and Kristensen 1986). The distinction between heterotardigrades and eutardigrades is based on: a)

claw structure (Figure 3 - c), b) shape and form of the sclerified structures within the pharynx (Figure 3 - p), and c) a separate gonopore and anus in heterotardigrades, verses a common cloaca and "Malpighian tubules" in eutardigrades (Figure 3 - r).

The phylogenetic position of the phylum Tardigrada within the Kingdom Animalia is still in debate. Most studies place the Tardigrada in the super-clade Ecdysozoa but the actual position, and nearest neighbour, are unclear. The group could be associated with arthropods and onychophorans, or related to the nematodes and nematomorphs (see: Aguinaldo *et al.*, 1997; Dunn *et al.*, 2008; Edgecombe, 2009).
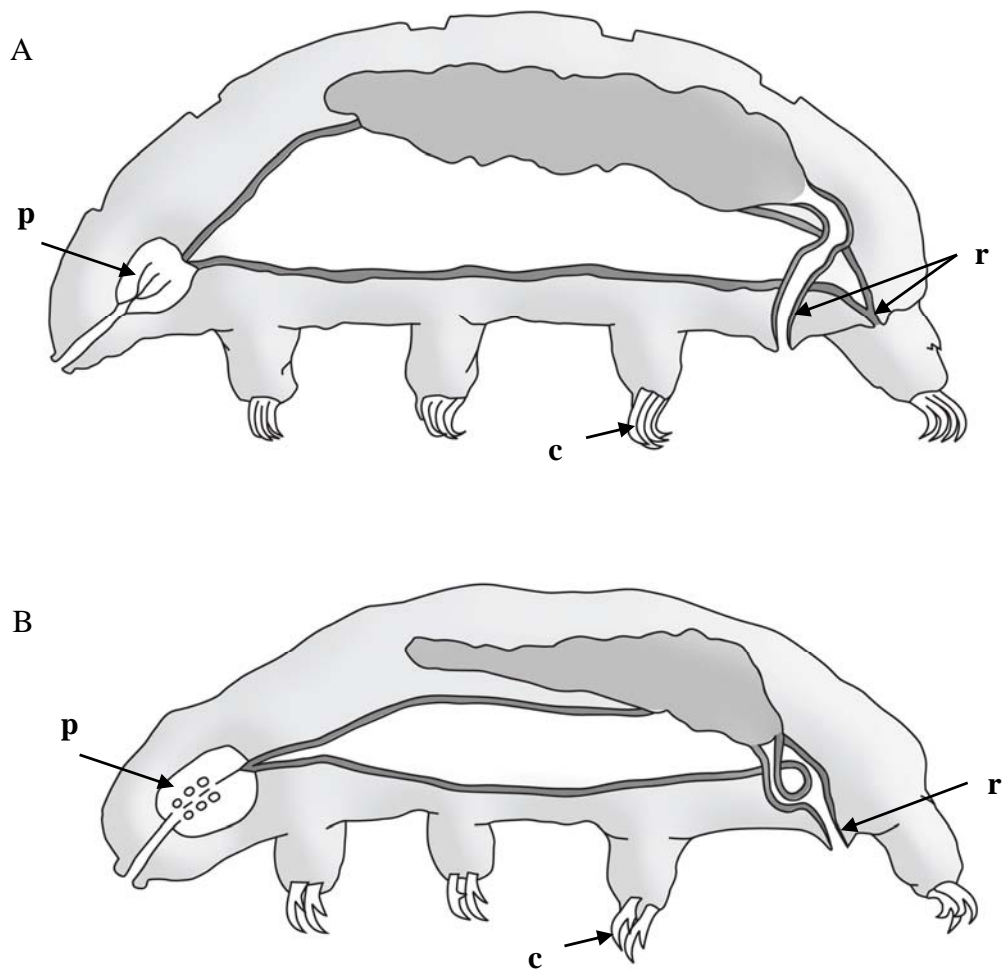
Figure 3. A. Stylised diagram of Heterotardigrada. B. Stylised diagram of Eutardigrada. Noted differences that define the classes are: c - claws - four simple claws in heterotardigrades and two claws divided into two branches in eutardigrades; p - placoids – tri-radiate fused bars in heterotardigrades and tri-radiate individual placoids in eutardigrades; r - reproductive system – separated into anus and gonopore in heterotardigrades and fused system into single cloaca in eutardigrades.
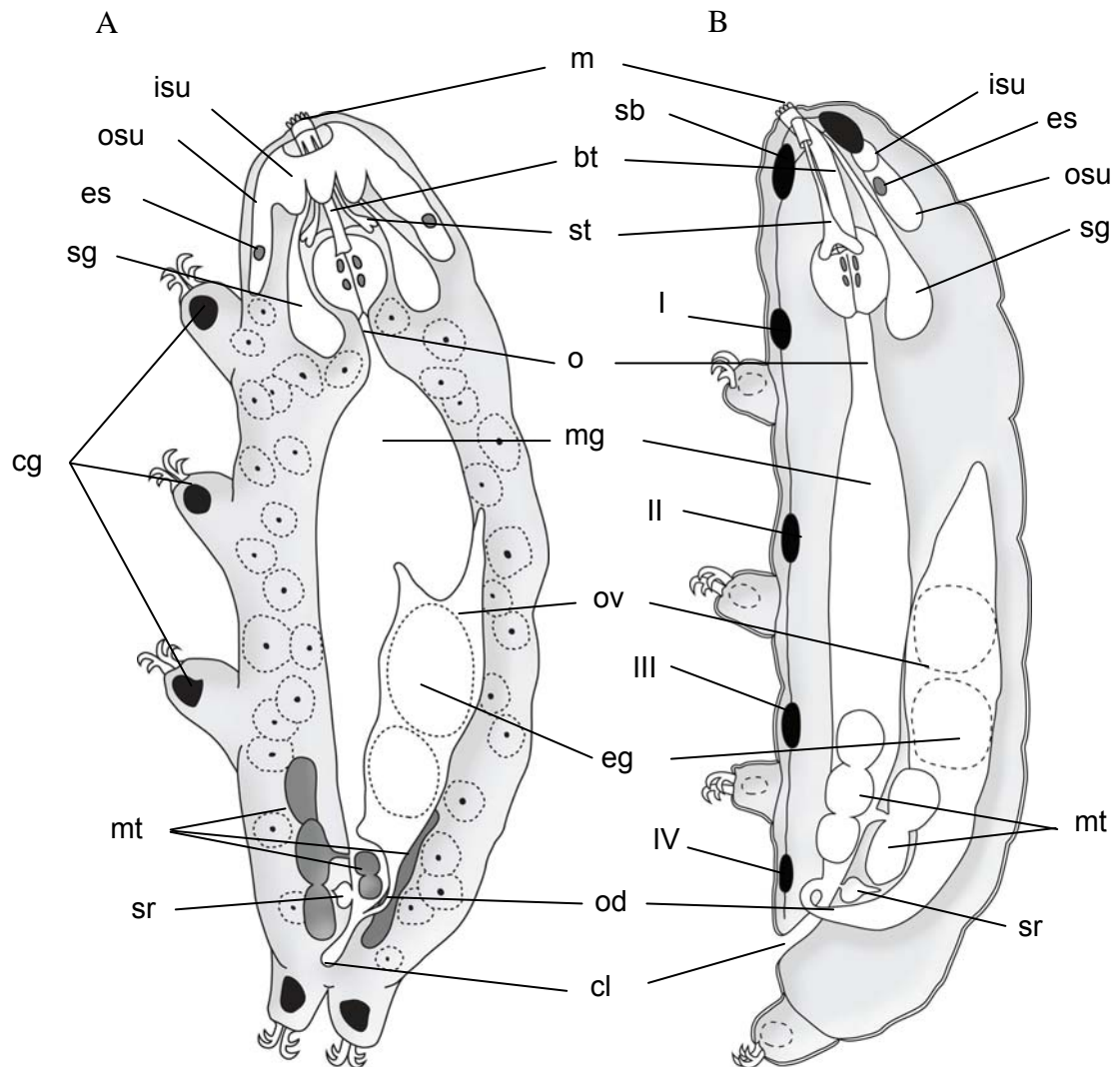
Image: Original.

Figure 4. A. Stylised dorsal view of *Macrobiotus* type eutardigrade showing internal structures. B. Stylised lateral view of *Macrobiotus* type eutardigrade showing internal structures. (Both diagrams adapted from Marcus, 1928)
m - mouth; bt - buccal tube; st - stylets; es - eyes; cg - claw glands; o - oesophagus; mg - mid gut; ov - ovary; eg - eggs; mt - malpighian tubules; od - oviduct; cl - cloaca;
sg - salivary gland; sb - suboesophageal ganglion; isu – inner section of the supraoesophageal ganglion; osu – outer section of the supraoesophageal ganglion; I, II, III, IV - ventral ganglia; sr - seminal receptacle.

Image: Original.

Throughout the Twentieth Century there has been a constant slow trickle of studies relating to the Tardigrada. The majority of the work was morphological with few physiological studies and latterly more discussion on evolutionary and systematic status. Most of the current higher subdivisions in the phylum are based on the monographs by Marcus (1929, 1936), who in turn established his work on that of Thulin (1928). More recent monographs by Ramazzotti (1962, 1969) with the final edition III (Ramazzotti and Maucci 1983) have been the main starting point for most subsequent studies on tardigrades.

Early Antarctic tardigrade literature

Both classes of the phylum Tardigrada, the Heterotardigrada and Eutardigrada, are present in the Antarctic. The earliest record of a continental Antarctic tardigrade (*Acutuncus antarcticus*, originally described as *Macrobiotus antarcticus*) by Richters (1904) who, in a preliminary report, listed the species found by the *Deutsche Südpol Expedition* of 1901-1903. A broader description, of the tardigrades found, is in the expedition report (Richters, 1907). This 'Heroic Age' of expeditions provided a number of samples from the Antarctic and sub-Antarctic. Richters received samples from the *Schwedischen Südpolar Expedition* 1901-3 (Richters, 1908) and from the *National Antarctic Expedition* 1901-4, via samples from Jules Cardot (Richters, 1909). Murray, a contemporary of Richters, received material from the *Scottish National Antarctic Expedition* (Murray, 1906), and was a member of the *British Antarctic Expedition* of 1907-9 (Murray, 1910). Tardigrades by this time had been reported from two sites on the Antarctic continent: Gaussberg (66º 50'S, 89° 11'E) and Victoria Land (77-78ºS), along with the Antarctic Peninsula, South Orkney Islands, South Shetland Islands, and sub-Antarctic islands of South Georgia, Crozet, Kerguelen and Heard (see Fig 6).

There followed a gap of many years before interest in the meiofauna of the Antarctic provided more information about Antarctic tardigrades. Most of these reports were the product of opportunistic studies and restricted to the coastal region of the continent and peninsula or islands, with most in the vicinity of the various stations (e.g. Sudzuki, 1964, 1979; Dastych, 1984; Jennings 1976a; 1976b). The most recent guide to Antarctic Tardigrada (Dastych, 1984) is limited and (now) dated. Figure 5 shows a timeline and Figure 6 the sites where tardigrades have been reported around the Antarctic.
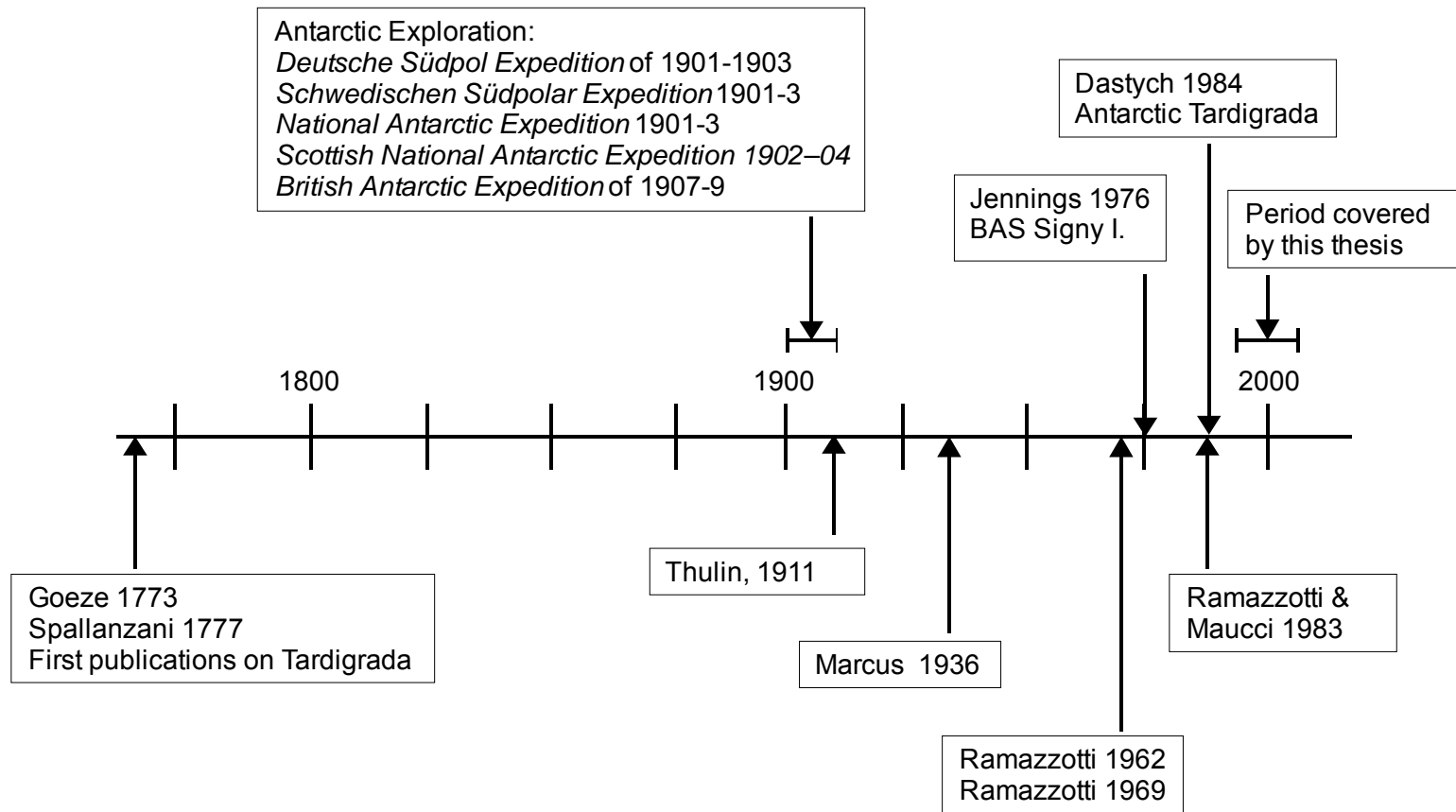
Figure 5. Timeline: Study of Tardigrada.

Figure 6. Map of the Antarctic with some of the sites from which tardigrades have been identified. Blue dots are areas mentioned in this thesis, red dots sites mentioned in tardigrade literature. The red line at the base of the Antarctic Peninsula divides the northern maritime Antarctic from the southern continental Antarctic.

Image: Original, BAS MAGIC map.

i) __Taxonomy__

Traditional taxonomy: Tardigrada

The Tardigrada are a challenging phylum with a limited suite of morphological characters upon which to base alpha-taxonomic studies. Classical morphology of this group has increasingly incorporated more characteristics, reflecting the effectiveness of the microscopes and techniques used, from basic Köhler illumination (Figure 2D) through phase contrast (Figures 2A & B) to differential interference contrast (DIC) (Figure 2C). More unusual techniques such as darkfield cardioid condenser and confocal microscopy are not always available to general taxonomists but can offer additional insights to the problem of visualising structures. Similarly, scanning electron microscopy (SEM) (Figure 1A –D) provides excellent images of surface structures and, with freeze fracture (Figure 7A - C), a window to the internal structural organisation.
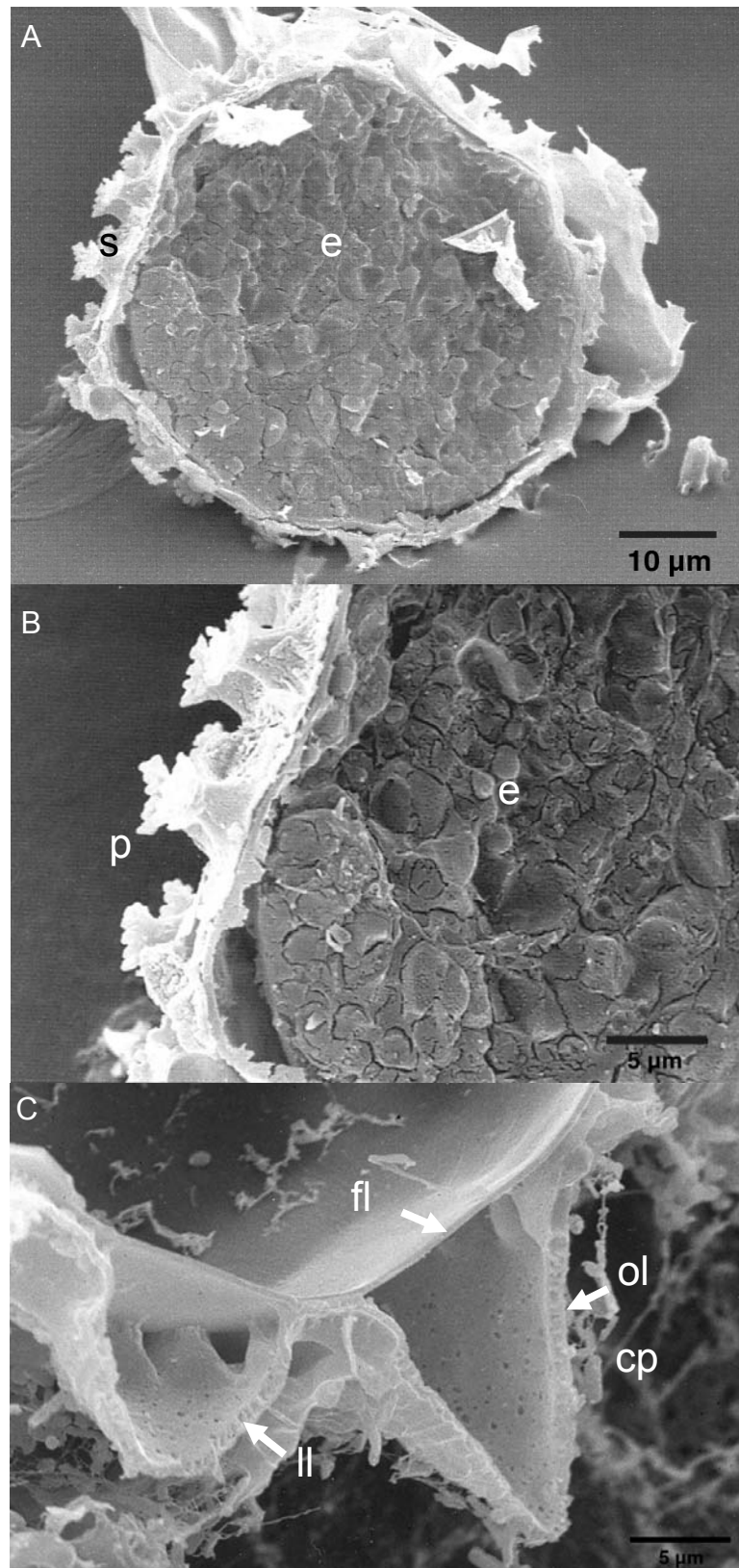
Figure 7. Freeze-fractured eggs of: A & B *Macrobiotus sandrae* Bertolani & Rebecchi, 1993 and C *Paramacrobiotus* sp.
e - embryonate material (apparently undifferentiated); s - chorion or egg shell (egg shell morphology is a useful taxonomic character); p - egg processes; cp - conical process, ol - outer dense layer; fl - fibrillar layer; ll - labyrinthine layer.
Images: Original.

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I have described eight new tardigrade species using classical morphology (e.g. McInnes, 1991; 1995; Dastych & McInnes, 1994; 1996; Dastych *et al* 1998), two of which are represented in this thesis. *Echiniscus corrugicaudatus* (Heterotardigrada; Echiniscidae) described by McInnes (2009), is placed within the *Echiniscus* '*arctomys* group' based on the characteristic of ''a lack of body appendages other than cirrus A'' (Figure 8A - C). It has less well-defined edges to the dorsal plates (Figure 8B) than other species within the group and distinct ridges on the caudal plate (Figure 8C). In this paper I took the opportunity to tabulate the characters of the '*arctomys* group' from the original reports to assist further studies on this group (see: McInnes 2009, table 3). *Echiniscus corrugicaudatus* was found in the limited vegetation from the inland nunataks of Ellsworth Land, West Antarctica, a rarely explored region that shows limited overlap with the tardigrade fauna from the maritime or coastal continental Antarctic. Males were found in the population indicating gonochoristic reproduction, and a large number of juveniles that suggested an egg over-wintering strategy or a biennial population over-wintering as both adults/sub-adults and eggs.

The presence of males is considered a plesiomorphic condition in the Echiniscidae that is rarely seen in the more advanced genus *Echiniscus* (Kristensen 1987). There is, however, mounting evidence to suggest a Gondwanan link to gonochoristic reproduction as all literature references to male *Echiniscus* spp. were reported from current land mass sites that were originally part of Gondwana (e.g. Miller & Heatwole 1996; Claxton 1996). In reviewing the current literature on the existence of gonochoristic reproduction within *Echiniscus* species (McInnes 2009) the number of positive identifications of males moves to a slightly broader geographic range (Table 1). Most of these references are still for localities that fall within the previously recognised ''Gondwanan'' zone (Figure 9). However, reports for Alabama (USA), Costa Rica, Cuba and China have extended the boundaries, but may still be explained by dispersal from a Gondwana origin.
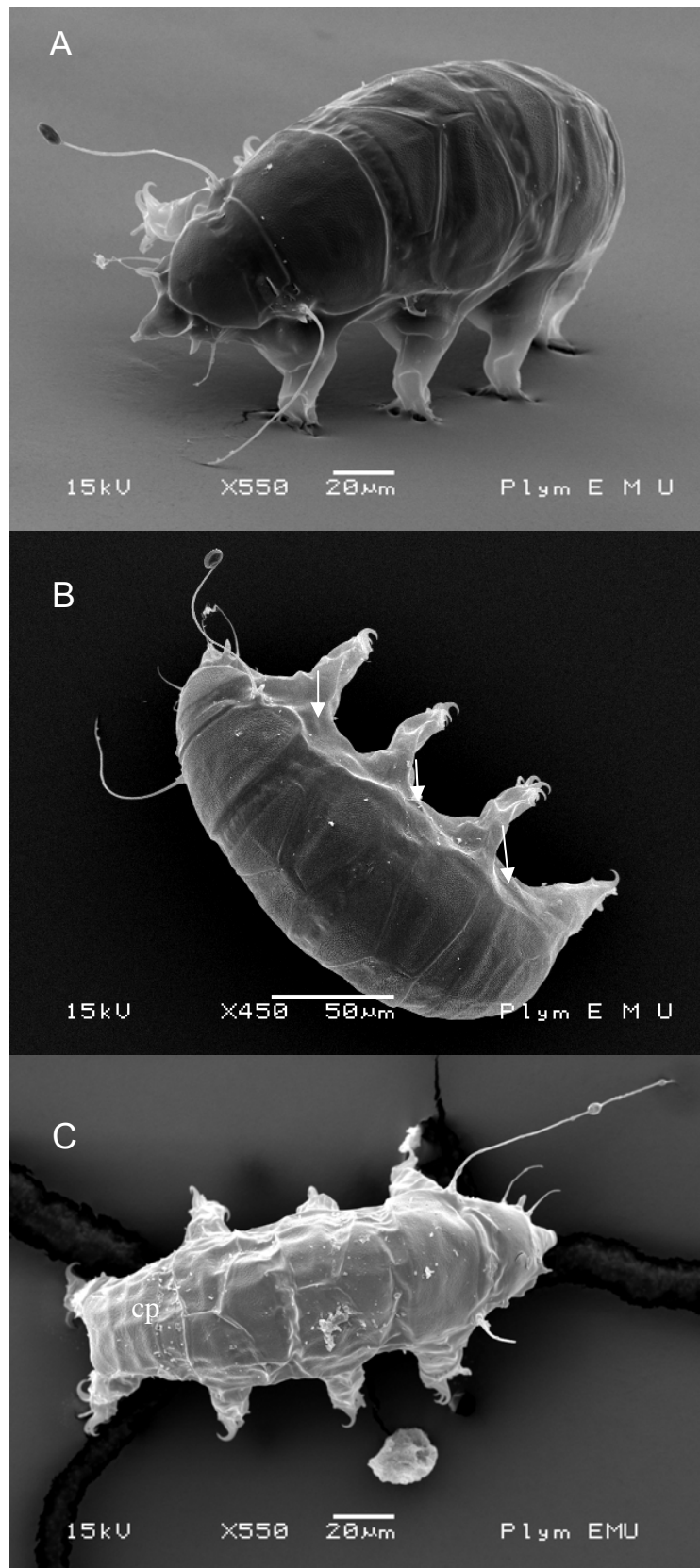
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Figure 8. Images of *Echiniscus corrugicaudatus* McInnes, 2009. A. Dorsal cuticle (SEM); B. Dorsal cuticle showing weak definition to edges of all plates (arrows) (SEM); C. Dorsal cuticle showing sculpturing and ridging of caudal plate (cp) (SEM). Images: Original, with acknowledgements to the Plymouth Electron Microscopy Unit.

Table 1. Examples of data recording males and females found in the genus *Echiniscus*.

| Species	Totals *n*	Males numbers (*n*) or ratio (*r*)	Females numbers (*n*) or ratio (*r*)	2-claw/ Juveniles	Location	Study
Echiniscus jenningsi	765	296 (n)	395 (n)	p	Antarctica	Miller and Heatwole 1996
Echiniscus jenningsi	65	2 (n)	63 (n)		Antarctica	Dastych 1987
Echiniscus pseudowendti	330	p	p		Antarctica	Dastych 1987
Echiniscus merokensis	50	6 (n)	44 (n)		Sub-Antarctic	Miller, *et al*, 1999
Echiniscus sp. A	44	24 (n)	20 (n)		Sub-Antarctic	Miller, *et al*, 1999
Echiniscus jamesi	44	19 (n)	23 (n)	p	Australia	Claxton, 1996
Echiniscus rodnae	77	36 (n)	34 (n)	3/4	Australia	Claxton, 1996
Echiniscus curiosus	330	9 (n)	9 (n)	33/29	Australia	Claxton, 1996
Echiniscus cfr. *vinculus*	113	10 (n)	10 (n)		Australia	Claxton, 1996
Echiniscus duboisi	297	1 (r)	2 (r)		Australia	Claxton, 1996
Echiniscus sp 1		p	p		Australia	Claxton, 1996
Echiniscus sp 2		p	p		Australia	Claxton, 1996
Echiniscus sp 3		p	p		Australia	Claxton, 1996
Echiniscus palmai	18	4 (n)	4 (n)		Australia	Dastych 1997
Echiniscus nepalensis		1 (r)	10 (r)		Nepal	Dastych 1987
Echiniscus aff. *testudo*		1 (r)	3 (r)		Nepal	Dastych 1987
Echiniscus ehrenbergi	195	72 (n)	48 (n)	p	Himalayas	Dastych and Kristensen 1995
Echiniscus clevelandi	58	10 (n)	10 (n)	a	China	Beasley 1999
Echiniscus taibaiensis	3	1 (n)	2 (n)		China	Wang and Li 2005
Echiniscus ganczareki	25	6 (n)	19 (n)	0	Costa Rica	Michalczyk and Kaczmarek 2007
Echiniscus barbarae	17	3 (n)	12 (n)		Cuba	Kaczmarek and Michalczyk 2002
Echiniscus maucci	1329	87 (n)	135 (n)	107	Alabama USA	Mitchell and Romano 2007

p = present
a = absent
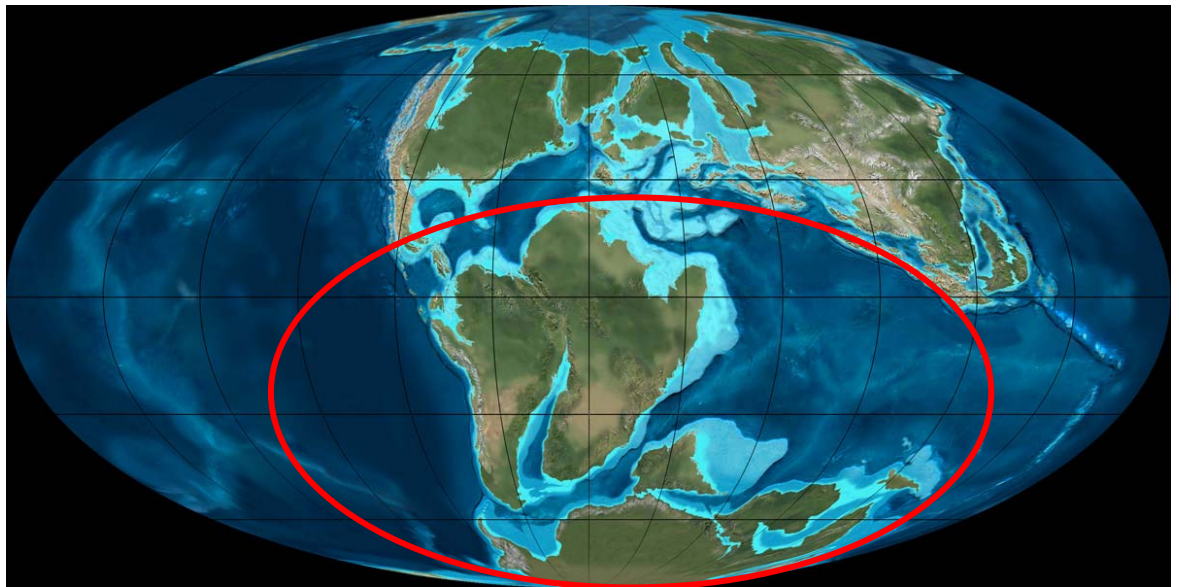
Figure 9. Mollewide (oval globe) projections showing global paleogeography reconstruction of the Earth in the early Cretaceous (120 Ma). From Table 1 only 'China', 'Costa Rica', 'Cuba' and 'Alabama, USA' would fall outside the 'Gondwanan' zone – red circle.

Image: from Wikimedia.

In a second paper, Miller, McInnes & Bergstrom (2005) I was asked, as a senior taxonomist, to describe the new species of *Hypsibius* from samples collected during the Australian National Antarctic Research Expeditions (ANARE) 1986-87 expedition to Heard Island, in the southern Indian Ocean (Figure 10). We described *Hypsibius heardensis* (Eutardigrada: Hypsibiidae) (Figure 11) as belonging to the '*dujardini* group' based on the characters of smooth cuticle, two rod shaped macroplacoids of similar length and an obvious septulum (rather than a microplacoid). This species differs from similar sibling species by the absence of eyes, buccal tube length to insertion point of stylet muscles ratio (after Pilato, 1981) of 56-63%, large pharyngeal apophyses, near equal length macroplacoids, lack of a microplacoid, presence of a small septulum, and cuticular bars near the base of the claws on all four legs.
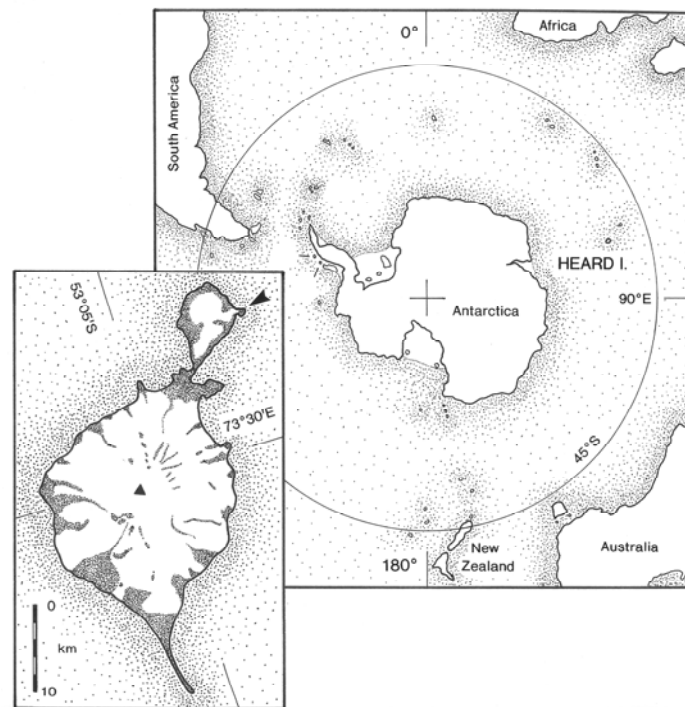

Figure 10. Antarctica and the sub-Antarctic Islands. Inset: Heard Island. Red Island to the north and Laurens Peninsula (arrow).
Image: Original; drawn by P.J.A. Pugh.

This taxon was found in a range of habitats from discontinuous bryophyte dominated vegetation, which though never dry could not be described as 'water-logged', to a continually 'wet' site bordering a meltwater lake. The original description of *Hypsibius dujardini* (Doyère, 1840), is from small pools

in the forests of Fontainbleau (France), in association with the rotifers *Brachionus* sp. and *Floscularia* sp. (Doyère, 1840), and has therefore been considered hygrophilous or hydrophilous. The type locality of *Hypsibius heardensis* was a shallow bryophyte layer of predominantly *Sanionia uncinata* (Hedw.) Loeske, over a very free draining lava substrate, which received wind-driven mist. This ability to cope with dryer habitats, though still active in continuously wet sites, indicates *Hypsibius heardensis* is a more eurytropic or euryhygric taxon.

Figure 11. *Hypsibius heardensis* Miller McInnes & Bergstrom 2005. A, dorsal view buccal apparatus. B, lateral view buccal apparatus. C, claws of legs III – arrow indicating cuticular bar at the base of the internal claw. D, claws of legs IV – arrow indicating the cuticular bar between the internal and external claws.
Scale bars = 10 µm.

Images: from Miller, *et al.*, 2005.

A third paper, Dastych, McInnes, & Claxton (1998), combined the skills, knowledge and samples of three equally eminent tardigrade taxonomists. The problem caused by the confused descriptions of the genus *Oreella* was being experienced by all three authors from different perspectives. In discussion we found we were all working towards the same conclusion and so opted to pool our resources. In this paper we re-examined *Oreella mollis* Murray, 1910 (Heterotardigrada; Oreellidae), using specimens collected from close to the type locality in Australia designating a neotype for this taxon (in lieu of lost type specimen). We compared the neotype and Australian examples with fresh samples from the maritime Antarctic and slides from various collections. We concluded that *Oreella mollis* is a *bona fida* species with a Gondwanan distribution, but that *Oreella vilucensis* Rham, 1931, with a disjunct Chile and Germany distribution, should be recognised as a *nomen dubium* and, *Oreella minor* Ramazzotti, 1964 recognised as a junior synonym of *O. mollis.* On re-examining slides of *Oreella breviclava* Grigarick *et al* 1983 we proposed that this species was actually a junior synomym of *Hypechiniscus exarmatus* (Murray 1907). *Oreella* has a number of characters that link it to both the heterotardigrades (presence of cirrus A, clavae and head appendages) (Figure 12A&B) and eutardigrades (ornamented eggs) (Figure 12C & D) such that it is a potentially important taxon in constructing the phylogeny of terrestrial Tardigrada.
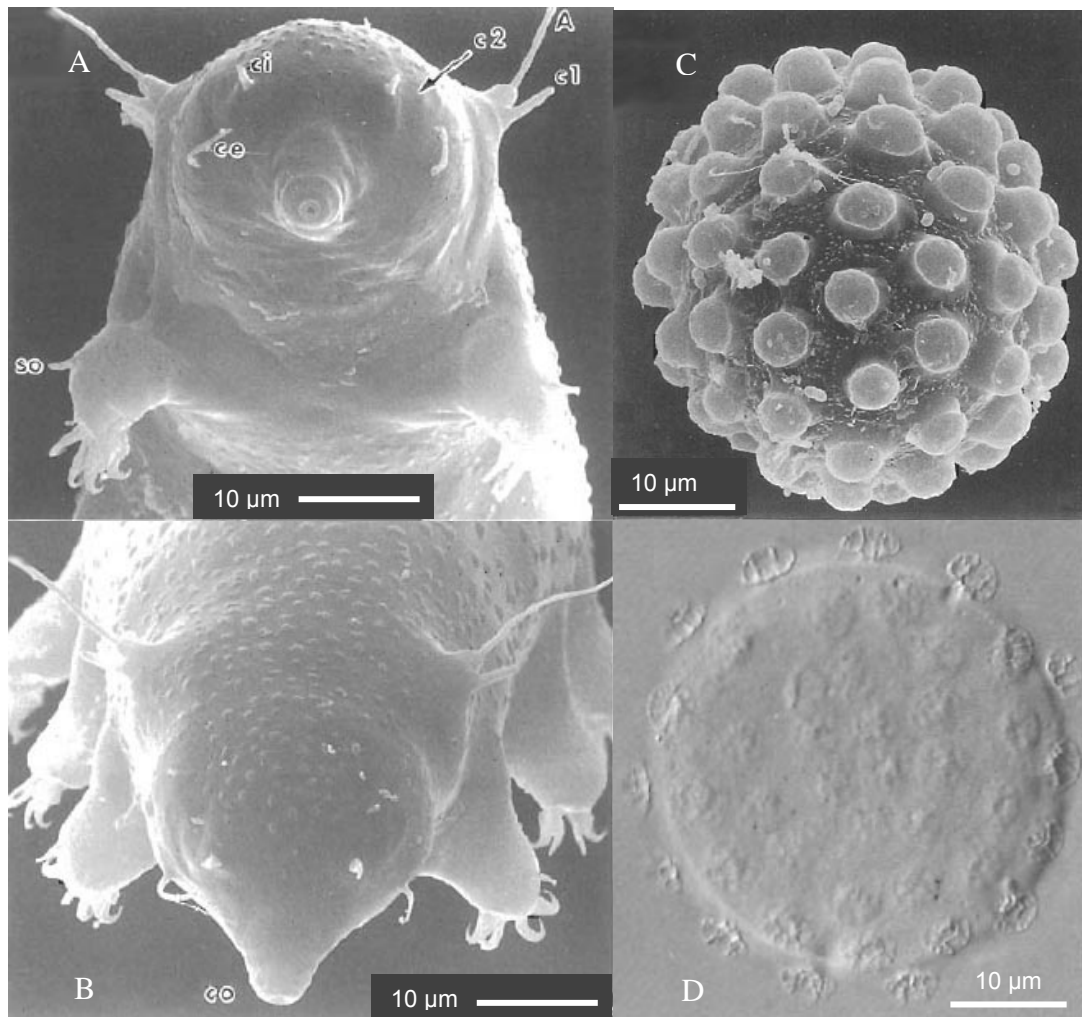
Figure 12. *Oreella mollis.* A & B. SEM images showing head and appendages; C & D. SEM and DIC images respectively, showing the ornamented egg.

Images: from Dastych, *et al.*, 1998.

Traditional taxonomy: Fungi-imperfecti

A few of the benthic samples from the shallow margins of Signy Island lakes revealed the presence of a predaceous fungus of tardigrades and bdelloid rotifers. Despite trying to find a mycology expert, none were available to identify this obscure taxon. I found the only way to answer the question of what was preying on tardigrades and rotifers in the lake margins was to acquire a basic understanding of mycological systematics and taxonomy. This was achieved by extensive reading of the literature and gaining access to specific papers on this group.

The tardigrade and rotifer trapping fungus belonged to the Fungi-imperfecti, or Deuteromycota, which are fungi that do not fit any of the commonly established fungal taxonomic classifications due to an asexual reproductive strategy and no observed sexual structures. The asexual, non-motile spores are termed conidia and are formed on a structure referred to as a conidiophore. Classified in the Deuteromycota are the Hyphomycetes and in McInnes (2003) I identified the Signy Island taxon as a member of the genus *Lecophagus* Dick, 1990, and concluded that there were sufficient differences to separate it from its nearest relatives, *L. muscicola* (Barron et al. 1990) and *L. longispora* (Barron et al. 1990). Specifically, the new species *L. antarcticus* (Figure 13) had broader vegetative hyphae and larger adhesive pegs. Also, the conidia were longer, broader and less curved than those of *L. muscicola* and, although the lengths of the conidia fell within the range for *L. longispora*, they are much broader, and less distally curved. *L. longispora* was reported to have a slightly different growth habit in water, producing long, aseptate conidiophores (<200 μm), though this did not affect hyphal morphology. However, it should be noted that this record was for cultured samples as both *L. muscicola* and *L. longispora* were found in wet terrestrial habitats associated with moss, organic debris and lichens (Barron et al. 1990; Hofstetter et al. 2007). Unlike *L. antarcticus*, neither *L. muscicola* nor *L. longispora* produce either septate conidiophore branches or terminal, lateral vegetative hyphae conidiophores (cf. Barron et al. 1990).

In contrast to the publication of zoological new species, fungi, as for all new botanical species, requires a Latin description. The lack of a Latin description and the location of the type material in the original report (McInnes 2003) are being rectified. Recent information (pers com Drs Baral and Webber – August 2009) indicates this genus is being revised using molecular techniques (e.g. Tanabe et al. 1999). For *L. antarcticus* to be included further live samples from Signy Island would be required as the original cultures sent to CABI with the type material were not preserved.
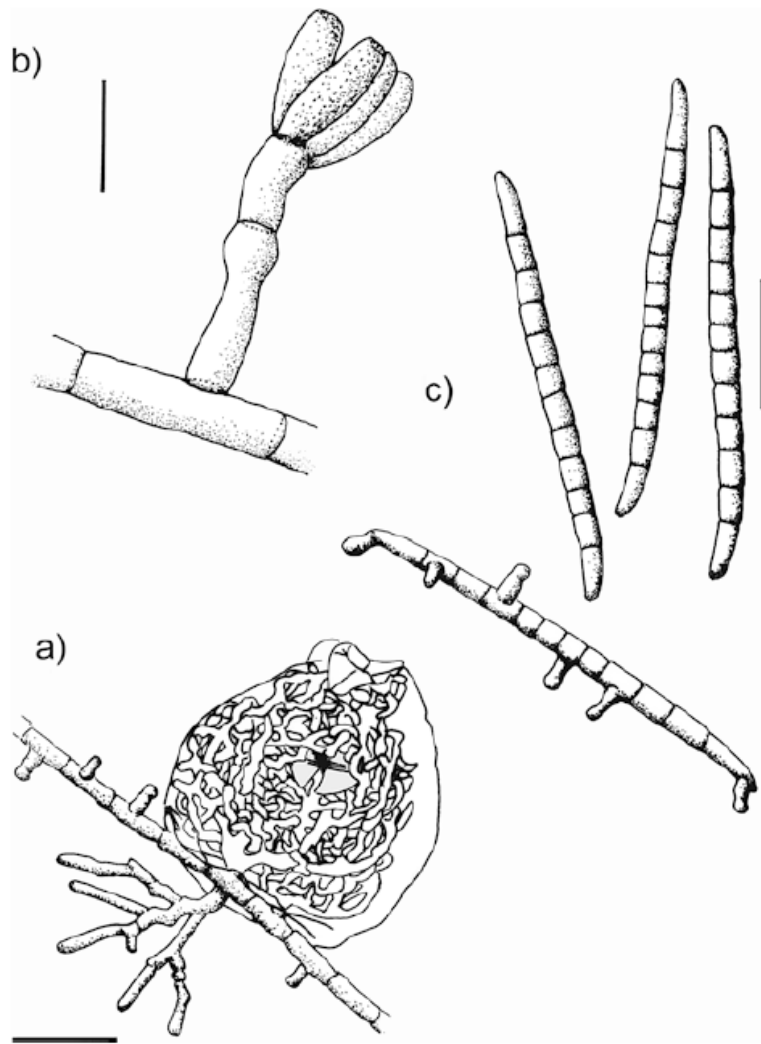
Figure 13. *Lecophagus antarcticus* McInnes 2003: (a) Assimilative hyphae inside captured bdelloid rotifer re-emerging to continue as vegetative hyphae while the remaining prey husk is further reduced by bacterial action (bar = 20 μm). (b) Septate conidia bearing branch with immature conidia (bar = 50 μm). (c) Mature and germinating conidia (scale bar = 50 μm).

Images: Original.

Combining molecular and traditional taxonomy: Tardigrada

In the early 1990's molecular biology began to be used for deep phylogenetic analysis of the animal kingdom including the position and affinities of the Tardigrada. More recently molecular analysis has been used to explore relationships within groups. With the advent of the BAS molecular laboratory and employment of experienced staff I have been able to develop this aspect of a taxonomists' skills arsenal. Sands, Convey, Linse & McInnes (2008) described the methods we used from sample collecting through extraction techniques, specimen identification and initial preparation for molecular work, to gene amplification and sequence alignment analysis using maximum likelihood methods. The first requirement was to standardise a method of DNA extraction from a single individual tardigrade. The very small size of the Tardigrada and limited DNA content meant most earlier papers had used samples of 10-50+ individuals. Despite the ability of molecular techniques to amplify the most common source within a sample, multiple samples can result in worthless chimera products. Furthermore, pooled habitat samples can mask rare or cryptic species. We pointed out the problems of chimera results by comparing a small (*ca.*100 base) anomalous region of two aberrant *Echiniscus testudo* (Doyère, 1840) 18S specimens, which proved a perfect match for the yeast *Candida* sp. Working with low power microscopes in order not to compromise material for subsequent molecular study (we used 400× inverted microscope), affords the potential for misidentification. The need, therefore, for voucher specimens from each sample was stressed.

In Sands, McInnes, Marley, Goodall-Copestake, Convey & Linse (2008) we explored an expanded database that combined data from GenBank with our own study, almost tripling the known tardigrade taxa within GenBank. Using maximum parsimony and Bayesian analyses of the 18S rDNA gene we evaluated the combined data of 343 individual tardigrades. This was the largest contemporary tardigrade dataset analysed. It provided evidence that the Parachela (Eutardigrada) comprises three (super)-families, rather than

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the accepted and well documented two families. It also highlighted several other generic level relationships indicating many of the genera, e.g. *Macrobiotus*, *Pseudechiniscus* and *Milnesium* require re-evaluation and the probable establishment of new genera (Sands *et al*. 2008, Figures 1 & 2). It also showed that even experienced taxonomists can have difficulty distinguishing taxa, e.g. *Hypsibius* and *Acutuncus*, when restricted to 400x magnification, but that the techniques used allow the error to be noted, checked and corrected.

The results of the molecular work has involved not only ribosomal *18S* but also mitochondrial *cytochrome c oxidase subunit* 1 (*CO1*) and to a more limited extent *Wingless*. These studies suggest we need to re-evaluate several taxa including: the generic position of *Testechiniscus/ Echiniscus macronyx* (Richters 1907); Antarctic lineages within *Milnesium* (McInnes 2006 -presented at the 10th International Symposium on Tardigrada and 'work in progress'); the evidence of new species groupings within the Antarctic *Macrobiotus "hufelandi"* and "*furciger*" groups (some of which was presented at the Xth Scientific Committee for Antarctic Research (SCAR) International Biology Symposium; Sands, Convey, Linse & McInnes (2009) and 'work in progress'); and possible population differentiation or speciation within *Macrobiotus furciger* Murray 1907 along the maritime Antarctic Peninsula (presented at the 11th International Tardigrade Symposium (McInnes and Sands 2009).

Molecular taxonomic work should not stand in isolation and so we have returned to the classical taxonomic morphology to explain the molecular divergences. At the first International Conference for Invertebrate Morphology, we, McInnes, Marley & Sands (2008), presented the case for a re-evaluation of the Parachela and the morphology that would distinguish the three proposed super-families. In Marley, McInnes and Sands (2011), with additional data from GenBank, we reviewed the combined strength of molecular and morphological evidence to identify four super-families within the order Parachela. These are: Macrobiotoidea Thulin 1928, Isohypsibioidea Marley, McInnes & Sands 2011, Eohypsibioidea Bertolani

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and Kristensen 1987 and Hypsibioidea Pilato 1969. In addition, we raise two familial rank taxa: Isohypsibiidae Marley, McInnes & Sands 2011 and Ramazzottidae Marley, McInnes & Sands 2011. The alpha-taxonomy is defined by a combination of structural differences in the shape and form of the claws and the 'apophysis for the insertion of the stylet muscles' (AISM). Two major differences in claw structures are considered significant: (a) claw branch symmetry (2112) versus asymmetry (2121) of the paired claws (Figure 14), and (b) the distinction between a claw comprising rigidly joined basal section, secondary and primary branches, with a claw consisting of a flexible primary branch junction joining a rigid basal section and secondary branch (Figure 15). The development of the AISM can currently be grouped as either a 'ridge' or 'hook', or modified by the presence of the ventral laminar (Figure 16). The molecular data (Sands *et al* 2008) clearly indicated three super-families. Claw and ASIM morphology indicates the Eohypsibiidae shares characters with the Hypsibiidae and Calohypsibiidae, with which it was compared (Bertolani and Kristensen 1987), but adding new GenBank molecular data for *Berotolanius nebulosus* (Dastych, 1983) (Eohypsibiidae) suggests that four super-families are apparent (Figure 17 & 18).

We concluded with descriptions of the superfamilies:

Eohypsibioidea, superfam. nov. Bertolani and Kristensen
Diagnosis. Parachela; claws asymmetric (2121); Eohypsibiidae-type claw pairs; apophysis for the insertion of the stylet muscles (AISM) ridged with lateral caudal processes of crests and hooks.

Isohypsibioidea, superfam. nov. Marley, McInnes and Sands 2011
Diagnosis. Parachela. Claws asymmetrical (2121); Isohypsibius-type claw pairs; AISM ridged.

Hypsibioidea superfam. nov. Pilato
Diagnosis. Parachela; claws asymmetrical (2121); Hypsibius-type claw pairs; AISM hooked (or, if the buccal tube is elongated, AISM can be broad ridges)

Macrobiotoidea, superfam. nov. Thulin, 1928.

Diagnosis. Parachela; claw pairs symmetrical (2112); AISM generally asymmetrical, due to the ventral lamina, with lateral caudal processes of crests and hooks.
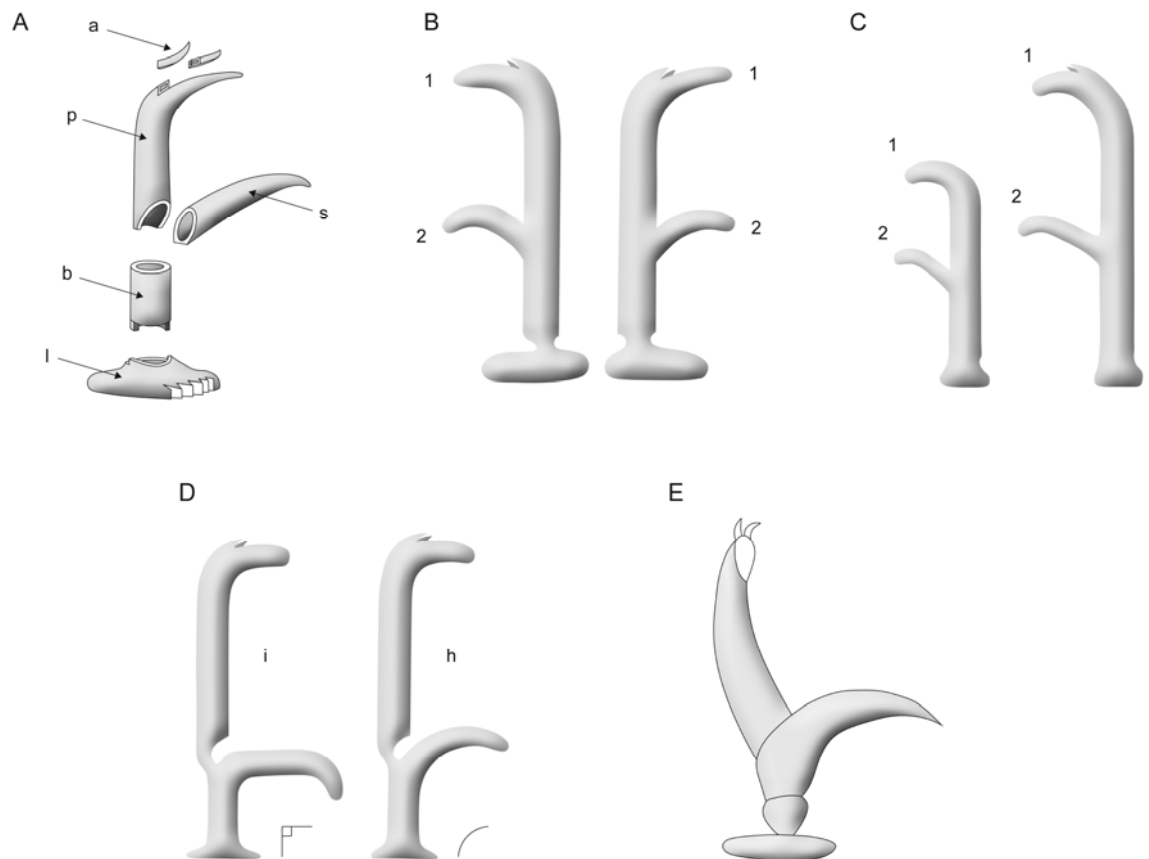

Figure 14. Diagrammatic representation of eutardigrade claw structure and nomenclature.
A Exploded diagram of a eutardigrade claw to explain component parts.
a – accessory spines; p – primary branch; s – secondary branch; b – basal tract;
l – lunule (edge may be smooth or dentate).
B Stylised diagram of macrobitid claw (2112). Secondary branch – 2; primary branch – 1; primary branch – 1; secondary branch – 2.
C Stylised diagram of a hypsibid claw (2121). Secondary branch – 2; primary branch – 1; secondary branch – 2; primary branch – 1.
D Stylised diagram of: i – isohypsibid (basal section and secondary branch at right angles) and h – hypsibid (basal section and secondary branch forming continuous arc) claws.
E Stylised diagram of Eohypsibiidae-type claw – claws are clearly delineated by septa into basal section, secondary branch and primary branch
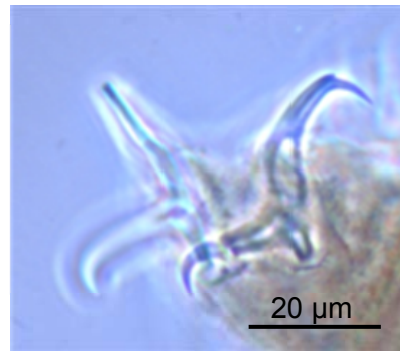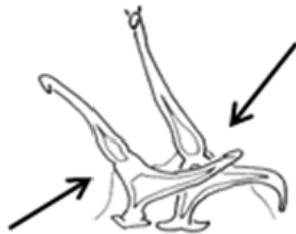
Images: Original.
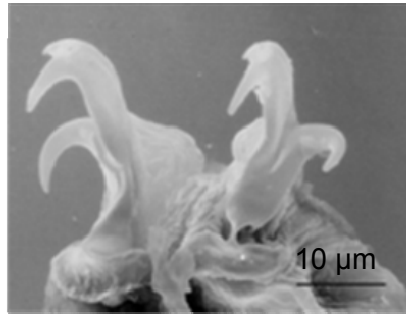
Macrobiotoidae:
Two claws on each leg divided into primary and secondary branches.
Symmetrical with respect to the median plane of the leg (2112).
Two claws of each leg are similar size and shape.
Branches of both claws are rigid, without articulation (broken line arrow).

Isohypsibioidea:
Two claws on each leg divided into primary and secondary branches.
Asymmetric with respect to the median plane of the leg (2121).
Both external and internal claws usually of similar size and shape.
Secondary branch and basal section forming a right angle.
Both claws articulated (solid line arrow).
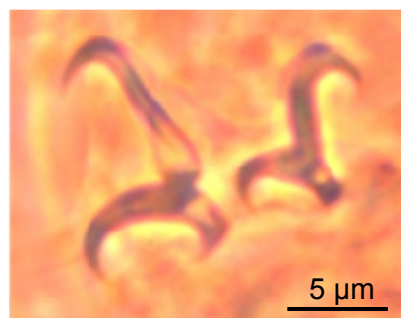
Hypsibioidea:
Two claws on each leg divided into primary and secondary branches.
Asymmetric with respect to the median plane of the leg (2121).
External and internal claws usually of dissimilar size and shape.
One or both claws are rigid (broken line arrow).

Figure 15. Showing the differences in claw structures between three of the four super families. (NB. Images for Eohyspibioidea not available from personal collection.
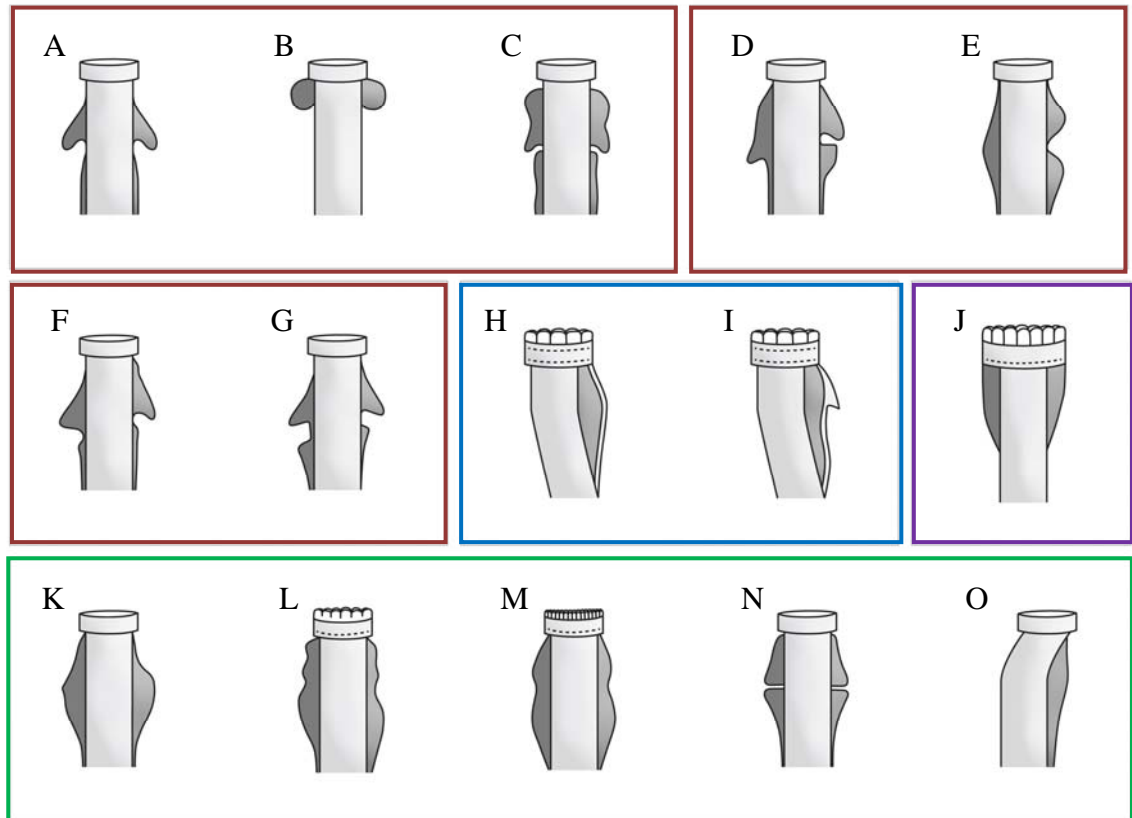
Images: Original.

Figure 16. Stylised diagrams of the apophysis for the insertion of the stylet muscles (AISM)

Hook shaped AISM of Hypsibioidea, Hypsibiidae, Hypsibiinae:
>   A *Hypsibius*, simple hooks,
>   B *Borealibius*, modified spherical hooks,
>   C *Acutuncus*, accentuated hooks.

Modified hook shaped AISM of Hypsibioidea, Calohypsibiidae:
>   D *Microhypsibius*, accentuated hook and hook with ridge.

Modified hook shaped AISM of Hypsibioidea, Microhypsibiidae:
>   E. *Calohypsibius*, triangular hook and triangular hook with triangular ridge.

Modified hook shaped AISM of Hypsibioidea, Ramazzottidae:
>   F *Hebesuncus*, asymmetric triangular and basic hook,
>   G. *Ramazzottius*, asymmetric hooks.

Ventral crest ASIM of the Macrobiotoidea, Macrobiotidae:
>   H *Macrobiotus*, asymmetric crest, 10 peribuccal lamellae present.

Ventral crest ASIM of the Macrobiotoidea, Murrayidae:
>   I *Dactylobiotus*, asymmetric crest with hook, 10 peribuccal lamellae present.

Ridge shaped AISM of Eohypsibioidea, Eohypsibiidae:
>   J. *Eohypsibius*, Broad ridges widening mouth with 14 peribuccal lamellae.

Ridge shaped AISM of Isohypsibioidea, Isohypsibiidae:
>   K *Isohypsibius*, simple triangular ridges,
>   L *Thulinius*, simple undulating ridges, 12 peribuccal lamellae present,
>   M *Pseudobiotus*, simple undulating ridges, 30 peribuccal lamellae present,
>   N *Mixibius*, modified ridges with small gap, and
>   O *Doryphoribius*, asymmetric crest ridge.

Images: Original.

A

Class   Order   Family

```
                                                          ┌──────── Archechiniscidae
                                                          ├──────── Batillipedidae
                                                          ├──────── Coronarctidae
                                                          ├──────── Halechiniscidae
                    ┌── ARTHROTARDIGRADA ─────────────────┤──────── Neoarctidae
                    │                                     ├──────── Neostygarctidae
                    │                                     ├──────── Renaudarctidae
  HETEROTARDIGRADA ─┤                                     └──────── Stygarctidae
                    │
                    │                                     ┌──────── Echiniscoididae
                    └── ECHINISCOIDEA ────────────────────┤──────── Carphaniidae
                                                          ├──────── Echiniscidae
                                                          └──────── Oreellidae

  MESOTARDIGRADA ──────────────────────────────────────────────── Thermozodiidae

                    ┌── APOCHELA ──────────────────────────────── Milnesiidae
                    │                                     ┌──────── Beornidae
                    │                                     ├──────── Eohypsibiidae
                    │                                     ├──────── Calohypsibiidae
  EUTARDIGRADA ─────┤                                     ├──────── Microhypsibiidae
                    └── PARACHELA ────────────────────────┤──────── Hypsibiidae
                                                          ├──────── Macrobiotidae
                                                          ├──────── Murrayidae
                                                          ├──────── Necopinatidae
                                                          └──────── incertae sedis
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B

Class   Order   Super Family   Family   Subfamily

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                 ┌── APOCHELA ──────────────────────── Milnesiidae
                 │
  EUTARDIGRADA ──┤              ┌── Eohypsibioidea ── Eohypsibiidae
                 │              │                            ┌── Ramazzottidae
                 │              │                            ├── Calohypsibiidae
                 │              ├── Hypsibioidea ──┤── Microhypsibiidae
                 │              │                            │                ┌ Diphasconinae
                 └── PARACHELA ─┤                            └── Hypsibiidae ─┤ Hypsibiinae
                                │                                            └ Itaquasconinae
                                │
                                ├── Isohypsibioidea ── Isohypsibiidae
                                │
                                │                        ┌ Macrobiotidae
                                └── Macrobiotioidea ─┤
                                │                        └ Murrayidae
                                ├ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ Beornidae
                                ├ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ Necopinatidae
                                └ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ incertae sedis
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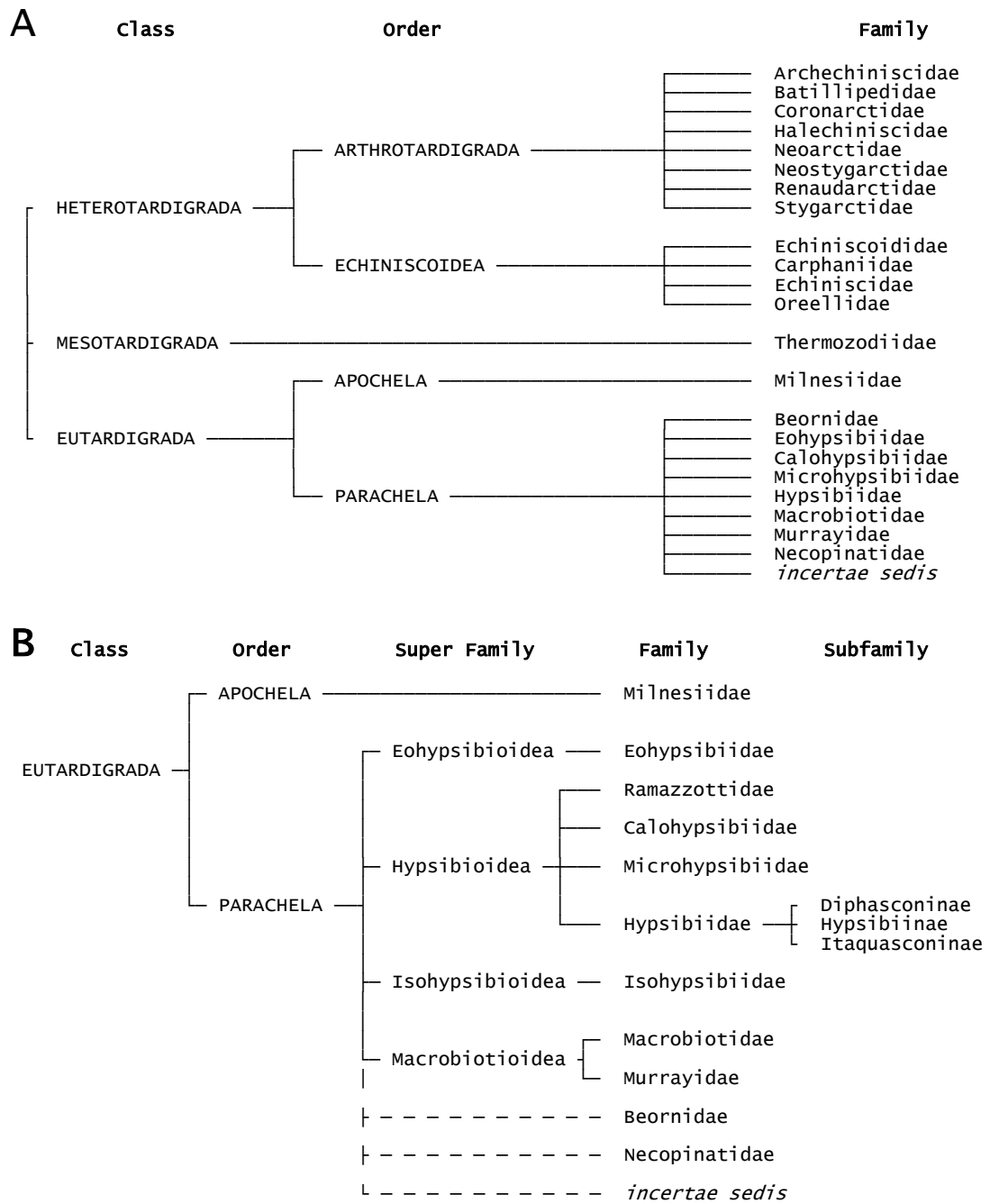
Figure 17. Phylogenetic topology of the Tardigrada.
A Phylogenetic topology of the Tardigrada based on the current systematic knowledge.
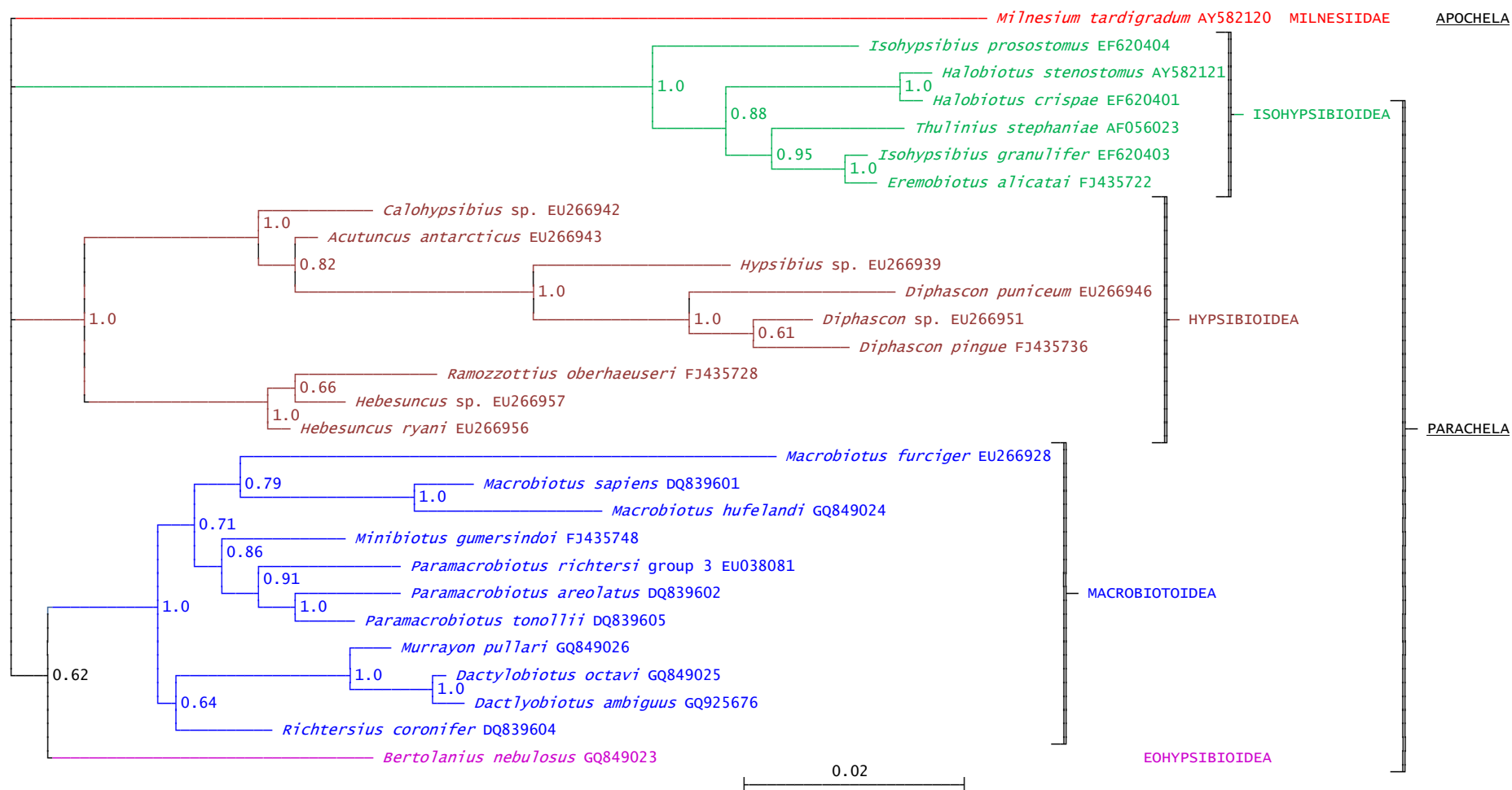B Proposed phylogenetic topology of the Parachela, with the new super families.

Figure 18. Tardigrade Bayesian phylogeny estimated using nearly complete18S rRNA sequence showing superfamily groups within the Parachela. Milnesiidae, Apochela was used as the out group. All data used are available from GENBANK. The analysis was conducted in 'Mr Bayes' using a 6 parameter model; node support values are 'posterior probabilities' based on 2×10^6 MCMC chains with a 25% burn-in; and line lengths are proportional to genetic distances.

ii) **Biodiversity**

Based at the British Antarctic Survey I have been able to conduct studies on the biodiversity of the depauperate Polar Regions. Antarctic terrestrial ecosystems in particular are relatively simple with the fauna limited to microarthropods, nematodes, tardigrades, rotifers and protozoans (Block 1984, Convey 2001). We have seen that the fauna can be very restricted, with the tardigrade fauna providing the main grazers and predators (Convey & McInnes, 2005).

In 1996 I was invited to join the 'Inlandsis' expedition organised by Janot Lamberton (Expéditions sous-glaciaires), which included a French team of spelaeologists, glaciologists and a botanist studying the cryoconites and moulins of the Inlandsis Glacier on Greenland (Figure 19). In the field, I collected samples and identified *Diphascon (Diphascon) recamieri* (Richters, 1911) as the most common tardigrades species, with bdelloid rotifers and protozoa, of the cryoconite habitat (unpublished notes). I also trialled two forms of aerial samplers, which initial field observations indicated they had not captured the larger propagules of tardigrade tun or eggs, but may have had smaller algal, fungi and chytrid spores. Sadly, the baggage with all samples was lost on the return journey.

The field trip did give me an understanding of the environment, so I was able to help when, shortly after returning from the field, a colleague who had been on the trip, and involved in earlier work on the Greenland glacier, asked for assistance writing-up the work for a Symposium which was presented in Grøngaard, Pugh, & McInnes (1999). In this paper we discussed the depauperate meiofaunal communities associated with cryoconite holes, supraglacial lakes and moulins of the Greenland Ice Sheet and noted that all the colonists of these habitats were generalists pre-adapted to cope with such extreme environments. We hypothesised that these colonists, though able to seed local similar habitats, were effectively trapped and survived *in situ* until washed into the glacier drainage system.
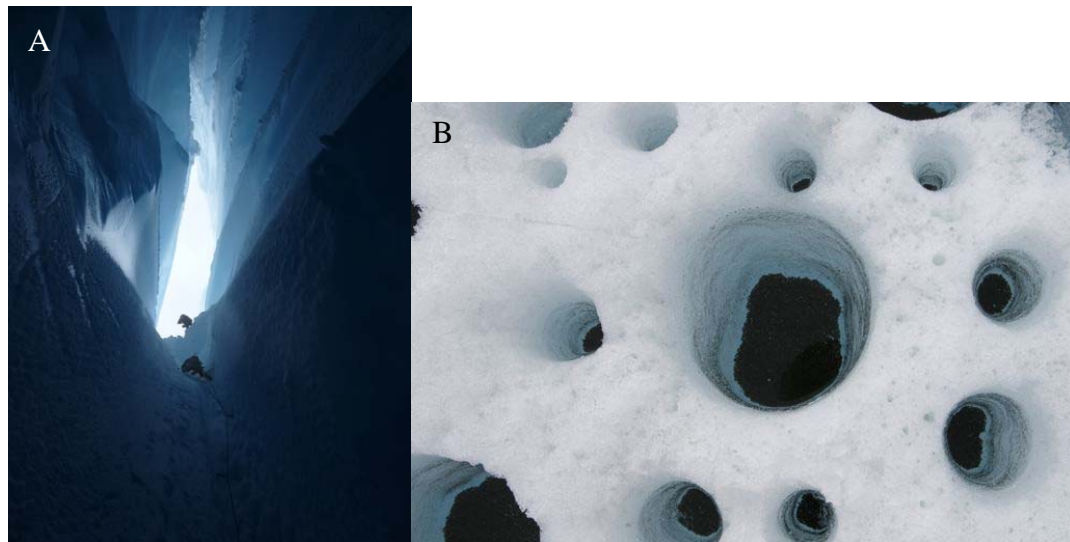
Figure 19. A Moulin, or glacier-mill, is formed by melt water on the surface forming streams and rivers which find holes or crevices and, by gravity, enlarge and deepen. Moulins can go all the way to the bottom of the glacier several hundreds of meters deep. B Cryoconite holes are water filled cylindrical melt-holes on glacial ice surface. Cryoconite is the dark material that absorbs solar radiation and promotes melting of the ice beneath it to form the cylindrical holes.
Images: Photo A: L'expédition *INLANDSIS* 2007, Janot Lamberton.
Photo B: website: icestories.exploratorium.edu.

While based at BAS I have been involved in several projects. In Downie, Convey, McInnes, & Pugh (2000) we created a baseline inventory of invertebrates for Deception Island, South Shetland Archipelago, from a small collection of terrestrial and freshwater habitat samples. Deception Island (62° 57'S, 60° 38'W) is a basaltic volcano formed ca. 100,000 years ago and now a horseshoe-shaped flooded caldera that still shows small scale activity, the most recent seismic event was in 1992. Despite this islands' activity it is a favourite tourist location where the geothermally heated ground and shallow water can create hot 'bathing pools'. My involvement in this project included the analysis of the freshwater samples, crustaceans and meiofauna, noting four (two tardigrades and two rotifers) species new to the island and several new additions that were not determined to genera (one harpacticoid copepod, three bdelloid rotifers, one gastrotrich, two nematode and two enchytraeids). The Deception Island samples yielded a total of 14 invertebrate taxa (the majority from the aquatic habitat), which when combined with the literature provided a faunal inventory of 57 invertebrate taxa (Downie *et al* 2000, table 3).

We discussed how the fauna, like the flora, show evidence of post-eruption colonisation by Antarctic and South American immigrant species, and historical human impact (Lewis-Smith 1988; Convey *et al* 2000). We concluded that this baseline inventory would allow future monitoring of natural and anthropogenic changes on Deception Island. Indeed, this paper will become the baseline from which to monitor the effects of tourism on maritime Antarctic soils as part of a grant programme run by a German research group for which I have been contracted to identify the Tardigrada.
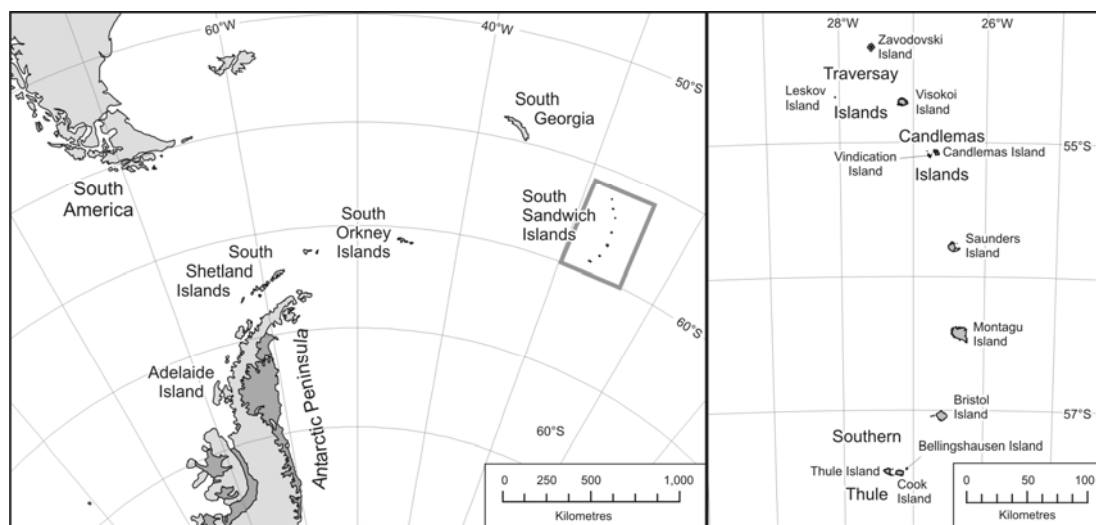
Figure 20. Map indicating the major islands in the South Sandwich archipelago, and the position of the archipelago relative to the Antarctic Peninsula and southern South America.

Images: Original, from BAS MAGIC.

Samples brought back by Pete Convey from an extensive biological field trip to the South Sandwich Islands (Figure 20) provided moss and lichen samples from which I extracted tardigrade fauna for McInnes & Convey (2005). These islands are, geologically, relatively recent (maximum age 0.5-3 million years), with some still active (Figure 21A), and lie on the outer edge of the Scotia Arc. This arc of islands, which includes South Georgia, South Orkney and South Shetland islands, link the Andean mountain chain of South America to the mountains of the Antarctic Peninsula. While species richness was low, concomitant with the recent age and isolation of these islands, population numbers were similar to those found at Signy Island, yet higher than those described for more temperate habitats. The biodiversity

showed no endemics and reflected the source of the colonists to have been from both sub-Antarctic, and maritime Antarctic, e.g. *Ramajendas* cf. *frigidus* (Figure 21B), which is in keeping with the results for arthropod fauna and bryophyte flora.

Figure 21. A Professor Pete Convey collecting samples on Saunders Island; and B *Ramajendas* cf. *frigidus* one of the species recovered from in the samples (scale bar 75 µm).

Images: Photo A, P. Convey; Photo B, Original.

A second collaboration with Pete Convey on samples he collected from Ellsworth Land at the continental end of the Antarctic Peninsula (Figure 22) resulted in Convey & McInnes (2005). There were no previous biological records from Ellsworth Land and therefore provided the first records for the 'Ronne Sector'. These samples revealed a community that included only

tardigrades and bdelloid rotifers, the most simple Antarctic metazoan community yet described. Only five species of tardigrade (Table 2) (three new to science – one: *Echiniscus corrugicaudatus* McInnes 2009, reported above) and two species of rotifer (*Adineta* sp. and *Philodina* sp.) were present. The absence of nematodes and micro-arthropods could be attributed to the extraction techniques. However, those techniques employed (Baermann (wet), Tullgren (dry) extraction and a floatation technique) are usually highly effective at removing these taxa from other similar habits. We concluded our evidence strongly supported the contention that arthropods and nematodes are absence or, at most, a very minor element of the fauna. The tardigrades included the predatory *Milnesium* cfr. *tardigradum* representing the tertiary tropic level of the habitat. Tardigrades e.g. *Diphascon sanae* Dastych, Ryan & Watkins 1990 and *Hebesuncus ryani* Dastych & Harris, 1994, indicate this area has links with continental Antarctic, and while *Milnesium* cfr. *tardigradum* has a pan-Antarctic distribution, comparative morphological analyses suggest the presence of distinct local forms. I am current working, in collaboration with molecular experts, to analyse whether these morphological differences in the *Milnesium* cfr. *tardigradum* group are distinct new species or population variants. The third group, *Echiniscus corrugicaudatus* and *Ramazzottius* sp., are, in the light of limited exploration and published literature, endemic to the area.
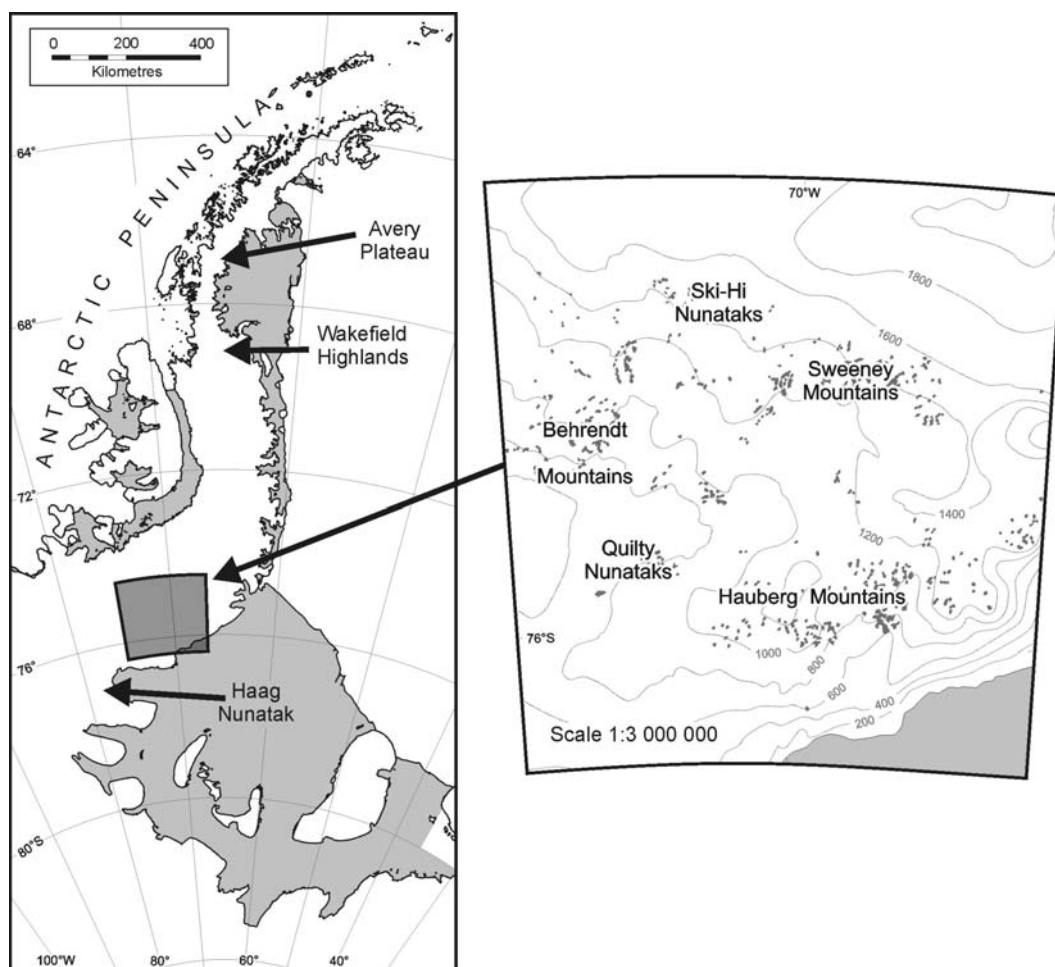
Figure 22. Map of the Antarctic Peninsula and inset of the mountain ranges samples in Ellsworth Land.
Light grey areas indicate the current extent of permanent sea ice.
Image: original, from BAS MAGIC.

Table 2. Tardigrades found at the Ellsworth Land sites (see above).

Tardigrade species	Sky Hi Nunataks	Behrendt Mountains (N)	Behrendt Mountains (S)	Quilty Nunataks	Haag Nunataks
Echiniscus pseudowendti			x	x	
Echiniscus corrugicaudatus			x		
Acutuncus antarcticus		x	x	x	x
Hypsibius sp			x		
Diphascon sanae			x	x	
Diphascon (Adropion) sp			x	x	
Hebesuncus ryanii	x	x	x	x	x
Ramazzottius sp				x	
Milnesium cfr. tardigradum			x		

Underlined species locally endemic

36

Investigation of Antarctic life has resulted in a number of extraordinary discoveries in environmental limits. In, Newsham, Maslen, & McInnes, (2006) we reported and discussed the survival of nematodes, tardigrades and a bdelloid rotifer which had been kept at -80°C for a little over six years. On 1 January 1999 a sample of the liverwort *Cephaloziella varians* (Gott.) Steph. was collected at Rothera Point, Adelaide Island, western Antarctic Peninsula and partially dried before being stored at -80°C. Six years later the sample was removed from storage and rapidly defrosted to *ca.* 10°C. Examination under the microscope yielded 159 nematodes of which 49 (31%), 15 tardigrades with two (13%) and 48 rotifers with two (2%) alive (Figure 23). Of the four species of tardigrade all eleven *Macrobiotus furciger* Murray 1907 were dead, as was the single *Echiniscus* sp. One of the two *Hypsibius* cfr. *dujardini* and the single specimen of *Diphascon* sp. both survived. Microscopic examination of the tardigrades after mounting showed no evidence of decomposition, in contrast with the bdelloid rotifers where most (*ca.* 90%) showed evidence of breakdown. Whilst some of the tardigrades formed the environmentally resistant 'tun', in the partial drying process before freezing, those that survived recovered almost immediately suggesting these were freeze-tolerant rather than freeze-avoidant animals.
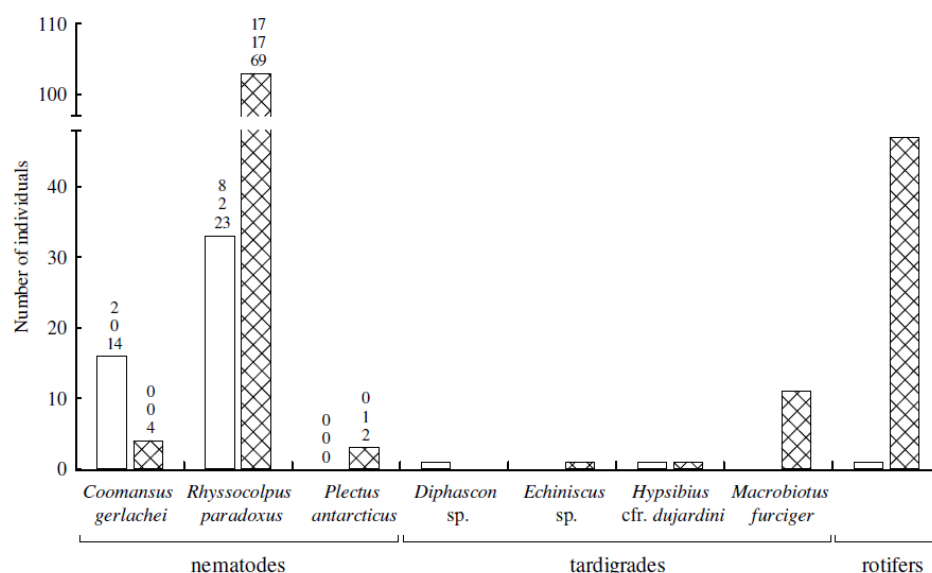
Figure 23. Numbers of live (□) and dead (▨) nematode, tardigrade and bdelloid rotifer individuals following storage at −80°C for six years and two months. Numbers above bars for nematodes are counts of adult males (top), adult females (middle) and juveniles (bottom) in each group. Note the split *y*-axis.

Image: from Newsham *et al* 2006.

During the period over which I have studied tardigrades changes in the methods we use have been constantly occurring. One such change was marked by a major divergence from traditional analogue film photography to the digital imaging. I became involved in organising digital imaging facilities for the BAS microscopes and was appalled to observe how easy it was to manipulate the new images. To highlight this aspect I wrote a short article, McInnes (2001), exposing the potential hazards of this technology with respect to the potential of forging "new" species (Figure 24). I concluded that editors and referees should be allowed access to original images if these had been in any way manipulated and then used to establish or verify a taxon.
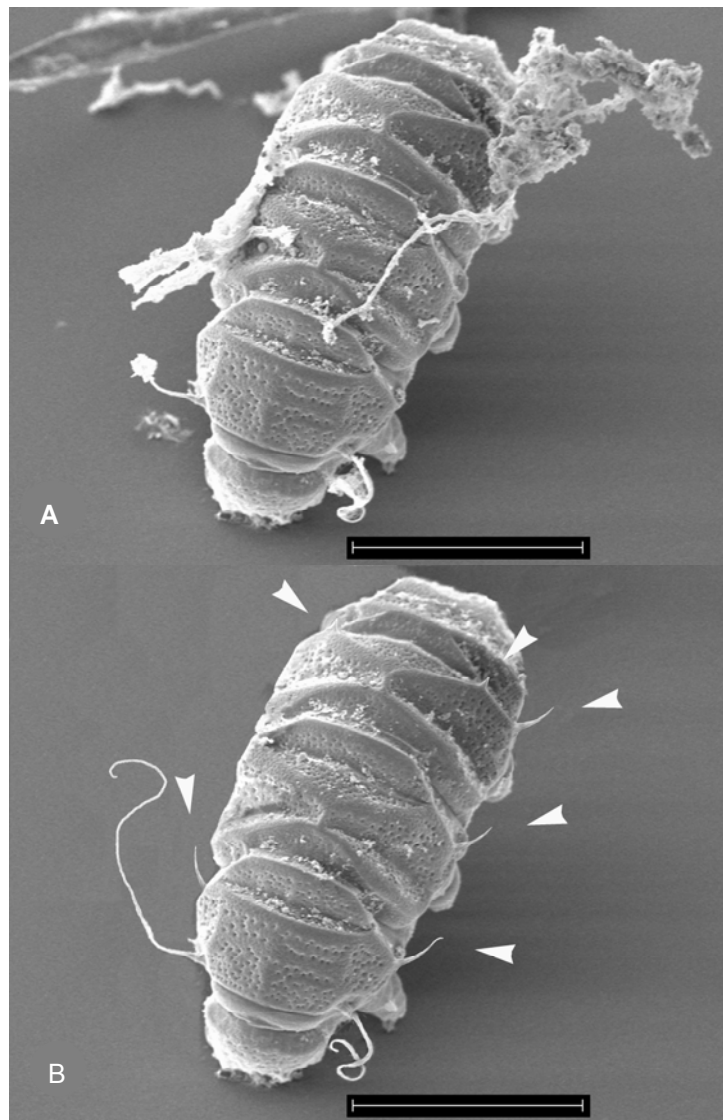
Figure 24. Using an SEM image to show how the original (A), can be altered to create a new species (B) (white arrows indicating additional features to create the apparent new species). Scale = 50 µm.   Images: Original.

Collaboration with the South African National Antarctic Programme (SANAP) provided samples collected from two high altitude sites on Marion Island, Prince Edward Archipelago (Figure 25), which offered additional information on the Tardigrada. We reported in McInnes, Chown, Dartnall, & Pugh (2001) that the carnivorous tardigrade *Milnesium* cfr. *tardigradum* (Figure 26) was present in reasonably large numbers despite a rather crude sampling method, though few if any of the smaller taxa were apparent. Relatively high numbers (43%) of these tardigrades were found with visible remains of prey in their guts (Figure 26B & C), providing 'forensic' evidence of smaller prey species missing from the original sample sorting. Using a more efficient technique we were able to increase the provided sample diversity and confirm the presence of the *Milnesium* prey taxa along with other co-occurring micro-fauna.
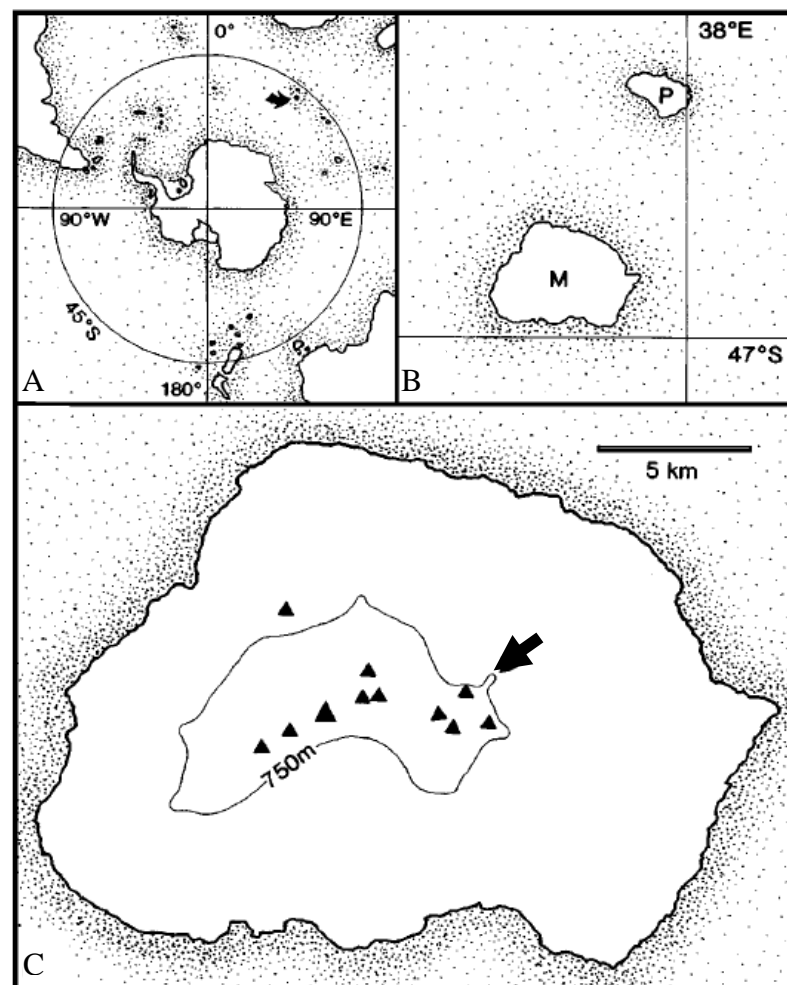
Figure 25. A Antarctica showing location of Prince Edward Islands (arrow). B Marion and Prince Edward Islands. C Marion Island sites with Katedraalkrans (arrow). Images: Original, drawn by P.J.A. Pugh.
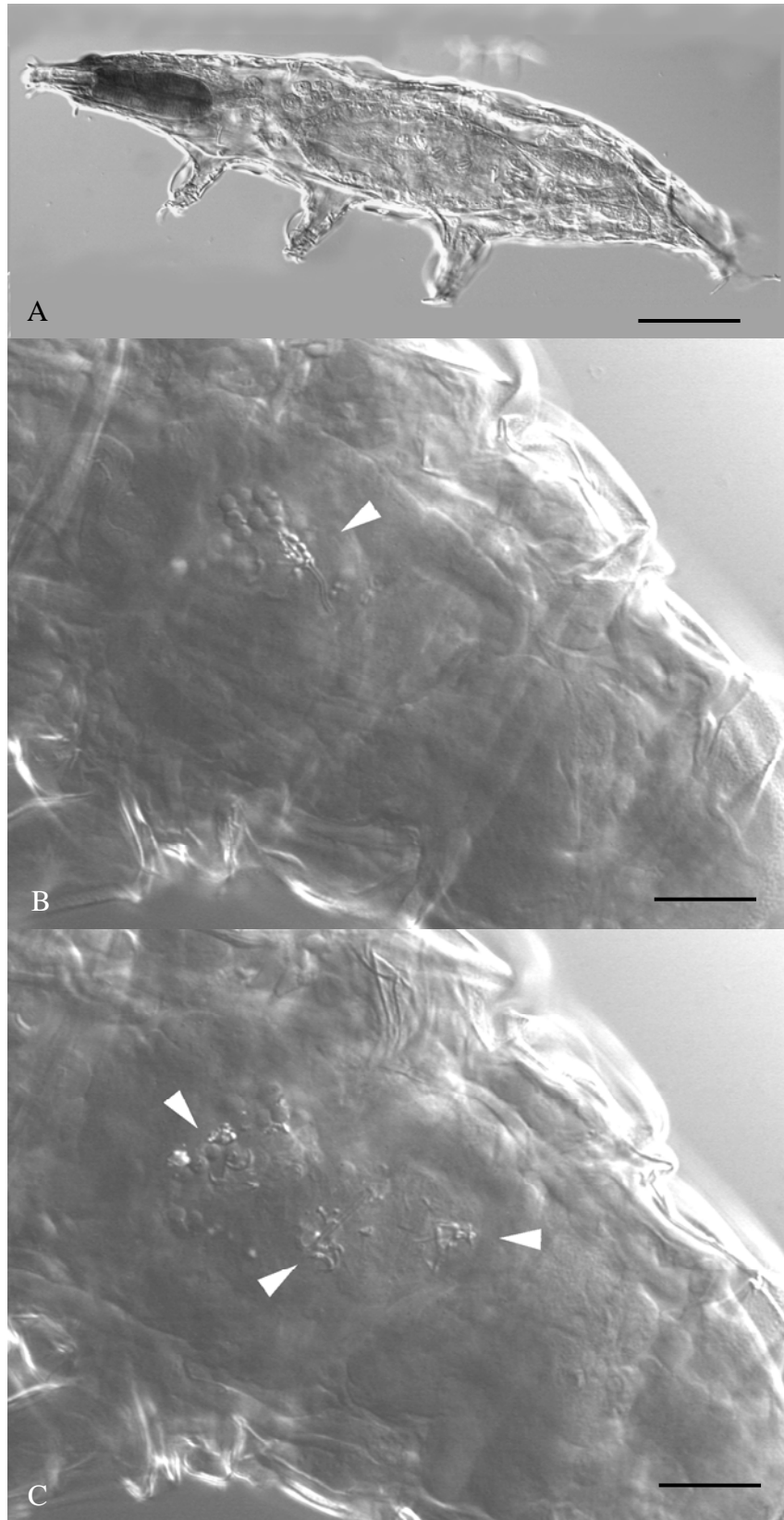
Figure 26. A. *Milnesium* cfr. *tardigradum* from Marion Island, Stained individual with bdelloid rotifer trophi in the stomach, DIC. Scale bar = 100 μm; B & C. Stomach contents of *Milnesium* cfr. *tardigradum* showing (B) placoids and (C) claws and mouthparts (arrows) of a new species of *Diphascon*. Scale bars = 20 μm.

Images: Original.

I was approached by Herbert Dartnall, of Macquarie University, Australia, to examine samples he had collected from freshwater lake sites at Commonwealth Bay, Wilkes Land, Antarctica, which we discussed in Dartnall, Scott, & McInnes (2007). This yielded two tardigrade species: *Acutuncus antarcticus* (Richters 1904) with an Antarctic wide distribution in permanent wet and semi-wet environments and its presence was not unexpected. Yet *Isohypsibius* sp. was more unusual as only *Isohypsibius improvisus* Dastych 1984 has been reported from mosses, lichen and algae on bird guano in Enderby Land, continental Antarctica. These examples from Commonwealth Bay differ in terms of morphology and by habitat preference from those from Enderby Land but the scarcity of samples precluded establishing a new species.

More recently I was approached by John Gibson, University of Tasmania, Australia, to identify tardigrades in lake sediment cores collected from maritime and continental Antarctica (Figure 27). In Gibson, Cromer, Agius, McInnes & Marley (2007) we discussed how the preservation of tardigrade eggs and exuviae in Antarctic lake sediments provided an opportunity to assess post-glacial colonisation and Holocene tardigrade dynamics. Tardigrade eggs were recovered from five lakes - two in the maritime Antarctic and three from continental Antarctica. I was able to identify the five ornamented egg types to: *Dactylobiotus* sp. (cf. *ambiguus*) (Figure 28A), *Macrobiotus furciger* Murray 1907 (Figure 28B), *Macrobiotus blocki* Dastych 1984, *Minibiotus weinerorum* (Dastych 1984) and *Acutuncus antarcticus* (Richters 1904). Other, unornamented eggs were also observed but not identified to species.
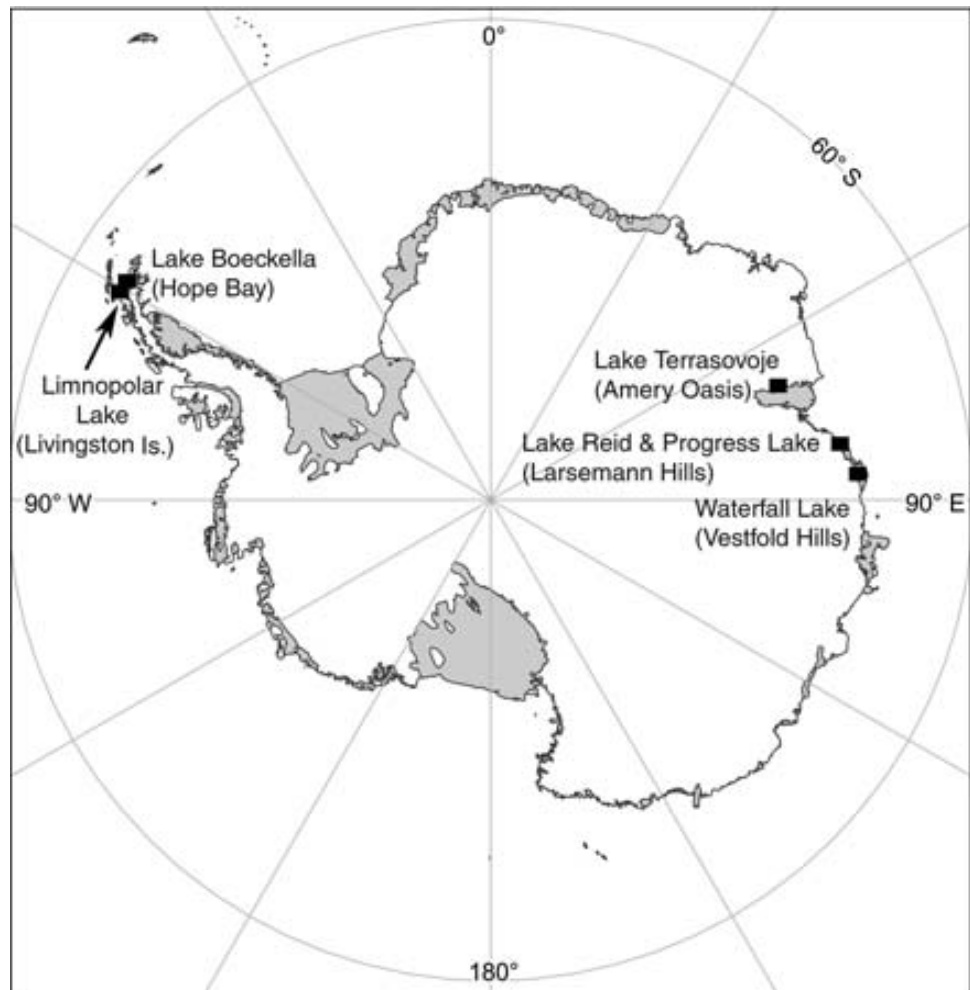
Figure 27. Map of Antarctica showing the locations of the lakes from which cores were obtained. (Grey areas are regions of permanent sea ice).
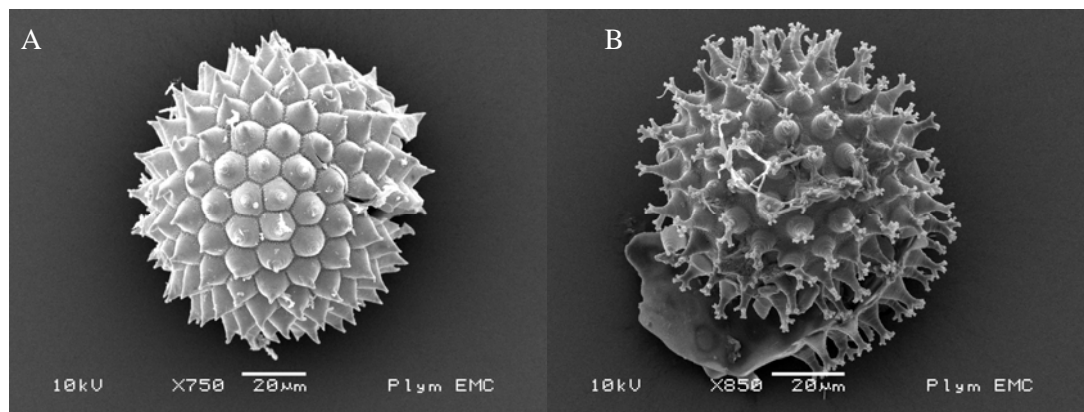
Image: from Gibson, *et al.*, 2007.

Figure 28. A. Example of *Dactylobiotus* cfr.*ambiguus* egg from Boeckella Lake, maritime Antarctic (no longer present in the lake); and B. *Macrobiotus furciger*, which shows subtle differences when compared with modern eggs of this species.

Images: original, with acknowledgement to the Plymouth Electron Microscopy Unit.

It seemed surprising that tardigrades generally did not become abundant for a considerable period after the onset of biological sedimentation within the lakes. For example, tardigrade remains are present in samples dating to *ca.* 20,000 yr BP in Lake Reid, Larsemann Hills, Enderby Sector, for which the earliest deposits are *ca.* 130,000 yr BP. Scattered occurrences of eggs prior to this increase in abundance suggests that tardigrades were present in the lake basins soon after their formation, but that it took considerable time for the correct ecological conditions to establish and support significant populations of animals. However, the earliest presence of *A. antarcticus* eggs (*ca.* 42,000 years BP lakes Reid and Progress - earliest sediments from both these lakes were deposited *ca.* 130,000 yr BP) and the absence of tardigrade eggs in the older sediments could reflect bacterial breakdown rather than absence of the animals from the lake, and this therefore provides an estimate of the longevity of the eggs in the sediment. Significant variations were observed in egg abundance within the sediment of each lake including, the more recent deposits, suggesting that *Dactylobiotus* cf. *ambiguus* became locally extinct in Boeckella Lake, Antarctic Peninsula, while other species continued. Unpublished data from my collection included samples collected from the shallow regions around Boeckella Lake which showed no evidence of *Dactylobiotus* cf. *ambiguus* thus corroborating this evidence. We concluded that the extinction probably followed eutrophication of the lake and increasing anoxia within the benthic sediments, as the decline of *Dactylobiotus* cf. *ambiguus* occurred inversely to the rise in penguin numbers at nearby rockeries (Gibson & Zale 2006).

This low level endemism is consistent with slow colonisation from local Antarctic sources rather than airborne tardigrade propagules transported from extra-continental sites. Tardigrade eggs appear to be abundant in high-latitude lake sediments, and greater use could be made of these records when evaluating Holocene tardigrade dynamics. This subject was revisited in Cromer, Gibson, McInnes, & Agius (2008) with emphasis on how the conservation of tardigrade eggs and exuvia in lake sediments provides a unique insight into the understanding of paleo-distributions and effects of environmental changes.

As an internationally recognised expert on Tardigrada I have been asked to write several chapters for books and encyclopaedias. McInnes (2007 and accepted) are two such examples. The former was specifically Antarctic-centred view point and the later a more general introduction. More recently, I have been approached for sections within books (Steven Chown - SCAR Terrestrial Biodiversity Review; Diego Fontaneto (Artois et al., in press) - Biogeography of microorganisms. Is everything small everywhere?). In a chapter Nelson & McInnes (2002) we wrote in depth about the Tardigrada in general and more specifically about freshwater taxa. In this publication I provided a dichotomous key to all terrestrial tardigrade families, the first since Ramazzotti & Maucci (1983), including major changes in terms of new families and taxonomic issues, which required a review of all the literature, revising the earlier familial descriptions, genera and taxonomic issues, before building a new key from basic principles.

In the recently published paper by Hodgson, *et al.* (2010) on the limnology and biology of the Dufek Massif (82° 24' S; 52° 12' W) in the Transantarctic Mountains (Figure 6), my role was to identify the tardigrades found in this extreme habitat and supply details of their distribution and biology, as well as assisting in writing the paper. Three species were present, *Acutuncus antarcticus* (Richters, 1904), *Diphascon sanae* Dastych, Ryan and Watkins, 1990 and *Echiniscus* (cf) *pseudowendti* Dastych, 1984. There were sufficient numbers of *Acutuncus antarcticus* to allow molecular analysis of the ribosomal 18S gene, which proved almost identical to populations from the Peninsula and islands.

Both flora and fauna of this area was recorded as impoverished, with diversity and abundance more limited than the coastal and lower latitudes. The Dufek Massif, like the Ellsworth Land samples, had a very limited fauna of tardigrades and bdelloid rotifers. All the tardigrades have Antarctic biogeographic distributions, with *Acutuncus antarcticus*, having the broadest range being continental, maritime and sub-Antarctic, *Diphascon sanae* has been reported from Dronning Maud Land (Robertskollen), Enderby Land

(Prince Charles Mountains and Mawson Station) and Ellsworth Land, and *Echiniscus (cf) pseudowendti* from maritime regions of the Antarctic Peninsula, Dronning Maud Land (Heimefrontfjella) and Enderby Land (Thala Hills). However, the flora indicated a mix of more cosmopolitan taxa with some endemics.

The very limited diversity of all the species in this study supports the hypothesis that, in the more extreme and remote regions of continental Antarctica, species assemblages present are characterised by a low biodiversity, taxa tolerant of extreme cold and dry conditions, and a certain degree of endemism. Such results imply that the Dufek Massif has not functioned as a biological refuge over long timescales (cf. Convey et al., 2008, 2009), but has been colonised during the Quaternary by a combination of Antarctic endemic and cosmopolitan taxa whose distribution, dispersal and establishment has been dependent upon life cycle characteristics (e.g., formation of resting spores and resistance to the extreme environmental conditions).

iii) <u>Databases and data based studies</u>

<u>Tardigrada</u>

My earlier publication on terrestrial tardigrade biogeography (McInnes, 1994) was the first attempt to place tardigrades in a global context, caused major interest amongst tardigradologists and is still used as a reference for new studies (Web of Science citations = 81) despite being somewhat dated. This very simple database was held as a MS Word document (see Figure 29), with associated files for references and countries. This has proved a
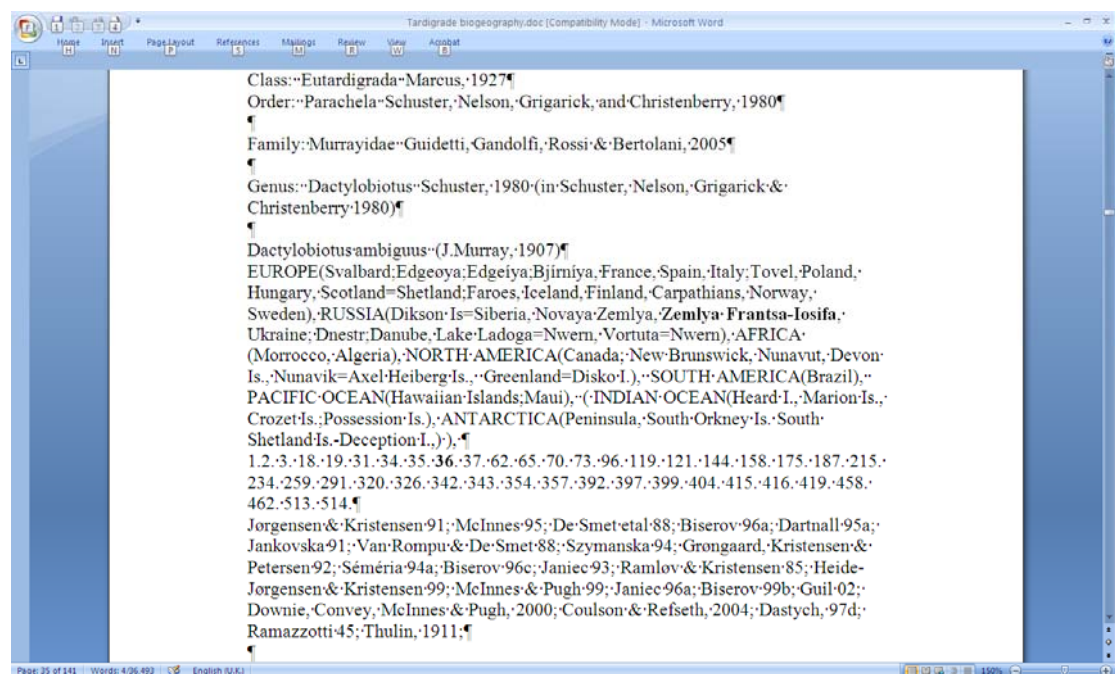
Figure 29. Screen print of original tardigrade database in MS Word, showing *Dactylobiotus ambiguus* (Murray, 1907).   Image: Original.

rather unwieldy form from which to retrieve data, though it is still being kept up to date with new references as they are received. My database is well known amongst tardigradologists, so I am often solicited directly for up-to-date information about the tardigrade data for different countries. I am presently in the process of transferring the database to MS Access (See Figure 30) which offers more scope for interrogation of the data and the isolation of more fields, e.g. habitat type.

Figure 30. Screen print of current tardigrade database in MS Access, showing *Dactylobiotus ambiguus* (Murray, 1907). Image: Original.

It was directly as a result of McInnes (1994) and the database that I was asked to be the 'Group Co-ordinator' of Tardigrada for Fauna Europaea (McInnes 2004) (Figure 31). This, in turn, lead to a paper based on the entire Fauna Europaea dataset (Fontaine *et al*, 2007) to review the aims of the European Union to halt the loss of biodiversity by 2010 in light of the indicators chosen to assess the progress. We pointed out that many of the European fauna are "rare", in that they have restricted ranges, which are precisely those most at risk. The European directives' indicator species that had been chosen were more representative of 'protected species' than 'threatened species', implying a significant number of extinctions could occur unnoticed. In a second manuscript (Fontaine *et al*, in prep.) we discuss that
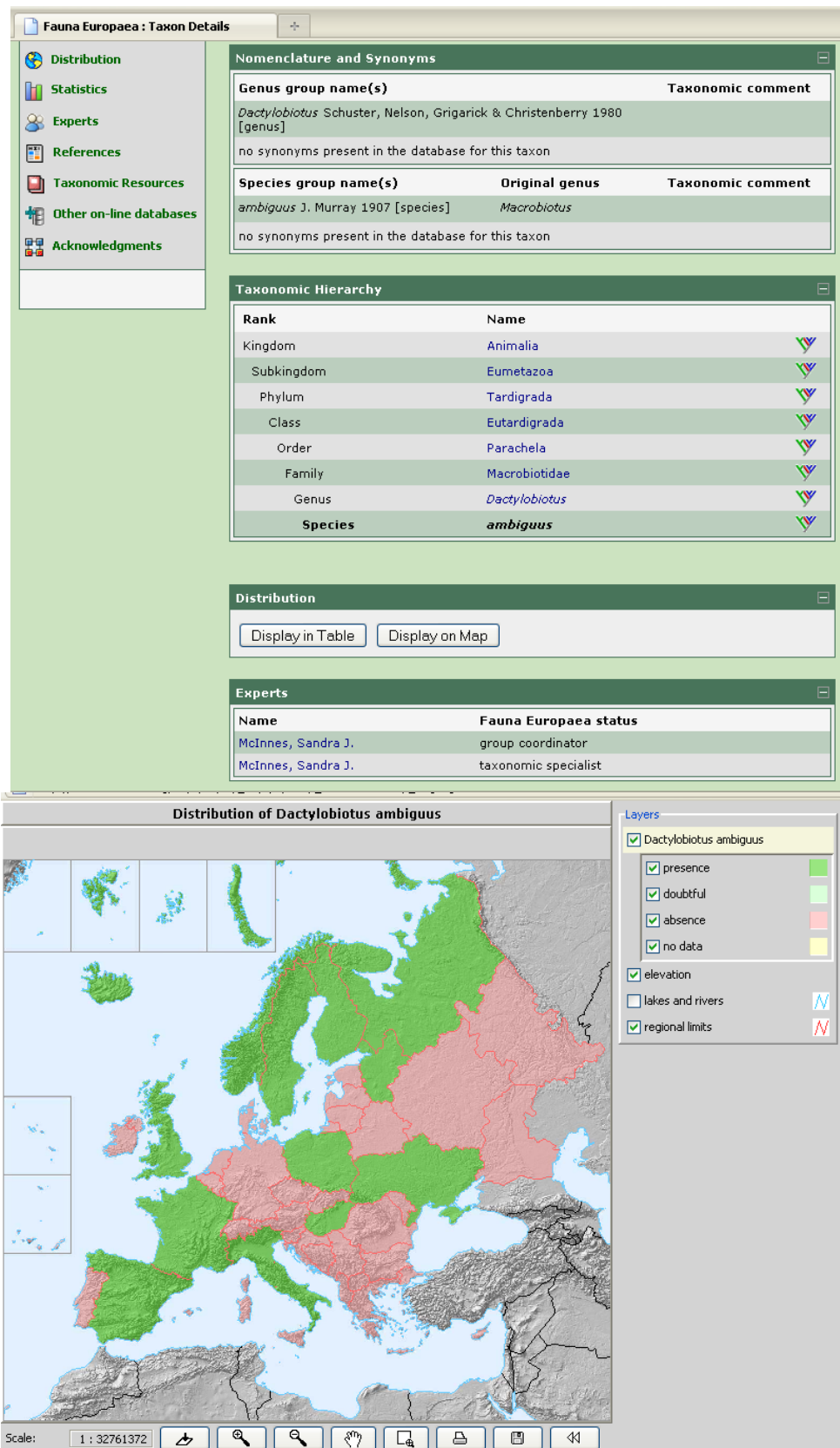
Figure 31. Screen shots of Fauna Europaea showing European data and distribution for *Dactylobiotus ambiguus* (Murray, 1907).
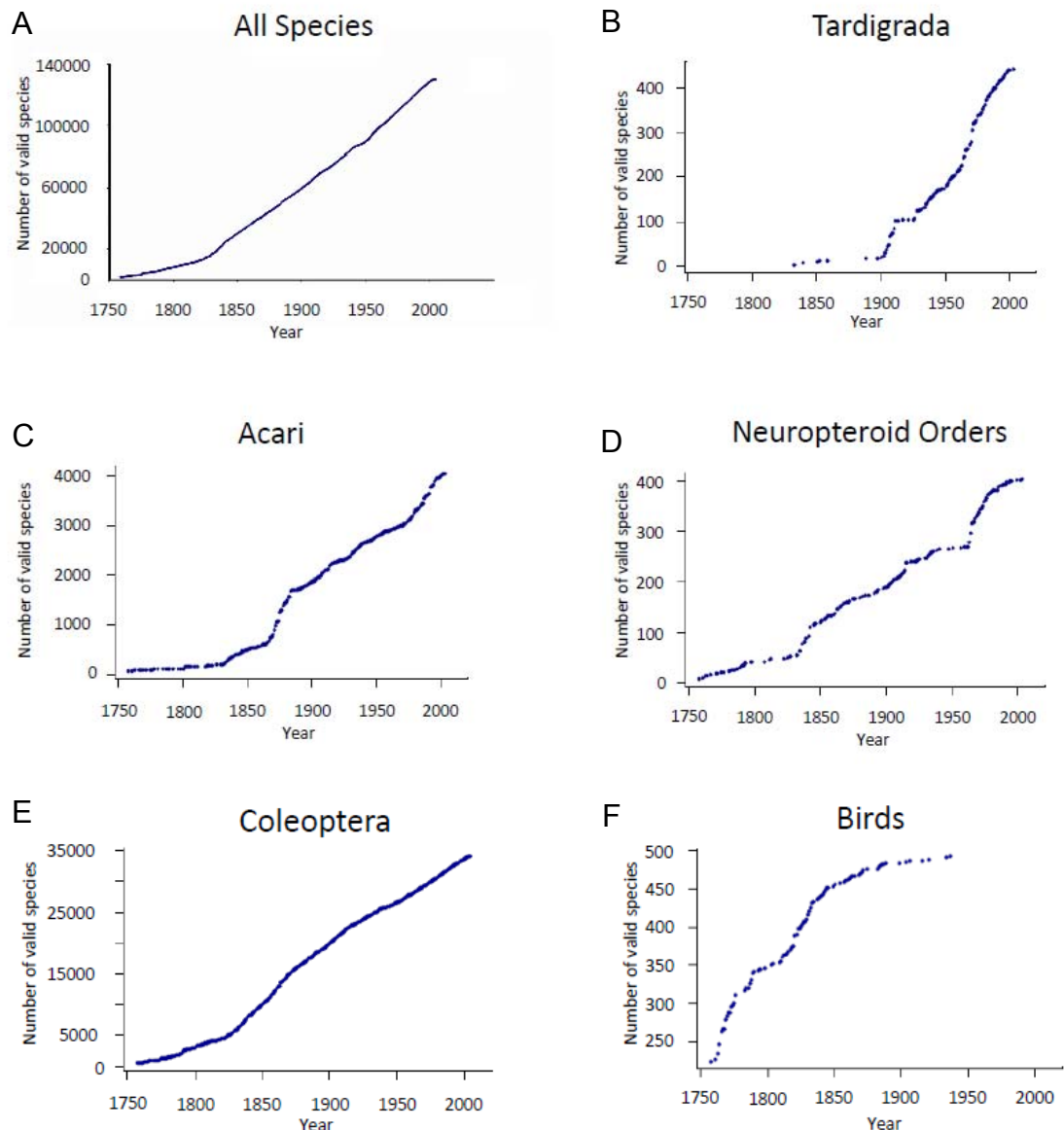
Image: from, www.faunaeur.org

Figure 32: Growth of the European taxonomic inventory. Cumulative number of valid species of terrestrial and freshwater multicellular animals recorded in Europe since Linnaeus (1758).
A: All species.
B: Tardigrada, a taxon which, because of the small size of individuals, remained neglected for two centuries, and is now exhibiting an exponential discovery rate.
C: Acari, another taxon which is exhibiting an exponential discovery rate.
D: The neuropteroid orders of insects, for which the rate of description is erratic and reflects bursts of activity by a handful of taxonomists.
E: Coleoptera, a taxon where the number of valid species has steadily increased and shows no sign of levelling.
F. Birds, a virtually complete inventoried compartment of European biodiversity.
Image: from Fontaine, *et al,*. In press.

while it is generally perceived that the frontiers of biodiversity exploration and discovery are in the tropics and/or the developing countries, contrary to this conventional wisdom, developed countries also remain seemingly inexhaustible reservoirs of unknown species. An analysis of the cumulative number of valid species reported through time shows that, despite the most formidable geographical concentration of taxonomical expertise over two and a half centuries, the European data have not yet reached a plateau (Figure 32). These discovery curves suggest that the increment pattern is limited by the size of the taxonomic workforce, rather than by the size of the reservoir of new European species. Over 60% of the new species are currently described by non-professional or retired professional taxonomists. We suggest that, rather than lament this state of affairs, more attention should be given to ways of encouraging, harnessing and guiding this redoubtable resource of 'citizen science'. Just published is a correspondence note in *Nature* (Fontaine *et al.* 2010) summarising this work.

Freshwater crustaceans

My familiarity with databases and my experience in the Antarctic freshwater fauna has resulted in collaborative work on the freshwater crustaceans of the Antarctic, which could be used to explore biogeography of an altogether different set of animal groups which are more dependent on water and potentially more limited in terms of dispersal than the Tardigrada. As with the early tardigrade database this was organised as simple MS Word documents. We presented in Pugh, Dartnall & McInnes (2002) 250+ records for 105 verified taxa from eight ordinal groups, the first time this data had been brought together in one publication and is still well cited in Antarctic limno-terrestrial crustacean studies (Web of Science citations = 34). We reported that the impoverished and depauperate fauna represented two distinct ecological / taxonomic cohorts. The freshwater taxa, incorporating the 'microcrustaceans', which occur throughout the continental, maritime and sub-Antarctic, with a few endemics, and the mainly terrestrial taxa from the Isopoda and Amphipoda, which include no Antarctic species. It would

appear that the Holocene sea level rises created a fragmented, largely vicariant set of species ranges across the Southern Ocean Islands, while the freshwater faunas of the 'Maritime' and 'Continental' Antarctic are entirely dissimilar. We proposed the argument that the presence of *Boeckella poppei* (Mrázek 1901) on the continent was probable due to anthropogenic alien importation, as there were no data at the time to support even a single lake as a viable refugium for a pre-glacial aquatic fauna. Though this opinion has now been challenged with new data (Bayely *et al.* 2003; Gibson & Zale 2006). We pointed out that many of the Crustacean propagules are tenacious and a number of taxa have been introduced via anthropogenic mechanisms to some Southern Ocean islands and possibly Continental Antarctica. This was an important observation, which provided a base-line from which current reviews and preventions of alien introductions could be based. This database and paper is due for revision as the more recent studies have corrected some of the significant distribution and/or systematic data anomalies we highlighted and filled some of the gaps in the overall knowledge.

iv) <u>Biogeographic analysis</u>

My database with all the details from the literature provided a unique source of information on which to base biogeographic studies of the Tardigrada. Following the 'Inlandsis 1996' expedition, to study tardigrades in Greenland, despite the lost samples, I returned to my database of tardigrade literature to review the possible biogeographic signals within the data. Pugh & McInnes 1998, explored three possible scenarios to account for what appears to be an indistinct biogeographic distinction between Nearctic and Palaearctic extant tardigrade fauna of the Arctic regions (Figure 33). The first possibility is that this represents the survival of many relict pan-Arctic tardigrades. The second that Pliocene Arctic tardigrades were driven south into Europe and North America by the advancing ice-caps, but returned north during the post-Pleistocene (= Holocene). Alternatively, all Pliocene/earlier Arctic tardigrades were obliterated by Pleistocene glaciation and replaced, during the Holocene, by a common non-Arctic fauna.

We considered seven Arctic locations (Axel Heiberg I. (44 recorded tardigrade species), west Greenland (including Disko I.) (79), east Greenland (33), Iceland (55), Svalbard (65), Novaya Zemlya (39) and the Taimyr Peninsula (68)). To these we included the Alps, Northern Europe, and Northern North America. Antarctica was used as an outgroup. A simple cluster analysis of the data (Figure 34) indicated a crown cluster of the Arctic sites with close affinity with Northern North America, Taimyr Peninsula and Novaya Zemlya. The Alpine / Northern European cluster is more distant and, as expected, Antarctica formed an outlier. We did find the Hypsibiidae are a significant and dominant family in the polar Tardigrada but are not radically different from the Macrobiotidae, Isohypsibiidae or Echiniscidae in terms of cold tolerance (cryptobiosis), aerial dispersal or parthenogenesis (vegetative reproduction), factors that are common to all cryoconite fauna and flora. We suggested that the dominance of the Hypsibiidae was more likely to be related to food source, many are hydrophylic, bacteriophages and/or algivores (e.g. Hallas and Yeates 1972),

advantageous factors for colonising nunataks, cryoconite holes or similar damp glacial habitats.
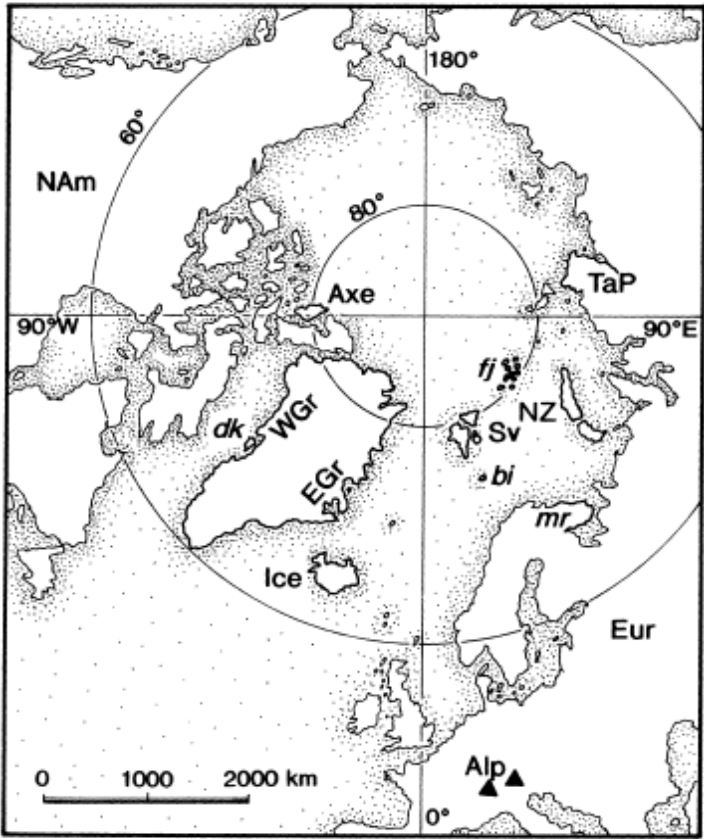

Figure 33. Map of localities. Localities shown in roman letters were included in cluster analysis (see Fig. 2); those in lower case italics were not (Alp The Alps; Axe Axel Heiberg I., bi Bear I.; dk Disko I.; EGr East Greenland; Eur northern Europe; fj Franz Josef Land; Ice Iceland; mr Murmansk area; Sv Svalbard; NAm northern North America; NZ Novaya Zemlya; TaP Taimyr Peninsula; WGr West Greenland). Image: from Pugh & McInnes, 1998.
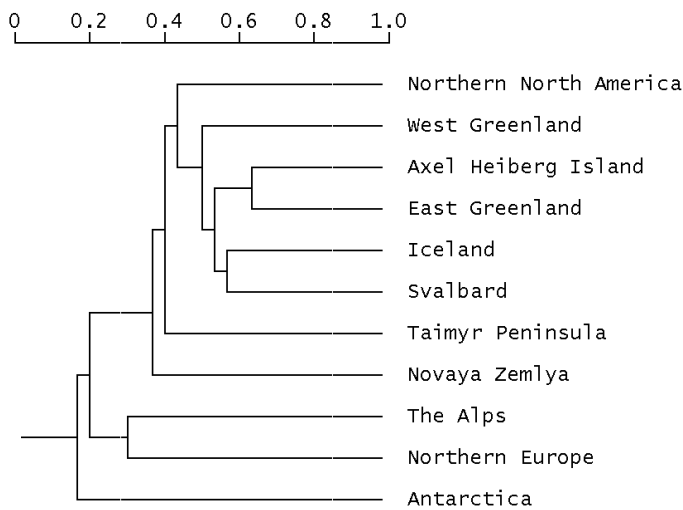

Figure 34. WMPGA cluster analysis of Sorensen (Ss) coefficients.
Image: Original.

The records indicate that it was probably rare for any of the extant tardigrade fauna to have survived in peri-glacial habitats through the Pleistocene glaciation. No 'specialist' glacial habitat tardigrades have been reported from the high Arctic, though recent work (e.g. Dastych, 1993; 2004; 2009) suggests there may be 'specialist' glacial tardigrade species in the Alps and Himalayas. We concluded that tardigrades must have colonised, or re-colonised, the Arctic during the Holocene. However, there is only limited indication of 'local' fauna, i.e. North American species in the Nearctic and European species in the Palaearctic. Nearly, three quarters of the Arctic tardigrades occur in the temperate Holarctic, with many 'American' species occurring throughout the Arctic while 'European' species are largely confined to the Palaearctic. The prevailing winds during the Holocene were, and continue to be, circumpolar from the west. With light airborne propagules (eggs or adults in resistant stage 'tuns') tardigrades were, and still are, dispersed into deglaciated Arctic terrain particularly those from Northern America.

As a direct result of my database (McInnes 1994) and biogeographic publications (McInnes & Pugh 1998; Pugh & McInnes 1998), Jim Garey and Brent Nichols requested that I joined with them to provide an invited paper for a dedicated journal issue on the global diversity of freshwater fauna. While all limno-terrestrial tardigrades are reliant on freshwater, very few are truly hydrobiotic. In Garey, McInnes, & Nichols (2008) we listed the 62 species from 13 genera which represent four families and genera (*Carphania*, *Dactylobiotus*, *Macroversum*, and *Pseudobiotus*) that were found to be exclusively aquatic. *Thermozodium* was included but, as discussed earlier, this is a dubious taxon. The limited literature on these animals precluded any analysis, of distribution within a habitat or habitat requirements, as most of the studies were essentially species lists from different regions. Figure 35 shows that the northern hemisphere appears to have the greatest diversity, particularly the Palaearctic region, which we suggested resulted from the more intensive sampling in Europe compared to elsewhere, a hypothesis supported by the literature database (McInnes, 1994 and unpublished updates).
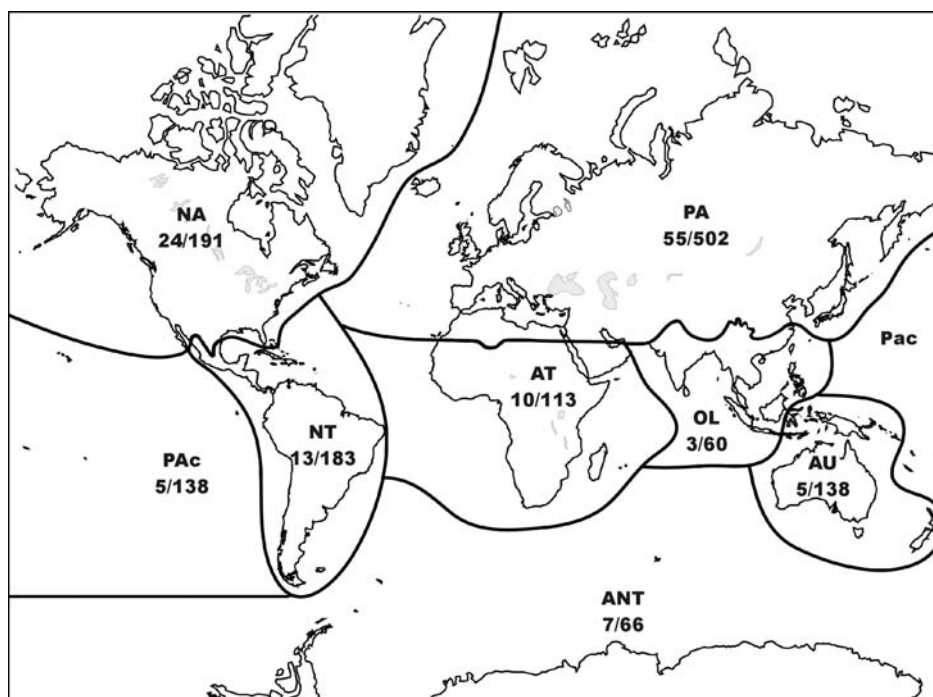
Figure 35. Biogeographical map divided into zones. The number preceding the slash represents the number of tardigrade species that are found exclusively in freshwater aquatic habitats. The number after the slash represents the number of genera with at least one species known to be found exclusively in freshwater aquatic habitats.
PA = Palaearctic, NA = Nearctic, NT = Neotropical, AT = Afrotropical, OL = Oriental, AU = Australasian, PAC = Pacific Oceanic islands, ANT = Antarctic.

Image: from Garey, *et al*., 2008.

Drawing together the geographic details within the database (McInnes 1994) opened the way to exploring the global biogeography of the non-marine Tardigrada. In the original paper (McInnes and Pugh 1998), this was analysed using a simple 'weighted mean pair-group analysis' (WMPGA) of taxa present or non-recorded (Sokal & Sneath, 1963), which provided the first attempt at studying the global biogeography of such a dataset.

The Tardigrada is a cosmopolitan phylum of pre-Pangaean origin, yet tardigrade families and genera showed distinct biogeographic components isolated by two major geological events. Separate Laurasian and Gondwanan familial clusters correlate with the Triassic disintegration of Pangaea, while discrete Antarctic, Australian and New Zealand familial/ generic clusters relate to the subsequent Jurassic/ Cretaceous disintegration of Gondwana. We interpreted the results of this analysis as indicating an ancestral origin in the Laurasian region with the spread southwards to a

derived Gondwana fauna, as being the best fit to plate tectonics. A larger database (70% increase) and more widely accepted analyses prompted a re-evaluation of the dataset (McInnes & Pugh 2007). Again, we used a simple clustering algorithm of taxa present or non-record (1/-), being unable to distinguish between species absence and lack of records. Instead of analysing the data by hand, as had been done in the earlier paper, we exported Excel 'species' and 'generic' spreadsheets to the Multi-Variate Statistical Package (MVSP – Kovach 1999) for the cluster analysis and principal components analysis (PCA) (Figure 36). We checked the results by repeating the cluster analysis with alternative but valid cluster algorithms, such Jaccard coefficient (S_J) for species data and the simple matching coefficient (S_{SM}) for more robust numerical generic data. Similarly, replacing UPGMA clustering with single-linkage (= nearest neighbour) and complete linkage (= furthest neighbour) clustering also yielded virtually identical clustergram topologies. The analysis produced very similar results which proved the robustness of the data and confirmed the original work (McInnes & Pugh, 1998).
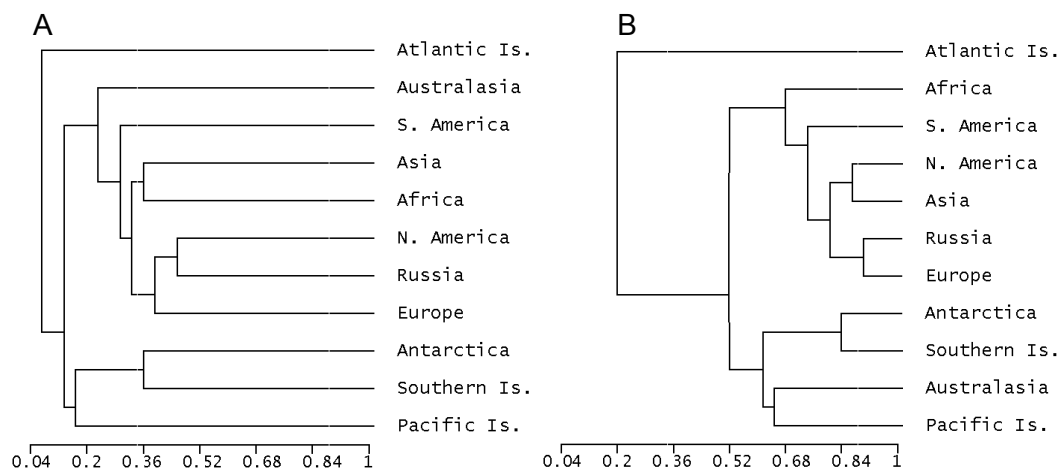

Figure 36. WMPGA cluster analysis of Sorensen (Ss) coefficient of limno-terrestrial tardigrades. A Species; B Genera.   Image: Original.

Though, in theory, easy to interpret, clusters are not necessarily statistically valid (Pillar 1999), so we applied 'parsimony analysis of endemism' (PAE) (Rosen 1988) using the method of De Grave (2001) to two bootstrapped parsimony routines within the 'PHYLIP' suite of programmes (Felsenstein

1989, 2005). We used 'MIX', a Wagner-parsimony routine that equal weights forward changes and reversals (Kluge & Farris 1969), thus providing a dispersal proxy; and second 'DOLPENNY', a Dollo-parsimony branch and bound routine, that minimises (1 → 0) reversal (here emigration or extinction) relative to (0 → 1) forward change (here immigration) (Farris 1977), providing a non-dispersal (i.e. vicariance) proxy. PAE, unlike cluster analysis, implies a direction from a basal regional clade to a more derived crown clade.
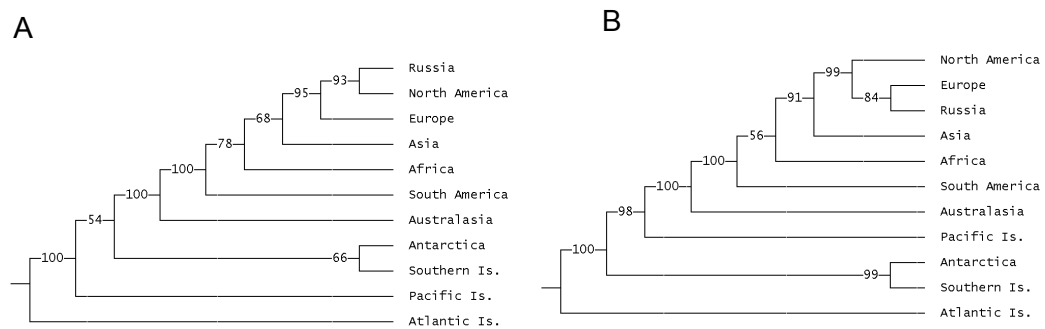

Figure 37. A PAE regional cladogram of limno-terrestrial tardigrade species under Dollo parsimony (vicariance proxy) criteria. B PAE regional cladogram of limno-terrestrial tardigrade species under Wagner parsimony (dispersal proxy) criteria. Numbers indicate percentage bootstrap consensus values of each regional clade.
Image: Original.

Figure 38. A PAE regional cladogram of limno-terrestrial tardigrade genera under Dollo parsimony (vicariance proxy) criteria. B PAE regional cladogram of limno-terrestrial tardigrade genera under Wagner parsimony (dispersal proxy) criteria. Numbers indicate percentage bootstrap consensus values of each regional clade.
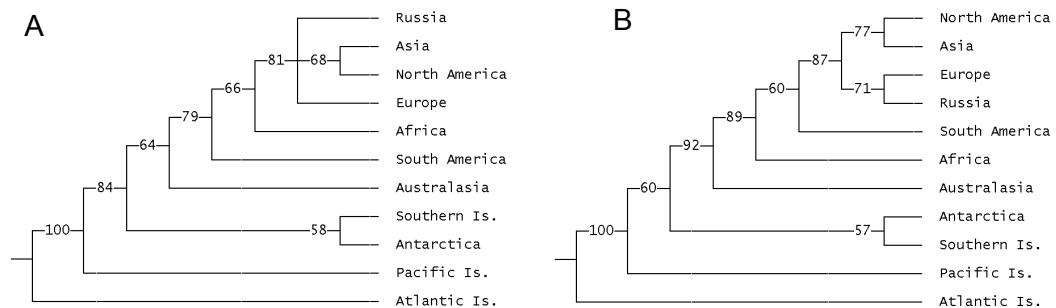Image: Original.

The results of this analysis, at both species and generic level (Figs 37-38), produced a stable Holarctic (North America, Russia, Europe) crown cluster and more basal southern (Antarctica, Southern island) Pacific and Atlantic island basal regional clades under both Dollo and Wagner parsimony

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routines. The similarity in the results of both 'dispersal' and 'vicariant' proxy analyses shows no major evidence for either process. Vicariance could take precedence over dispersal but, either way, PAE relies on 'shared endemism' and reads the paucity of shared taxa from the southern regions as ancient endemism.

Endeavouring to evaluate the disparate interpretations of the results we focused on the endemic taxa. The four Holarctic divisions support much richer tardigrade faunas of 216 to 456 species with modest (25% to 45%) regional endemism (compared with Antarctica with only 64 species, most of which (58%) are endemic), and hence more numerous shared species thus forming a relatively stable series of crown regional clades. This is further supported at familial level with three (Eohypsibiidae and Necopinatidae: Eutardigrada and Carphaniidae: Heterotardigrada) Laurasian but only one (Oreelliidae: Heterotardigrada) Gondwanan. With Heterotardigrada seen as being older than the Eutardigrada (Kristensen 1987), this would corroborate the PAE data with a basal Gondwanan fauna and a truly derived Laurasian regional crown clade.

Obviously, further study is required on this subject. The dearth of data for some regions of the world does not equate to absence yet influences the analysis. The Miocene-Pleistocene global cooling that ended 20,000 years ago would have wiped out the majority of Arctic and Antarctic Tertiary palaeo-endemic taxa (e.g. Liebherr & Schmidt 2004; Peck *et al.* 2006). In the Holarctic region limno-terrestrial tardigrades could slowly withdraw southwards ahead of the glacial advance and subsequently recolonise high latitude terrain following glacial retreat (see Pugh & McInnes 1998). In contrast, Antarctica was by this time isolated, under glaciers and surrounded by sea, so must have derived its fauna from a very different mechanism. This could be either vicariant fragments of a truly pre-glacial relict fauna, post-glacial immigration across the Southern Ocean, or, a combination of both. We felt that new analytical tools and further analysis of the data would be required for a better understanding of limno-terrestrial tardigrade biogeography.

Conclusions

I believe my papers constitute a significant contribution to tardigrade biology, most notably biodiversity, especially for the Antarctic, higher taxonomy and biogeography. I am considered an expert on the Tardigrada and therefore approached to write chapters for books and encyclopaedias, both for general knowledge and specific regions. I was, for example, asked to consolidate recent progress in global limno-terrestrial tardigrade descriptive morphology by producing a new dichotomous key to tardigrade genera (Nelson & McInnes, 2002). My research has highlighted the problems associated with the identification of Antarctic tardigrades, including both the diagnoses of new species and the clarification of older, dated, species descriptions (e.g. McInnes 1995 - 13 citations, according to the ISI Web of Science citation system). I have published on new taxa and I am regularly contacted to identify or verify tardigrades, particularly Antarctic taxa, some of which are reported in this thesis. More recent collaborations with molecular biologists have provided me with a new perspective on the problems associated with defining species, throwing the higher level taxonomic structure of the phylum into a sharp focus (Sands *et al*, 2008a, b). This new perspective has permitted me to both re-evaluate some of the 'established' species complexes and affiliations, and moreover re-examine alpha-characters that have been 'traditionally' used to define species and higher taxa in order to define new ones that are more consistent with the evolutionary lineages suggested by molecular systematics (McInnes *et al*, 2008; Marley *et al*, 2011).

My work on Antarctic fauna has contributed to a developing understanding of localised distribution patterns in the Maritime Antarctic from South Georgia to Alexander Island and Continental Antarctica (e.g. Convey & McInnes 2005 – 30 citations). With collaborators I have moved to explore the palaeolimnology of pan-Antarctic lakes (Cromer *et al*, 2008 - 2 citations). I have broadened my research to consider other tardigrades, particularly the comparative biology of species from other challenging (mostly European)

high altitude and Arctic regions. My published work in this area has addressed the more global issues of (particularly Arctic) dispersal and colonisation (Grøngaard et al, 1999 - 11 citations; Pugh & McInnes, 1998 - 16 citations). For the Antarctic freshwater Crustacea, the biodiversity database we published has acted as a major baseline for the Antarctic studies of these groups (Pugh et al, 2002 - 35 citations).

The literature survey of limno-terrestrial Tardigrada, published as a monograph (McInnes 1994 - 82 citations), proved to be both pivotal in my understanding the taxonomy and distribution of the Tardigrada and is still used to today as the definitive source and starting point for any studies in tardigrade systematics or zoogeography. This publication has been a key component of my recognition within 'Tardigradology', indeed my database has been used to assist students from a variety of countries as well as colleagues exploring new territory. I am also recognised as a source of tardigrade literature and am forwarded all new references to tardigrade species, changes in taxonomic status and site records, which are incorporated into an ever expanding and continually updated global tardigrade biology resource. This database was the principal source for the Tardigrada section within the Fauna Europaea project, for which I am the Group Co-ordinator for the phylum. This first stage is now complete and on-line (McInnes, 2004; Fontaine et al, 2007 - 15 citations).

I was the first worker to publish a paper on the global terrestrial biogeography of a phylum and this work is still being cited by the biogeography community (McInnes & Pugh, 1998 - 33 citations). The initial work indicated that the extant limno-terrestrial tardigrade fauna reflected the early tectonic break-up of the super continents of Laurasia, West and East Gondwana between 65 and 135 million years ago. The more recent reprise of a much larger (70% more species) database using parsimony analysis of endemism (PAE), an alternative cladistic technique, corroborates the original biogeographic divisions but suggests a new and contrary hypothesis, namely that ancient tardigrades may have migrated globally from South to North rather than vice versa (McInnes & Pugh, 2007 – 2 citations).

My work has lead to an understanding of tardigrades in depauperate polar ecosystems. I have an understanding of how new methods can result in resolution of phylogeny – and have warned of the need for care in applying these new methods. This thesis shows that I have contributed significantly to the understanding of the phylum Tardigrada within the fields of taxonomy and biogeography.

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STATEMENT REGARDING THE RESEARCH

The research on which the published papers were based was carried out at the British Antarctic Survey over the period 1996-2010 while I was employed as a research scientist.

THE CONTRIBUTION OF COLLABORATORS

Within the British Antarctic Survey there are no other staff working on the Tardigrada or as a taxonomist. I have field experience of both the Antarctic and the Arctic but a large proportion of my samples were provided by other scientists or have been collected for me by field support staff.

On all the papers for which I am first author the overall design of the research project, determination of the methods and writing of the paper was carried out by me. Where I have been invited to collaborate on a project, either with BAS staff or external collaboration, I have been involved in setting up the research ideas; I have had total control of the study and write-up for anything involving the Tardigrada, with the exception of molecular methodology.

Sandra J. McInnes

DECLARATION REGARDING THE WORKS SUBMITTED

I confirm that the work presented here has not been submitted in whole or in part for a

research degree at any other university.

Sandra J. McInnes