Antarctic bdelloid rotifers: diversity, endemism and evolution

Introduction

Antarctica's ecosystems are characterized by the challenges of extreme environmental stresses, including low temperatures, desiccation and high levels of solar radiation, all of which have led to the evolution and expression of well-developed stress tolerance features in the native terrestrial biota (Convey, 1996; Peck et al., 2006). The availability of liquid water, and its predictability, is considered to be the most important driver of biological and biodiversity processes in the terrestrial environments of Antarctica (Block et al., 2009; Convey et al., 2014). Antarctica's extreme conditions and isolation combined with the over-running of many, but importantly not all, terrestrial and freshwater habitats by ice during glacial cycles, underlie the low overall levels of diversity that characterize the contemporary faunal, floral and microbial communities of the continent (Convey, 2013). Nevertheless, in recent years it has become increasingly clear that these communities contain many, if not a majority, of species that have survived multiple glacial cycles over many millions of years and undergone evolutionary radiation on the continent itself rather than recolonizing from extra-continental refugia (Convey & Stevens, 2007; Convey et al., 2008; Fraser et al., 2014). With this background, high levels of endemism characterize the majority of groups that dominate the Antarctic terrestrial fauna, including in particular Acari, Collembola, Nematoda and Tardigrada (Pugh & Convey, 2008; Convey et al., 2012).

The continent of Antarctica is ice-bound, and surrounded and isolated from the other Southern Hemisphere landmasses by the vastness of the Southern Ocean. The 1000 km Drake Passage separates it from South America, and distances of 4–5000 km from Australia/New Zealand and South Africa. Terrestrial ecosystems reach their greatest development in the coastal regions, where most of the continent's biodiversity is found, most evidently along the Antarctic Peninsula and parts of the coastline of East Antarctica. Terrestrial communities are also present on isolated nunataks and the major mountain ranges inland, as well as in the 'dry valleys' of southern Victoria Land, which are the single largest ice-free areas of the continent (Convey, 2013). However, most ice-free areas are small, and isolated by tens to hundreds of kilometres from neighbouring areas.

Bdelloids, microscopic water-dwelling invertebrates belonging to the Subclass Bdelloidea of the Phylum Rotifera, account for 11-100% of all rotifer species recorded in Antarctic waterbodies and for 40-100% of species from terrestrial habitats (e. g. Dougherty & Harris, 1963; Sudzuki, 1964; Everitt, 1981; Sohlenius et al., 1996; Smykla et al., 2010). The evolutionary success of Bdelloidea in the extreme Antarctic environment is underlain by their parthenogenetic mode of reproduction, and their ability to survive drying and/or freezing in an anabiotic state (cryptobiosis). Populations of bdelloids usually consist of a mix of reproductively isolated clonal lineages, often apparently morphologically uniform, but which are genetically distinguishable evolutionary entities (Birky et al., 2005). At least some clonal lineages can be identified by detailed examination of external morphological characteristics (Birky et al., 2011), and/or by the size and shape of hard parts of the masticatory apparatus (Fontaneto et al., 2007). To date only seven bdelloid morphospecies have been recognized as being endemic to the Antarctic and sub-Antarctic (Segers, 2007), although a recent preliminary molecular analysis has suggested that this number should be considerably greater (Velasco-Castrillón et al., 2014a). Unfortunately, many studies (including recent) use only superficial identification of rotifers, often incomplete or misleading when based on identification keys (Donner, 1965; Kutikova, 2005) for mostly European fauna. Much of the early literature on Antarctic Bdelloidea is inevitably in journals with limited access, and hence much relevant information is not easily accessible to contemporary researchers.

With this background, the aims of this study are: (1) to review contemporary knowledge of aspects of diversity, ecology and reproductive biology of Antarctic bdelloid rotifers, (2) to advance knowledge of morphological and molecular diversity of Bdelloidea in Antarctica, and (3) to evaluate the level of endemicity of Antarctic bdelloids.

Current state of knowledge of bdelloid diversity and biology in Antarctica

Early studies

The history of bdelloid research in Antarctica and the sub-Antarctic dates back more than a hundred years. Early records of Bdelloidea date to the start of 20th Century, obtained from material collected by the First German Antarctic (1901-03), Swedish (1901-1904), British (1907-09) and Second French (1908-10) Antarctic Expeditions. Richters (1907, 1908) was the first to record bdelloids from terrestrial mosses. However, the only two species unequivocally recognizable from his records, *Callidina angusticollis* (=*Habrotrocha angusticollis* Murray, 1905) and *C. longirostris* (=*Rotaria sordida* (Western, 1893)), were found further north, between 35º and 40º S (St. Paul and Amsterdam islands). The remaining 13 bdelloids, also attributed to the genus *Callidina*, are now unidentifiable to species. The illustrations available, depicting contracted bodies, jaws and foot appendages, suggest that these rotifers are most likely correctly referred to the genera *Habrotrocha* and/or *Macrotrachela*.

Scottish biologist, microscopist and polar explorer J. Murray was the first to describe new species of Antarctic Bdelloidea. In the excellently illustrated report on the British Expedition, Murray (1910) listed 12 bdelloid species from mosses and pools of Ross Island, and one species (*Callidina tridens* = *H. tridens* (Milne, 1886)) from terrestrial moss from the

Stranded Moraines of McMurdo Sound. Five species of the 12 found were previously unknown: *Philodina gregaria* Murray, 1910, *Ph. antarctica* Murray, 1910, *Ph. alata* Murray, 1910, *Habrotrocha* (as *Callidina*) *angularis* (Murray, 1910), and *Adineta grandis* Murray, 1910. Four species that Murray identified as cosmopolitan, *A. barbata* Janson, 1893, *A. longicornis* Murray, 1906, *Callidina constricta* (=*Habrotrocha constricta* (Dujardin, 1841)) and *C. habita* (=*Macrotrachela habita* (Bryce, 1894)), were noted to have morphological differences from the original descriptions of these species as found in Europe. One further species, *Philodina* sp., while apparently new to science, was not further described.

Murray (1910) also discussed the tolerance of bdelloids to desiccation, salinity and extreme temperatures, their habitat and possible feeding preferences, presumed cosmopolitanism and possible dispersal mechanisms, and the origin of the Antarctic rotifer fauna. He noted the predominance of Bdelloidea over other rotifers in the habitats examined, and the remarkably high proportion of species that appeared to be known only from Antarctica, which were fully adapted to the conditions of the Antarctic environment. He noted that the two most abundant species, *A. grandis* and *Ph. gregaria*, were both viviparous (possibly a means of increasing progeny survival under extreme conditions), although the only exclusively viviparous bdelloid genus, *Rotaria* Scopoli, 1777, would not be found in Antarctica for some time yet. Murray (1910) considered wind to be the main vector of bdelloid dispersal, also noting that the characteristics of air currents around the Antarctic continent made transportation of rotifers from sources to the north impossible. Waterbirds, along with wind, were also considered as dispersal vectors on the local scale, between different water bodies on Ross Island. Murray (1910) also included the first report of “watermelon snow”, a phenomenon caused by aggregations of *Ph. gregaria*, a large bdelloid rotifer with bright-red colored stomach.

Early taxonomic studies of the Bdelloidea of the maritime Antarctic and sub-Antarctic islands were carried out by de Beauchamp (1913, 1940), who investigated terrestrial habitats of Jenny Island and Îles Kerguelen. However, the description of a new viviparous bdelloid *Philodina* (?) *jeanelli* Beauchamp, 1940 from Kerguelen was based only on contracted individuals and has possibly hampered identification of this species by subsequent researchers.

Further taxonomic studies

More recent taxonomic studies were made by Donner (1972a, 1980) using collections made by Dougherty and Harris (1963) on Ross Island, and by Jennings (1976a) on Signy Island (South Orkney Islands; material initially erroneously attributed to the Falkland Islands). The Ross Island material allowed redescription of three species previously found by Murray – *A. grandis*, *Ph. gregaria* and *M. insolita* var., the latter apparently being identical with *M. habita* as described by Murray (1910). The Signy Island material included the previously undescribed species *Mniobia ostensa* Donner, 1980, and 11 other bdelloid species thought to be cosmopolitan. Sudzuki (1964), examining material from Langhovde on the continental Antarctic coastline, depicted 11 unidentified bdelloids from the genera *Adineta*, *Habrotrocha*, *Macrotrachela* and *Mniobia*, which cannot now be reliably attributed to any known species since many important characters (corona, trophi shape, oviparity/viviparity) were missing in the images presented. Dartnall (1983, 1995a, b) and Dartnall & Hollowday (1985) reported a total of 32 bdelloid species, depicting and redescribing 15 species from the maritime Antarctic and the continent (Princess Elizabeth Land), among which were nine previously unknown representatives of *Adineta*, *Habrotrocha*, *Macrotrachela* and *Philodina*. Notwithstanding some uncertainty over details of the corona, most of the specimens described were clearly different from known species, while specimens identified as *A. gracilis* and the viviparous

Rotaria rotatoria (Pallas, 1766) showed morphological inconsistencies with the original descriptions of non-Antarctic material. *Macrotrachela* (=*Callidina*) *papillosa* (Thompson, 1892) was erroneously listed as *Habrotrocha papillosa*, and *M. insolita* de Koning, 1947 as *M. insolata*.

Life cycle

Several studies have described the life cycles of endemic Antarctic bdelloids. Dougherty (1964) investigated reproductive features of *Ph. gregaria* cultivated in the laboratory, and found its maturation time to be 28-110 days (in a laboratory refrigerator). This suggests a life span considerably longer than in any other cultivated bdelloid, including another Antarctic endemic, *A. grandis* (Dartnall, 1992; Ricci, 2001). The fecundity of the viviparous *Ph. gregaria* (up to 24 offspring; Dougherty, 1964), was also much lower than those of various oviparous bdelloid species in cultures maintained at room temperature (Ricci & Caprioli, 1995). Dartnall & Hollowday (1985) recorded that *Ph. gregaria* could produce up to 32 young per female, a number close to that of many oviparous bdelloids but still lower than others. Dougherty (1964) stated that most *Ph. gregaria* offspring started to reproduce 27-90 days after birth. Dartnall (1992) confirmed the unusually long life span of *Ph. gregaria* – up to 89 days at 4ºC, and twice that of *A. grandis* at the same temperature (40-50 days). Ruttner-Kolisko & Kronsteiner (1979, cited in Dartnall, 1992) reported that at 6ºC *Ph. gregaria* lived longer than at 10ºC (60 days vs 26) and produced more offspring (15 vs 7). Also, Dartnall (1992) found the age at the first reproduction to be 36-37 days for *Ph. gregaria*, about 10 times more than typical oviparous non-Antarctic bdelloids cultivated at room temperature (Ricci & Caprioli, 1995).

Ecology

A number of studies have described the interactions of Antarctic bdelloids with their substrata (moss, algal mats, and soil) comparing these with other terrestrial micro-invertebrates, while others have addressed seasonal changes in their populations. Davis (1981) evaluated the role of bdelloids in bryophyte communities of Signy Island, by estimating their dry biomass from data presented by Jennings (1976b, 1979) on density and species composition. Both average and maximum biomass of Bdelloidea were comparable with or higher than those of Nematoda, though considerably lower than those of Tardigrada. The biomass of bdelloids in mosses could reach up to 29.5 mg dry mass m^{-2} – fourfold greater than that of monogonont rotifers, with about a half of the bdelloid biomass being attributed to *Adineta* species. Davis' (1981) data on feeding preferences suggested that the diet of bdelloids consisted entirely of dead organic matter, contrasting with Dougherty (1964) who stressed the importance of unicellular algae in the diet of *Ph. gregaria*. However, members of the genus *Adineta* are also known to feed predominantly on dead organic matter elsewhere (Örstan, 1992). Everitt (1981) observed cyclical changes in abundance throughout the year in the bdelloid population of a saline continental lake in the Vestfold Hills. Rotifers overwintered in a cryptobiotic state, and during the summer reproduced with abundance peaks occurring at three week intervals. Dougherty (1964) and Dartnall (1992) reported that the time between recovery from cryptobiosis, or birth, and the first reproduction in *Ph. gregaria* was at least 1 month in the laboratory, but that it could be shorter in the natural environment. In the relatively stable lake environment, the abundance peaks observed could be successive new generations, especially as Bdelloidea, unlike other rotifer group, Monogononta, do not possess specific larval or programmed dormant stages. However, the largest abundance peak described by Everitt (1981), corresponded to a massive inflow of N and P compounds into the lake, indicating that environmental influences are also important. In the more unstable (in terms of water availability) terrestrial habitats environmental factors seem to be the major driver of bdelloid abundance dynamics (Iakovenko, 2004). Priddle & Dartnall (1978), investigating the microflora and microfauna of aquatic moss and algal communities in lakes of Signy Island, observed three to seven-fold decreases in the abundance of *Philodina* sp. during winter compared to summer. They also reported that two non-sessile bdelloid species showed distinctive space distribution pattern inside moss cushions, dominating in different zones of stems and leaves. Cathey et al. (1981) found *Ph. gregaria* and *Ph. alata* to be able to colonize artificial substrata (polyurethane foam) in eight lakes of southern Victoria Land, the former being present in all the lakes and the latter in only three lakes.

Based on recent studies, most or all of rotifer species present in Antarctic soil communities are bdelloids (Smykla et al., 2010). In soils of the McMurdo Dry Valleys, one of the driest places in the Antarctica, rotifers were present in all the sampled localities (Courtright et al., 2001). Confirming Murray's (1910) speculations of almost a century ago, Nkem et al. (2006) found the wind to play an important role in the dispersal of soil rotifers, and this has been proposed as the mechanism allowing them to colonize remote nunataks, where they can reach abundances of up to 135 ind g^{-1} dry substrate (Sohlenius et al, 1996). In certain types of soil at Edmondson Point, Victoria Land, Smykla et al. (2010, 2012) found bdelloid rotifers to be the dominant group of micro-invertebrates, reaching over 8000 ind 100 g^{-1} raw soil. Smykla et al. (2010, 2012) reported that bdelloids reached high abundances in wet soils under moss and algal and cyanobacterial mats, while being absent in both barren fellfields and heavily nutrient-enriched penguin colonies. The latter observation contradicts that of Porazinska et al. (2002), who reported rotifers to be present and even dominating in terms of abundance (> 4000 ind kg^{-1} dry soil) in ornithogenic soils collected on Ross Island. Sohlenius et al. (2008) noted that rotifers were the most frequently-encountered and abundant group of invertebrates in the ornithogenic soils and fellfields of Dronning Maud Land, in contradiction with the data of Smykla et al. (2010, 2012). Velasco-Castrillón et al (2014b) reported bdelloid rotifers to be the most widespread and abundant taxon in the soils of East Antarctica, being present in 87% of sampled sites and reaching 44 ind g^{-1} dry soil. They found bdelloids to be present in soils with various particle size (from fine to coarse), both with and without vegetation, and with a broad variety of abiotic and geochemical parameters, consistent with the high tolerance of this group towards extreme conditions.

As also noted in the Arctic (De Smet & Van Rompu, 1994), Bdelloidea play an important role in Antarctic cryoconite communities. In cryoconites on glaciers of the McMurdo Dry Valleys rotifers were dominant, reaching over 3500 ind 100 g^{-1} dry sediment, although abundance decreased with elevation and also changed in response to pH, nutrient concentrations and cryoconite area (Porazinska et al., 2004). Positive correlation was also found between rotifer and tardigrade abundances in cryoconites.

"Watermelon snow" and similar phenomena on the surface of water, ice or algal mats as the result of massive accumulation of the red-coloured *Ph. gregaria*, was originally described by Murray (1910) and later addressed briefly by Dougherty & Harris (1963), Dougherty (1964) and in more detail by Dartnall (1992). The rotifers can create very noticeable red patches on the surface of such substrata, ranging from a few centimetres to many meters in diameter (Dartnall, 1983). According to Dartnall, for a patch to grow to a size of about 10 m may take only a week, with the abundance of *Ph. gregaria* in the patches reaching up to over 20 million ind. m^{-2}.

Diversity and endemism

Studies that have included or provided compilations of the rotifer fauna of Antarctica and the sub-Antarctic, in particular terrestrial Bdelloidea, have been published by Dartnall (1983), Dartnall & Hollowday (1985), Sudzuki (1988), Adams et al. (2006), Segers (2007), Velasco-Castrillón et al. (2014a-c), and Fontaneto et al. (2015). These sources should be referred to for details on species diversity of particular regions. A few studies have been carried out at the same location over time. For example, Dougherty & Harris (1963), investigating Ross Island and the McMurdo Dry Valleys, found virtually the same species as recorded by Murray (1910). A number of previously unrecorded bdelloid species have been reported from Antarctica and sub-Antarctic by Jennings (1976a), Sudzuki (1979), Everitt (1981), and Sohlenius et al. (1996, 2005). These studies reported, along with the indigenous Antarctic bdelloids, some 20 morphospecies similar to species first described from Europe, thus considering Antarctic bdelloid fauna to include many cosmopolitan species. No Antarctic endemic bdelloid families or genera have been reported.

Velasco-Castrillón et al (2014a) consider that the known Antarctic Bdelloidea diversity comprises 36 morphospecies. However, this figure does not include three species-level taxa identified by Murray (1910), Jennings (1976a), and Cathey et al. (1981), or 10 further undescribed species reported by Dartnall & Hollowday (1985), Dartnall (1995a,b), and Sohlenius (1996): *Adineta vaga minor* Bryce, 1893, *Ceratotrocha cornigera* (Bryce, 1893), *Philodinavus* sp., *A.* sp., *Habrotrocha* sp., *Macrotrachela* sp. "A", *Macr.* sp. 1, *Macr.* sp.2., *Mniobia* sp. N, *Philodina* sp. “A”, *Ph.* sp. “B”, *Ph.* sp. 1., and *Ph.* sp. 2. Including these taxa, in total 49 bdelloid morphospecies have been recorded in Antarctica and the sub-Antarctic over the last century.

Based on classical taxonomy, only five endemic bdelloids (those originally described by Murray (1910)) have been reported for Antarctica, with the remainder being cosmopolitan and previously known from other continents including Europe (Donner, 1965; Segers, 2007).

In contrast, the application of contemporary molecular approaches (Velasco-Castrillón et al., 2014a) suggests that the bdelloid fauna of Antarctica comprises mostly endemic species, or at least species not yet recorded from any other continent.

Molecular approaches

A number of genomic and molecular phylogeographic studies have been performed during the last decade on various groups of Antarctic microfauna, mainly microarthropods and nematodes (Stevens et al., 2006; McGaughran et al., 2008, 2010; Stevens & Hogg, 2006; Velasco-Castrillón & Stevens, 2014), as well as various microbial groups (see Vyverman et al., 2010) and mosses (Pisa et al., 2014). However, the application of such studies to bdelloid rotifers in Antarctica remains at an early stage. Fragmentary sequence data on Antarctic bdelloids have been published in studies of the evolution and global biogeography of Bdelloidea (Barraclough et al., 2007; Fontaneto et al., 2008, 2012). Velasco-Castrillón et al. (2014a) recently evaluated molecular diversity of Antarctic and sub-Antarctic bdelloids across a wide area. Their study identified 47 putative species, counting both sequence clusters, and singletons (entities with only one sequence obtained). All of the putative species were designated as Antarctic or Tierra del Fuego endemics based on percentage sequence similarities in comparison with representatives of eight bdelloid genera from other continents. The study also indicated that the true number of taxa in the genera *Adineta* and *Philodina* determined from the sequence data analysed using the Poisson Tree Processes (PTP) model (Zhang et al., 2013), must be considerably higher than can apparently be determined by morphological approaches alone.

The current study shows that when appropriately analysed, the morphological diversity of Antarctic bdelloids is sufficient to reveal most diversity detected by contemporary molecular markers. We also re-evaluate previously published data on Antarctic bdelloid rotifer endemism, and determine the ratio of endemic to cosmopolitan bdelloid species in comparison with such from other continents.

Materials and Methods

Sampling and extraction of rotifers

New samples included in this study were obtained from both maritime and continental regions at sites between 63°60' S and 77°55' S (Fig. 1, Table 1). In the continental Antarctica 11 sampling locations were visited in the Ross Sea area, including the Victoria Land coast, Beaufort and Ross Islands (Fig. 1a, Supplementary file 1). The fieldwork and sampling in the Ross Sea area were conducted during five austral summer seasons between 2003/04 and 2011/12 within the project of J. Smykla (Smykla et al., 2010, 2011, 2012). Soil and moss were collected at all localities. The soil samples were obtained from barren fellfields, bryophyte communities, wetlands with algal and cyanobacterial mats, and from the vicinity of active and relict penguin colonies. Algal mats were collected in coastal areas and on Ross Island. Most of the collected material was stored frozen (─20ºC), but some terrestrial mosses were dried and stored at room temperature; details of collection methods and primary sample processing are given in Smykla et al. (2010, 2012, 2015).

In the maritime Antarctic 237 samples were obtained during the summers of 2004/05, 2006/07 and 2009/10 from the Argentine Islands archipelago, King George Island, and coastal areas of the Antarctic Peninsula under the projects of K. Janko, I. Kozeretska and V. Trokhymets. These included 50 soil and 183 moss samples, one sediment sample from a pool on King George Island, and three lichen samples from the Argentine Islands archipelago (Fig. 1b, Table 1, Supplementary file I). Methods of collection, storage and rotifer extraction were as used for the continental samples, except for mosses which were washed directly along with wet sieving and sugar gradient centrifugation as described by Freckman & Virginia (1993).

Alpha taxonomy procedures

Detailed procedures of rotifer sorting, identification, digital imaging, and the preparation of type material (glycerin jelly slides and SEM mounts) are described in Iakovenko et al. (2013). We used the keys of Donner (1965) and Kutikova (2005) as a primary guide for identification, but detailed taxonomic analysis was based on the first descriptions (cited in Donner (1965), and further specific studies (Donner, 1972a,b, 1980; Haigh, 1965, 1966; Koste, 1996a; Örstan, 1995; Ricci et al., 2001, 2003; Birky et al., 2011).

Rotifer trophi (hard parts of the mastax) were extracted using Savo®Perex bleach and prepared for SEM according to De Smet (1998). Trophi measurements (ramus length and trophi width) were made as described by Iakovenko et al. (2013). Type material for newly described species (holotypes, paratypes and additional specimens) are deposited in the collections of the Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Kyiv, Ukraine.

Additionally, we investigated and described rotifers depicted in photographs in Velasco-Castrillón et al. (2014a). To reliably distinguish, both morphologically and genetically, between several similar European and Antarctic species, we used material from our collections in Poland, Czech Republic, and Germany (Supplementary file I). Previously unpublished data on the morphometry of *A. gracilis* Janson, 1893, *A. vaga* (Davis, 1873) and *Habrotrocha thienemanni* Hauer, 1924 from these collections, as well as COX1 sequences of the voucher specimens from which the morphometric data were taken, were used for these analyses.

Morphometric analyses

External rotifer body dimensions were taken on screenshots from digital videos, and trophi were measured on SEM photos, as described in Iakovenko et al. (2013). Total length (TL) in the case of adinetid rotifers was taken as the distance between the middle of the anterior rim of the head and the posterior rim of the spur pseudosegment, i.e. not including the rostrum, as it was usually bent under the head (Fig. 2).

To distinguish some Antarctic species from morphologically similar European ones, we measured specimens from clonal cultures, from which we subsequently obtained some COX1 sequences: 113 specimens of Antarctic *Adineta*, 69 specimens of European *Adineta*, and 16 specimens of Antarctic *Habrotrocha*. We used the Linear Mixed Effects Model (LME) and Principal Components Analysis (PCA) to compare body and trophi measurements. The results of PCA were visualized as the two first principle components of variation plotted against each other. All statistical analyses were performed in R 2.15.1 following Crawley (2007).

DNA taxonomy procedures

The DNA extraction protocol followed Fontaneto et al. (2007) and Iakovenko et al. (2013). The target locus of the mitochondrial COX1 gene (355 bp in length) was amplified and sequenced from 192 bdelloid specimens (Supplementary file III) using universal primers LCO1490 and HCO2198 (Folmer et al., 1994) with the subsequent re-amplification to increase the outcome of the product, using bdelloid-specific primers Bdell_CO1_FW (5′-CGTACWGAGTTAGGAATRGTA-3′) and Bdell_CO1_Rev (5′-CCAAAATTWCGATCTAAYA-3′) (Robeson et al., 2011).

To construct phylogenies, we downloaded available sequences of the taxonomically assigned bdelloid species from GenBank, available from all continents except South America (976 COX1 sequences, their detailed descriptions are given in the Supplementary file II). We used EMBL online version of MAFFT software (Katoh et al., 2002) to construct one total alignment of both newly obtained sequences and those downloaded from GenBank, and four separate alignments for four genus-specific datasets (*Adineta* Hudson and Gosse, 1886, *Habrotrocha* Bryce, 1910, *Macrotrachela* Milne, 1886, *Philodina* Ehrenberg, 1830). The monogonont rotifer *Brachionus calyciflorus* Pallas, 1755 was used as outgroup in each of these alignments, and each genus-specific dataset also contained a member of another bdelloid genus an additional outgroup: *Bradyscela clauda* (Bryce, 1893) for *Adineta*, *M. ehrenbergii* (Janson, 1893) for *Habrotrocha*, and *H. constricta* (Dujardin, 1841) for *Macrotrachela* and *Philodina*.

We constructed phylogenetic trees in MrBayes 3.2.3 (Ronquist et al., 2012), running 8 to 20 million generations and sampling every 1000 generations. The optimal nucleotide substitution model (GTR+I+G) was chosen for each dataset in jModelTest 2.1.6 (Darriba et al., 2012). The analysis was stopped when the standard deviation of split frequencies was below 0.01, with the PSRF being 1.00 for all the parameters. Effective sample size (ESS) sufficiency for the model parameters, process stationarity, and the amount of burn-in trees were checked using both MrBayes and Tracer 1.6 software (Rambaut et al., 2013). The resulting consensus trees constructed in MrBayes were visualized using FigTree 1.4.2 (Rambaut, 2012), and the full-size Bayesian trees are included in Supplementary file IV.

Three independent approaches were used for species delimitation based on DNA sequence data: 4x rule (Birky et al., 2005; Birky & Barraclough, 2009), Generalized Mixed Yule Coalescent Approach (GMYC; Fujisawa & Barraclough, 2013), and Poisson Tree Processes with Bayesian support (bPTP; Zhang et al., 2013). The 4x rule identifies as putative species those monophyletic clades whose genetic distances (K) to other sequences on the phylogenetic tree are larger than four times the intra-clade divergence (θ). To assess this, we constructed matrices of mean pairwise correlated sequence distances for each clade in the Bayesian trees in MEGA6 software (Tamura et al., 2013), calculating θ, and estimating the K/θ ratio within and between the clades (Supplementary file V).

The GMYC method likewise identifies species as independently evolving entities represented by a number of clades on a phylogenetic tree. However, each clade is delimited by optimizing the tree nodes indicating transitions between inter- and intraspecific evolutionary processes. The maximum likelihood optimum is found between models of species diversification (based on the Yule model) and branching events within species (based on the neutral coalescent model). The initial tree should be time-calibrated (ultrametric), unrooted and not contain polytomies or zero-length branches. We used a single-threshold version of the method implemented in GMYC species delimitation software available online (http://species.h-its.org/gmyc/). The uploaded coalescent trees were produced from Bayesian unrooted trees in R 3.1.2 (http://www.r-project.org/) using the **chronopl** function of the “ape” package. This function utilizes a semiparametric method based on penalized likelihood (Sanderson, 2002) to estimate the tree node ages through a trade-off between contiguous and non-contiguous branches’ rates.

Unlike GMYC, the bPTP method does not require a time-calibrated and unrooted tree as input. In this method, the number of substitutions κ between intra- and interspecific events is used instead of time as a tree-calibrating parameter. Assuming that each substitution (which is independent of other substitutions) has a probability ρ of generating a speciation event, κ substitutions generate η speciations in a continuous process, and in a population of the size η the number of substitutions is sufficient, the process proceeds at rate ρ x η and follows a Poisson distribution. The number of substitutions is calculated from the branch lengths of the input tree. We used online implementation of bPTP (http://species.h-its.org/ptp/) and the trees produced in MrBayes as the input.

Results

In total, we identified 60 morphospecies, including 20 taxa currently identified to generic level only and still under investigation, and 10 listed as "conformis" that show minor morphological differences from known species. Only 13 of the morphospecies found occurred both in maritime and continental Antarctica. The material examined included six of the seven known Antarctic endemics: *A. grandis*, *H. angularis*, *Mn. ostensa*, *Ph. alata*, *Ph. jeanelli*, *Ph. gregaria*. We have identified 10 morphospecies reported by other researchers from Antarctica as *A. barbata* Janson, 1893, *A. vaga* (Davis, 1873), *H. gulosa* Milne, 1916, *H. vicina* Donner, 1980, *Macr. ambigua* Donner, 1965, *Macr. concinna* (Bryce, 1912), *Macr. habita* (Bryce, 1894), *Macr. musculosa* (Milne, 1886), *Macr. nixa* Donner, 1962, and *Rotaria rotatoria* (Pallas, 1766). These species are considered cosmopolitan, or at least are known from locations other than Antarctica. However, of these 10 species, those resembling *A. barbata* and *A. vaga* s. str. are shown to be distinct new taxa and therefore currently endemic to Antarctica, based on both minor but consistent morphological differences and molecular analyses.

In *Alpha taxonomy*, below, we describe 12 new for science Antarctic bdelloid species. For some of them we also provide statistical analysis of morphometric data confirming their delimitation from morphologically similar described species occurring in Europe (*Morphometric analyses*). New records for the Antarctic, yet to be verified by molecular analyses belonging to already described cosmopolitan species, included *H. angusticollis* (Murray, 1905), *M. nana* (Bryce, 1912), *Mniobia incrassata* (Murray, 1905), *Mn. scabrosa* Murray, 1911, and *Pleuretra lineata* Donner, 1962. The genus *Scepanotrocha* (*S.* cf *semitecta* Donner, 1951) is reported from Antarctica for the first time. The list of known Antarctic bdelloids is therefore extended to **66 morphospecies** (49 already known and reported in the existing literature, 12 new for science, and 5 new for Antarctica).

Phylogenetic trees constructed using 192 original COX1 sequences, and 976 sequences downloaded from GenBank, gave similar results on the delimitation of independently evolving entities (IEE) according to the 4x rule, GMYC and bPTP models. These results are discussed in detail below (*DNA taxonomy* sub-section). The 4x rule gave 140 IEEs: 44 of *Adineta*, 22 of *Habrotrocha*, 26 of *Macrotrachela*, and 48 of *Philodina*. In total, 132 IEEs were identified by GMYC: 44 of *Adineta*, 20 of *Habrotrocha*, 18 of *Macrotrachela* and 50 of *Philodina*. Finally, bPTP generated a somewhat higher number of IEEs (160): 47 of *Adineta*, 26 of *Habrotrocha*, 29 of *Macrotrachela* and 58 of *Philodina*. Most of the IEEs identified by GMYC and bPTP were confirmed by the 4x rule. Delimitation according to the GMYC approach gave the best correspondence with rotifer morphology, considering both major and minor external features, and morphometric data. Geographical distribution of the identified IEEs is discussed in *Biogeography*.

The integrity of most species identified by morphology, with the exception of *A. grandis*, *Ph. gregaria* and two new species of *Habrotrocha*, was confirmed by molecular analyses (*DNA taxonomy*). According to the molecular data, *A. grandis* consists of at least two cryptic species, one of which is described below as new for science. Ten putative species (IEEs) were identified from molecular data only, obtained both from the new material examined in this study and COI sequences downloaded from GenBank.

Alpha taxonomy

The list of locations is given both after the literature sources (cited in the Introduction), and our data (marked with *). Full descriptions of the examined samples, mentioned in Type material and Additional material below (as sample codes), are given in the Supplementary file I.

Abbreviations: BW – body width, HL – head length, HW – head width, NL – neck length, MinNW – minimal neck width, MxNW – maximal neck width, RL – rump length, RW – rump width, SL – spur length, SSW – spur pseudosegment width, TL – total length. The abbreviations of the localities are explained in the Fig. 1 and Table 1, with the exception of the data from literature: DM – Dronning Maud Land, EB – Enderby, FI – Francis Island, HI – Haswell Island, LH – Langhovde, MM – McMurdo Sound, QM – Queen Mary's Land, SI – Signy Island, TF – Tierra del Fuego, WK – Wilkes Land.

Phylum Rotifera Cuvier, 1817

Class Eurotatoria De Ridder, 1957

Subclass Bdelloidea Hudson, 1884

Order Philodinida Melone & Ricci, 1995

Family Adinetidae Hudson & Gosse, 1889

Genus *Adineta* Hudson and Gosse, 1886

***Adineta coatsae* sp. nov.**

Figs 3a, 4

Murray, 1910 (*A. barbata*?): 53-54, Pl. XII figs 9a-9c. Dartnall & Hollowday, 1985 (*A. barbata*): 30, Fig. 24a-b. Velasco-Castrillón et al., 2014a (*A.* sp. Bd24): 8 (main text), 2, Fig. 6 (Annex S1).

Type locality. Chocolate Point (Victoria Land), 20 m asl., S77° 56.400', E164° 30.693'. **Type habitat.** Algal and cyanobacterial mats.

Type material. Holotype: SIZ 55.1 (CzM3NCmatAC1, 23.1.2010, Leg. J. Smykla), mounted in glycerin jelly. Paratypes: SIZ 55.2-55.3 (CzM3NCmatAC2-3), trophi mounted for SEM.

Additional material. SIZ 55.4-10 (CzM3AS1-2, MPM4mossAC1, V10AC1-2, KG2AC1, V10AC1), digital photos and videos.

Etymology. Named in honor of a mountaineer and Antarctic researcher Dr Larry Coats who assisted in the fieldwork done in the Ross Sea area.

Barcodes: GenBank ID KJ543629-30.

Diagnosis. Similar to *A. barbata* (Fig. 4b) by flat laterally widened rostrum with two protrusions ending with a bundle of long thin sensory bristles, and long sword-like spurs. However, the protrusions are leaf-like while in *A. barbata* they are tubular. Frontal rim of the rostrum is concaved and has a notch in the middle, while in *A. barbata* the notch is absent and the frontal rostral rim is prominently convex. Spurs gradually tapering from their base to the points, shorter than in *A. barbata*.

Description. Body of moderate size, not very wide, flattened dorsoventrally, transparent, stomach usually of brown-yellow color. Dartnall & Hollowday (1985) report the color of this rotifer as greyish-brown. Integument smooth, thin, without sculpturation, spines, knobs or bolsters. Head trapezoid, wider in the posterior part, HL is 15-19% of TL, HW is 76-94% of HL. Distal rostral pseudosegment flat, lobe-like widened, with a V-shaped shallow and wide notch in the middle. Rostral lamella shaped as two lateral leaf-like narrow protrusions with a bundle of long sensory bristles under each protrusion. Eight rectangular teeth in each rake.

Neck of moderate length and width, NL is 12-17% of TL, antenna about ¼-1/5 of bearing pseudosegment. Trunk oval, BW 17-28% of TL. Rump conical, first pseudosegment slightly swollen, RL is 12-17% of TL, RW is 75-92% of RL. Slim foot of 5 pseudosegments, of moderate length, FL is 28-35% of TL, FW is 40-62% of FL. Spurs sword-like, long, gradually tapering from the base to the points; SL is 115-181% of SSW. Three short unsegmented toes. No eyespots. Throat and straight oesophagus of moderate size. Trophi small, round, 11-12 µm long and 13-15 µm wide; 2/2 major teeth and 26/26 minor teeth in unci. Oviparous; egg oval, 101 x 46 µm, smooth, 1-6 round knobs on both poles and the sides.

Measurements. See Table 2. Body length 120 µm (possibly in contracted state) according to Velasco-Castrillón et al. (2014a), and 325 µm according to Dartnall & Hollowday (1985).

Distribution. Maritime Antarctica: AI*, KG*, SI. Continental Antarctica: EB, VL (CR, CH, GH*, MP*), possibly also DM and MM (Dougherty & Harris, 1963; Sohlenius, 1996).

Habitat. Algal and cyanobacterial mats in wetlands; terrestrial moss, soil.

Adineta editae sp. nov. Iakovenko

Figs 3b, 5

Dartnall & Hollowday, 1985 (*A. gracilis*): 31, Fig. 24c. Fontaneto et. al., 2008 (*A. gracilis*): 3139. Velasco-Castrillón et al., 2014a (*A.* cf *gracilis* Bd8): 8 (main text); 1, Figs 2-5 (Annex S1).

Type locality. Rocka Islands (Argentine archipelago), 15 m asl, S65° 10.738', W64° 29.522'.

Type habitat. Soil.

Type material. Holotype: SIZ 53.1 (MRockaAED1a, 15.02.2010, Leg. K. Janko), mounted in glycerin jelly. Paratypes: SIZ 53.2-7 (MRockaAED2-7), in glycerin jelly on a separate slide; SIZ 53.8-21 (MRockaAED1b-e, MRockaAED8-18), trophi mounted for SEM. **Additional material.** SIZ 53.22-25 (870_1AED1, V12AED1-3), trophi mounted for SEM; SIZ 53.26-33 (870_1AED2, VRA01AED2-3, VS03AED1-3), digital photos and videos.

Etymology. Named after the Czech biologist Dr. Edita Drdová-Janková, wife of the collector and project leader Dr. Karel Janko.

Barcodes. Gen Bank ID EF173189-91, EF173193, KJ543598-600, see also Supplementary file III.

Diagnosis. Resembles *A. gracilis* s. str. Janson, 1893 (Fig. 5c, f) and *A. bartosi* Wulfert, 1960 known from Europe, by the short narrow rostrum, the arcuate rostral lamella not divided into lobes and without long sensory bristles underneath, and the structure of rakes. Unlike other *Adineta*, all three mentioned species have rod-like, V-shaped rakes with only two claw-like teeth in each rake directed towards each other. The new species differs from *A. gracilis* and *A. bartosi* by the shape of the head and spurs. The first head pseudosegment not bubble-like swollen as in *A. bartosi*. Head not elongated in the proximal part and not widened (hexagonal) in the distal part as in *A. gracilis* s. str. The head of the new species is larger and wider than in *A. gracilis* s. str. Differently from *A. gracilis* s. str., the new species has somewhat larger and stouter body. Spurs of the new species narrow conical, pointed, with short interspace, while *A. gracilis* s. str. has isoceles triangular spurs without interspace, and the spurs of *A. bartosi* are narrow, peg-like, and without interspace. Trophi much larger than in *A. gracilis* s. str. with larger number of minor teeth in unci.

Description. Body of moderate size, dorsoventrally flattened, transparent, colorless except the yellow-brown stomach. Integument smooth, thin, without knobs, spines or bolsters. Rostrum very short, of moderate width; its lamella wide, semicircular, not divided into lobes. Two claw-like sharp teeth pointing toward each other in each thin rod-like rake. Head wide oval, of regular shape or slightly narrowed towards rostrum, HL is 13-19% of TL. Neck rather short and wide, NL 34-62% of TL, antenna about 1/3 of the bearing pseudosegment width. Trunk wide, oval. Rump somewhat swollen in the middle part, RL is 11-16% of TL. Foot of moderate length, 5 pseudosegments, FL is 10-16% of TL. Spurs short, conical, pointed, divergent, with tiny interspace, SL is 60-83% of SSW. Three short unsegmented toes. No eyespots. Trophi round, 15-19 µm long and 16-20 µm wide; 2/2 major and 28-34 minor teeth in unci. Oviparous. Eggs oval, smooth without knobs or spines. Egg size 71-89 x 45-61 µm by our data and 70 x 50 µm as reported by Dartnall & Hollowday (1985).

Measurements. See Table 2. TL 300 µm by Dartnall & Hollowday (1985), and 220-300 µm according to Velasco-Castrillón et al. (2014a).

Distribution. Maritime Antarctica: AI*, SI, AP*. Continental Antarctica: DM, EB, LH, MM, VL (Cz*, CR*), WK. **Habitat.** Soil, terrestrial moss and lichens, pools.

Comments. Most likely all the researchers, except Murray (1910), have been reporting this species under *A. gracilis* – which, in spite of presumed cosmopolitanism, is very unlikely to inhabit dry and cold Antarctic, being a strict acidophile most common in sphagnum bogs (Bērziņš, 1987).

The head of the new species is 45±4 µm long and 34±4 µm wide, HW/HL is 69-90%. According to our data, *A. gracilis* s. str. has the head 40±7 µm long and 29±4 µm wide (N=42), HW/HL is 53-70%. By our data, *A. gracilis* s. str. has TL 247±45 µm, BW/TL 13-23%, RW/RL 54-82%, FW/FL 27-40% (N=42). The new species TL is 286±41 µm, BW/TL is 13-23%, RW/RL is 67-99%, FW/FL is 38-58%. By our data, the trophi of *A. gracilis* 11.1±0.4 µm long, 13.7±0.9 µm wide (N=14), 20-24 minor teeth in each uncus. The new species has trophi of 16.6±1 µm long and 18.4±0.7 µm wide, with 28-34 minor teeth in each uncus.

***Adineta emsliei* sp. nov.**

Figs 6a, b, d, e

Dartnall, 1995a (*A.* sp.): 13, Fig. 7A. Velasco-Castrillón et al., 2014a (*A.* sp. Bd1): 8.

Type locality. Cape Royds (Ross Island), 27 m asl, S77° 32.500', E166° 8.933'. **Type habitat.** Cyanobacterial mats in wetlands.

Type material. Holotype SIZ 52.1 (CR23matAE1a, 14.1.2010, Leg. J. Smykla) mounted in glycerin jelly. Paratypes: SIZ 52.2-52.16 (CR23matAE2-16) in glycerin jelly on a separate slide; SIZ 52.16-17 (CR23matAE1b-c), trophi mounted for SEM. **Additional material**. SIZ 52.18-52.22 (CR23matAE1e-j, CBM2AE1), trophi mounted for SEM; SIZ 52.23-31 (CR23matAE17-21, CBM2matAE1-4), digital photos and videos.

Barcodes. Gen Bank ID KJ543570-80, see also Supplementary file III.

Etymology. Named in honour of the leading Antarctic researcher Dr. Steven D. Emslie, for his invaluable support in the Ross Sea project.

Diagnosis. Resembles *A. grandis* by the bright orange body color, but it is smaller and not viviparous. By our data, the new species is larger than the similar oviparous species *A. vaga* s. str. (Davis, 1873) (Fig. 6c). Trophi size is intermediate between *A. vaga* s. str (Fig. 6f) and *A. grandis* (Fig. 6g). Spurs are needle-like with bulb-like swollen bases, while *A. vaga* s. str. has straight triangular spurs. From *A. vaga major* Bryce, 1893 and *A. vaga minor* Bryce, 1893 the new species differs by the shape of the spurs, and the intermediate head size (it is larger than *A. vaga minor*, but smaller than *A. vaga major*). From *A. vaga* s. lat. the new species differs by the orange body (*A. vaga* s. lat. is colorless inclusive stomach).

Description. Body of moderate size, wide, flattened, of bright orange color. Integument smooth, thin, transparent, without knobs, spines, bolsters or other appendages. Rostrum short, sickle-like, distal rostral pseudosegment not plate-like flattened. Two short semicircular rostrum lobes, no stiff sensory bristles, only short cilia under the lobes. Wide-oval head of a moderate size, HL is 13-18% of TL, HW is 71-94% of HL. Six thin peg-like teeth in each massive scoop-like rake. Neck of moderate length and width, slightly contracted behind the head, NL is 14-21% of TL, antenna about 1/3 of the bearing pseudosegment width. Trunk oval, wide, BW is 19-27% of TL. Rump conical, somewhat swollen in the middle, RL is 11-16% of TL, RW is 74-98% of RL. Relatively short slim foot of 5 pseudosegments, FL is 10-

16% of TL, FW is 29-45% of FL. Spurs short (SL 60-94% of SSW), pointed, needle-shaped with bulb-like swollen bases, divided by straight interspace of ~2 spur widths. Three short unsegmented toes. No eyespots. Trophi ramate, round, 15-18 µm long and 14-18 µm wide. Rami massive, the region of articulation is straight, protruding backwards, without incisure. Interior margins of rami with long numerous peg-like scleropili. Manubria thin, sickle-like. Two major teeth and 29-33 minor teeth in each uncus. Throat small, oesophagus short, straight. Stomach glands of moderate size. Eight nuclei (3-7 according to Murray) in each germovitellarium. Oviparous. Eggs oval, 60-70 x 39-44 µm, shell smooth, without knobs or spines.

Measurements. See Table 2. TL 350 µm according to Dartnall (1995).

Distribution. Maritime Antarctica: AI*. Continental Antarctica: EB, HI, VL (CR*, CB*, MP*), WK. **Habitat.** Cyanobacterial mats wetlands, terrestrial moss, soil.

Comments. According to our data, the new species has TL 294±44 µm, while TL is 414±61 µm in *A. grandis* (N=20) and 274±14 µm in *A. vaga* s. str. (N=15). The new species has trophi 15.7±1.1 µm long with 29-32 minor teeth in each uncus, while *A. grandis* has trophi 25.4±1.4 µm long with 36-44 minor teeth (N=53), and *A. vaga* s. str. has it 13±0.7 µm long with 25-27 minor teeth (N=14).

Adineta grandis Murray, 1910

Figs 3c, 6g, 7a

Murray, 1910: 51-53, Pl. XII fig. 10. Voigt, 1956-57: 71, Taf. 5 Abb. 24, Taf. 8 Abb. 19, Taf. 14 Abb. 16. Donner, 1965: 273, Fig. 200a. Donner, 1972a: 252, Abb.1. Koste, 1996b (as *A. grandis*, but most likely sibling species): 243, Abb.5. Dartnall & Hollowday, 1985: 31, Fig. 24d-f. Kutikova, 2005: 275, Ris. 299. Velasco-Castrillón et al., 2014a (*A.* sp. Bd2): 8 (main text); 2, Fig. 8 (Annex S1).

Type locality. Cape Royds. **Type habitat.** “Brown vegetation” (algae?) in lake.

Barcodes. GenBank ID KJ543581-88, see also Supplementary file III.

Material examined. BI11, 1 ind.; BI23, 10 ind.; CBM1CYmat, 6 ind.; CBM2mat, 4 ind.; CBC1mat, 5 ind.; CBPc2mat, 1 ind.; CRL21, 2 ind.; CR24, 2 ind.; CRL24, 1 ind.; CzM2Cymat, 8 ind.; CzM3CYmat, 9 ind.; EPL23, 11 ind.; MPM3, 1 ind.; MPM5, 34 ind.; MPM5CYmat, 10 ind.

Description. The largest species of the genus, and the only known viviparous one. Reported TL is 306-750 µm (Murray, 1910; Donner, 1965; Dartnall, 1985), and 304-505 µm according to our data. Its foot is shorter than in other species of *Adineta*. Trophi length 23-29 µm (our data). Body pale orange or brownish yellow, sometimes reddish ("light brown or yellowish, darker in the alimentary tract" according to Murray). Integument smooth, thin, transparent, without knobs, spines or other appendages. Rostrum short, of moderate width, distal rostral pseudosegment not strongly widened or flattened. Rostral lamella divided into two small semicircular lobes. No stiff sensory bristles under rostrum lobes, only short soft cilia. Head not large (HL is 13-19% of TL), wide-oval ("ovate" by Murray), tapering towards rostrum, HW is 66-97% of HL. 6-10 teeth in each massive scoop-like rake. Neck massive, long (NL is 11-27% of TL). Dorsal antenna thick, about 1/4 of width of the antennal pseudosegment. Trunk wide (its width depends on the number of embryos inside), BW is 16-31% of TL. Rump conical, with both pseudosegments somewhat swollen laterally (in some specimens the lateral swellings look like knobs), gradually tapering into a very short narrow foot. RL is 7-16% of TL, RW is 74-103% of RL. Foot short, of 5 pseudosegments, FL is 6-15% of TL, FW is 40-56% of FL. Spurs conical, widened at the base (according to Murray, "short broad cones", "stout and subacute"), pointed, narrow, divergent, divided by the straight interspace equal to 1-2 spur widths, SL is 60-98% of SSW. Three short unsegmented toes. No eyespots. Trophi ramate, large, round or elongate. Rami massive, interior margin with numerous peg-like scleropili. Articulation protruding to the ventral part, straight and without incisure.

Manubria wide, flat, crescent-shaped. Major uncinal teeth thick, dental formula 2/2; 38-41 minor teeth. Trophi unusually large for *Adineta*: 30 µm long according to Donner (1965), 24-31 µm long and width is equal to the length, according to our data. Throat voluminous, oesophagus short, straight. Stomach glands large. Eight nuclei in each of germovitellaria. Viviparous, up to 4 embryos with developed trophi can be seen inside trunk.

Measurements. See Table 2. TL up to 750 µm according to Murray (1910).

Distribution. Maritime Antarctica: SI. Continental Antarctica: EB, HI, MM, VL (BI*, CB*, CC*, CL*, CR, Cz*, EP*, MP*). Africa (questionable): Madagascar (Koste, 1996c). **Habitat.** Algal mats and sediment in pools and seepages, soil, terrestrial moss.

Comments. Velasco-Castrillón et al. (2014a) erroneously attributed this rotifer to „wheel-bearers" (although *A. grandis* has no trochi), and described it as „ovoviviparous" although the species is viviparous.

Adineta fontanetoi sp. nov.

Figure 7b-d

Type locality. Beaufort Island, 9 m asl, S76° 58.147', E166° 54.217'. **Type habitat.** Soil.

Type material. Holotype: SIZ 54.1 (BI27AG1a, 29.1.2010, Leg. J. Smykla), mounted in glycerin jelly. Paratypes: SIZ 54.2-4 (BI27AG1b-d), SIZ 54.5-22 (BI27AG2-19), trophi mounted for SEM.

Etymology. The species is named after colleague rotiferologist Dr Diego Fontaneto who first sequenced this species (as *A. grandis*).

Barcodes. GenBank ID EF173184-85, KP869896.

Diagnosis. By external morphology the new species does not differ from *A. grandis* (see the description above) and may be easily confused with the latter under the light microscope. However, it has somewhat larger trophi (ramus length mean±SD 26.8±1.2 µm in *A. fontanetoi* sp. nov. and 25.4±1.4 µm in *A. grandis*).

Description. Viviparous. 8 teeth in each rake. Trophi 24-28 µm long and 27-28 µm wide; 2/2 major uncinal teeth, 38-43 minor teeth in the left uncus and 39-42 in the right one.

Measurements. See Table 2.

Distribution. Maritime Antarctica: SI. Continental Antarctica: BI*. **Habitat.** Soil.

Order Philodinida Melone & Ricci, 1995

Family Habrotrochidae Bryce, 1910

Genus *Habrotrocha* Bryce, 1910

Habrotrocha antarctica sp. nov.

Figs 3d, 8

Murray, 1910 (*Callidina constricta*): 48-49, Pl. XII figs 13a,b; Dartnall & Hollowday, 1985 (*H. constricta*): 32; Fig. 25a-c. Velasco-Castrillón et al., 2014c (Bd12): 8.

Type locality. Cape Royds, 18 m asl, S77° 32.532', E166° 8.855'. **Type habitat.** Soil.

Type material. Holotype: SIZ 56.1 (CRL23HE1a, 14.01.2010, Leg. J. Smykla), mounted in glycerine jelly, encircled in green ink. Paratypes: SIZ 56.2-4 (CRL23HE1b-d), on the same slide as holotype, encircled in black ink; SIZ 56. 5-11 (CRL23HE1e-k), trophi mounted for SEM. **Additional material.** SIZ 56.12-23 (EPL24M51-5, CzL4CYmatHE1-7), digital videos and photos; SIZ 56.24 (CzL4CYmatHE8), trophi mounted for SEM.

Etymology. Named after the Antarctic continent where it was first found.

Barcodes. GenBank ID EF650588-90, KJ543609-11, see also Supplementary file III.

Diagnosis. Very similar to *H. elusa* s. lat. Milne, 1916, except of the rump shape and trophi structure. The foot is much wider and the spurs longer than in *H. elusa vegeta* Milne, 1916. Differently from *H. elusa* s. str. Milne, 1916, it has no lateral knobs on the first rump pseudosegment. The integument on the trunk and rump is not granulated or dotted, unlike reported for *H. elusa* s. str. by Donner (1965). *S*imilar to *H. constricta* by the size and body shape, however distinguished by the upper lip with a notch in the middle (so that the tip is divided into two small lobes), while in *H. constricta* the tip is whole. Dental formula 7/7 major uncinal teeth (the last 2-3 thinner than the rest), while in *H. elusa* s. str. it is reported to be 6/6, 7/7 or 8/8. In *H. elusa vegeta* it is 4+3/3+4, in, and *H. constricta* usually has 6/6 major teeth in unci and rarely 7/7 or 8/8 (Donner, 1965).

Description. Body of moderate size, transparent, spindle-shaped, colorless but usually with yellow-brown or bright orange stomach. Integument smooth, thin, without knobs, ribs or spines. No knob on the 1st foot pseudosegment. Rostrum short, lamella divided into two small semicircular lobes. Corona narrower than the oval head base, CW/HW 79-91%, HW is 96-100% of HL. Pedicels short, straight, divided by a narrow sulcus without membrane or ligula. Trochal discs kidney-shaped in apical view. No papillae or sensory bristles on trochi. Upper lip triangular, reaching plane of trochal discs, upper rim thickened by cuticular bolster, tip divided by a notch into two small rounded lobes. Lower lip not wide, not projecting laterally. Cingulum bolster very narrow. Neck of moderate length and width, NL is 15-35% of TL. Trunk slim, BW is 15-20% of TL. Rump conical, 1st pseudosegment swollen, RL is 11-15% of TL, RW is 80-110% of RL. Foot very short, 4 pseudosegments, FL is 10-12% of TL, FW is 49-74% of FL. Spurs short, triangular with elongated narrow tips and slightly swollen middle part, divergent, without interspace, SL is 55-74% of SSW. Three short unsegmented toes. No eyespots. Throat narrow, oesophagus short, straight. Stomach glands small, round. Food pellets rounded, small. Trophi ramate, heart-shaped, 15-19 µm long and 17-20 µm wide. Rami thick, with numerous short scleropili along the inner rims. Articulation straight, wide, without incisure. Manubria narrow, sickle-like. Dental formula 7/7, with 24-25 minor teeth in each uncus. Oviparous. Eggs oval, 65-70 x 33-41 µm, shell smooth, without knobs or spines.

Measurements. See Table 2. TL 250 µm by Murray (1910) or 375 µm (Dartnall & Hollowday, 1985).

Distribution. Maritime Antarctica: SI. Continental Antarctica: EB, MM, VL (CR, EP*, Cz*).

Habitat. Soil, algal mats, sediment in pools.

Comments. Murray (1910) first depicted this species, but erroneously identified it as *C.* (=*H.*) *contstricta*. In Murray's image the notch in the middle of the upper lip (absent in *H. constricta*) is clearly visible, and the dental formula seems to be 7/7 or 8/8 (though in the description Murray mentions only 4/4 major teeth). *H. antarctica* sp. nov. was identified as *H. constricta* by Dartnall & Hollowday (1985), but the specimen depicted by these authors has a two-lobed upper lip, while *H. constricta* has only one lobe.

***Habrotrocha devetteri* sp. nov.**

Figs 9a-d

Velasco-Castrillón et al., 2014a (Bd42): 8 (main text); 5, Fig. 21-22 (Annex S1).

Type locality. Cape Bird (Ross Island), 77 m asl, S77° 13.207', E166° 26.568'. **Type habitat**: Soil.

Type material. Holotype: SIZ 57.1 (CBM2HD2a, 19.01.2010, Leg. J. Smykla), mounted in glycerin jelly, incircled with green ink. Paratypes: SIZ 57.2-5 (CBM2HD1, CBM2HD3-5), mounted on the same slide as the holotype, incircled with black ink; SIZ 57.6 (CBM2HT2b), trophi mounted for SEM. **Additional material.** SIZ 57.7-14 (CBC4HD1, CBM2HD6-9, CBM2matHD1-3), digital photos and videos; SIZ 57.15 (CzL4CymatHD2), trophi mounted for SEM.

Etymology. Named after colleague rotiferologist Dr. Miloslav Devetter participating in this study.

Barcodes: GenBank ID KJ543668-74, see also Supplementary file III.

Diagnosis. Similar to *H. thienemanni* s. lat. by the shape and size of the corona, trunk and spurs. Alike *H. thienemanni* s. lat., its upper lip has two small lobes, however the lobes are rounded and divided by a broad interspace, while in *H. thienemanni* s. lat. the lobes are often pointed and divided by a notch. The new species has 2+2/2+2 major teeth in the unci, while

H. thienemanni s. lat. has 2+1/2+1 major teeth (Fig. 9d, e). It differs from *H. crassa* Donner, 1949, another species with two-lobed upper lip, by the corona wider than the head base, the smooth integument, body outline, and dental formula (4/4 in *H. crassa*). It differs rom *H. tranquilla* Milne, 1916 by its smaller size. TL of the new species is 209-282 µm, while for *H. tranquilla* it is 340-402 µm. The lower lobes of the upper lip are divided by an interspace, while in *H. tranquilla* they are higher and divided by a notch. Dental formula is not 7/7 - 9/9 as in *H. tranquilla*.

Description. Body of moderate size, spindle-shaped, colorless, transparent. Integument thin, smooth, without knobs, spines or bolsters. Rostrum short, lamella with two small semicircular lobes. Corona wider than the oval head base, CW is 103-118% of HW, HL is 22-31% of TL. Pedicels short, straight. Sulcus very narrow, half-covered with membrane. Trochal discs with papillae and sensory bristles. Upper lip goes up to a half of the pedicels, it is arcuate with two small semicircular lobes divided by an interspace. Lower lip slightly protruding laterally. Cingulum narrow. Neck of moderate length, NL is 15-26% of TL. The length of antenna is about 1/3 of the bearing pseudosegment width. Trunk plump, BW is 17-30% of TL. Rump conical, RL is 12-17% of TL, RW is 81-98% of RL. Foot short, slim, 4 pseudosegments, FL is 8-14% of TL, FW is 51-88% of TL. Spurs short, triangular, divided by interspace as broad as one spur width, SL is 44-76% of SSW. Three short unsegmented toes. No eyespots. Throat and oesophagus short, food pellets round, of moderate size. Stomach glands of medium size. Trophi ramate, heart-shaped, 16-19 µm long and 16-19 µm wide. Rami thin, with numerous short scleropili along the inner rim, articulation straight and without incisure. Manubria thin, sickle-like. 2+2/2+2 major teeth, 30-33 minor teeth in each uncus. Oviparous, egg oval, 67 x 39 µm, shell smooth, without knobs or spines.

Measurements. See Table 2. TL 250-360 µm (Velasco-Castrillón et al., 2014a)

Distribution. Continental Antarctica: EB, VL (CC*, CB*), WK. **Habitat.** Soil, algal mats.

Habrotrocha vernadskii sp. nov.

Figs 3e, 10

Type locality. Galindez Island (Argentine Islands archipelago), 4 m asl, S65° 15.060', W64° 14.558'. **Type habitat.** Soil.

Type material. Holotype: SIZ 58.1 (V12HE2a, 1.03.2010, Leg. K. Janko), mounted in glycerin jelly, encircled in green ink. Paratypes: SIZ 58.2-4 (V12HE2a-c), on the same slide as holotype, encircled in black ink; SIZ 58. 5-8 (V12HE4a-b, V12HE13a-b), trophi mounted for SEM. **Additional material.** SIZ 58. 9-15 **(**CCA2HE1-2, CCA4HE1-5), digital photos and videos.

Etymology. Named after the Ukrainian polar research base "Academician Vernadsky" in the vicinity of which it was found.

Barcodes. GenBank ID – see Supplementary file III.

Diagnosis. Resembles *H. elusa* s. str. Milne, 1916 by the yellowish body with thicker integument on trunk and rump, the triangular upper lip with the tip divided by a notch into two rounded lobes, by four lateral knobs on the rump, and by the short triangular spurs. However, the knobs seem to be smaller than in *H. elusa* s. str. Milne (1916) clearly states that *H. elusa* s. str. has "thick, leathery but smooth skin". Contradictory to this, the new species has trunk, rump and 1st foot pseudosegment covered with minute granulae, as in "*H. elusa* s. str." (possibly other species) depicted by Donner (1965). Corona distinctively narrower than the head base, CW/HW is 71-84%. Milne reports *H. elusa*'s corona to be equal or slightly wider than the head base (CW/HW is 100-111%). Dental formula 9/9 major uncinal teeth (the last 2 ones almost as thin as minor teeth), while in *H. elusa* s. str. it is 6/6, 7/7 or 8/8 (Donner, 1965). Differs from *H. crenata* s. lat. by the shape of the upper lip (in *H. crenata* s. lat. the tip of the upper lip is not divided into lobes), and by the type of sculpturation. In *H. crenata* s. str. the whole foot and spurs are granulated, and the 1st foot pseudosegment has a rounded knob absent in the new species. Dental formula of *H. crenata* is 7/7 or 8/8 (Donner, 1965). Differs from *H. antarctica* sp. nov. by the granulated integument, lateral knobs on rump, narrower corona, and slightly longer spurs. Also, it has a larger number of major and minor teeth in the unci (9/9) than *H. antarctica* sp. nov. (7/7).

Description. Body slim, spindle-shaped, yellowish. Integument granulated on the last neck pseudosegment, trunk, rump and the 1st foot pseudosegment, and smooth on the rest of the body. Four small pointed lateral knobs on rump (2 on the distal rim of the 1st and 2 on the 2nd pseudosegment). No knobs on foot. Rostrum very short, lamella with two small semicircular lobes. Corona narrower than rectangular head base, HL is 11-15% of TL. Upper lip triangular, reaches plane of trochal discs, tip divided by a notch into two small semicircular lobes. Trochi without papillae and sensory bristles. Pedicels short, straight. Sulcus very narrow, partly covered by prominent retractors of trochi. Lower lip not protruding laterally. Neck rather long, of moderate width, NL is 38-61% of TL. Antenna is 1/3-1/4 of the bearing pseudosegment's width. Trunk narrow, BW is 29-62% of TL depending on the amount of eggs in a female. The first rump pseudosegment swollen, RL is 10-15% of TL, RW is 71-99% of RL. Foot short, 4 pseudosegments, FL is 8-11% of TL, FW is 54-85% of FL. Spurs of moderate length, narrow triangular, bases merged but seem to form short interspace, SL is 56-84% of SSW. Three short unsegmented toes. No eyespots. Throat small, oesophagus short, straight. Food pellets small, of irregular shape. Oviparous, eggs oval, shell smooth, without knobs or spines. Trophi ramate, heart shaped, 15-17 μm long and 14-16 μm wide. Articulation straight, without incisure. Numerous short scleropili on inner rims of rami. Manubria narrow, sickle-like. Unci with 9/9 major teeth, gradually diminishing in thickness, the last ones hardly distinguishable from minor teeth (26-27 in each uncus).

Measurements. See Table 2.

Distribution. Maritime Antarctica: AI*. Continental Antarctica: CC*. **Habitat.** Soil, terrestrial moss.

Family Philodinidae Ehrenberg, 1838

Genus *Macrotrachela Milne, 1886*

***Macrotrachela donneri* sp. nov.**

Figure 11

Murray, 1910 (*Callidina habita*): Pl. IX fig. 3, Pl. XI fig. 8a. Donner, 1965 (*Macr. insolita* var. 3): 132, Fig. 96l, m. Donner, 1972a (*Macr. insolita* var.): 252, Abb.2. Iakovenko & Tyshenko, 2006 (*Macr. hewitti*): 2, Ris. 2.

Type locality. Marble Point (Victoria Land), 4 m asl, S77° 25.597', E163° 45.148'. **Type habitat.** Soil.

Type material. Holotype: SIZ 59.1 (MPM5MD1a, 25.01.2010, Leg. J. Smykla), on slide in glycerine jelly, encircled with green ink. Paratypes: SIZ 59.2-6 (MPM5MD2-6), in glycerine jelly on the same slide as the holotype, encircled with black ink; SIZ 59.7-9 (MPM5MD1b-d), trophi mounted for SEM. **Additional material.** SIZ 59.10-15 (KG1MD1, EPL25MD1, MPL1MD1, MPL3MD1-3), digital photos and videos; SIZ 59.15-18 (MPL3MD1-4), trophi mounted for SEM.

Barcodes. GenBank ID KP869998.

Etymology. Named after Austrian rotiferologist Dr J. Donner who first depicted this species as *M. insolita* var.

Diagnosis. Similar to *Macr. habita* (Bryce, 1894) and *Macr. insolita* de Koning, 1947 by the smooth integument, the upper lip with two rounded lobes, and the longitudinal knob on the 1st foot pseudosegment. However, ligula in the sulcus and knobs on rump are absent in the new species. The foot is rather stout (what distinguishes all three species from *Macr. plicata* s. lat.). Similarly to *Macr. insolita*, the dental formula of the new species is 2/2, while in *Macr. habita* it is 2+1/1+2 (with an additional thinner teeth). It is easily distinguished by the peg-like or narrow conical spurs – in *Macr. habita* and *Macr. insolita* the spurs are triangular, wide, and flat, with shorter interspace. Larger than *Macr. insolita* (TL 325±44 µm, TL of *Macr. insolita* is about 250 µm). CW/HW ratio (107-119%) is intermediate between *Macr. habita* (120%) and *Macr. insolita* (100-103%), and the head seems to be shorter and wider than in *Macr. insolita*. The upper lip is shorter, and the lobes are more separated from each other. The head base is rectangular, while it seems to be trapezoid in *Macr. habita*, and wide-oval in *Macr. insolita*.

Description. Body large, transparent, colorless, but often with bright-orange stomach. Integument smooth, without spines or bolsters, no knobs except a large longitudinal knob on the 1st foot pseudosegment. Rostrum short, thick, lamella with two semicircular lobes. Head wide, corona wider than head base, CW is 107-119% of HW, HL is 10-15% of TL. Pedicels short, straight. Sulcus as wide as ½ of a trochus, covered with protruding trochi retractors. No papillae or sensory bristles on trochi. Head base rectangular, shorter than its width. Upper lip arcuate with two large semicircular lobes not divided by interspace, reaching ½ of the pedicels' height. Lower lip protrudes laterally. Neck of moderate length and width, NL is 15-21% of TL. Length of antenna is about ¼ of bearing pseudosegment width. Trunk thick, BW is 15-25% of TL. Rump large, swollen, RL is 12-15% of TL, RW is 65-97% of RL. Foot short, of 4 pseudosegments, 1st pseudosegment with dorsal elongated knob. Spurs rather long, rod-like, pointed, gradually tapering from the base to tips, interspace equal to 2 spur widths, SL is 86-111% of SSW. Three thick unsegmented toes. No eyespots. Throat voluminous, oesophagus short, straight. Lumen long, thick, often with a loop. Stomach glands large, round. Trophi ramate, large, heart-shaped, 21-25 µm long and 24-28 µm wide. Rami massive, inner rim with numerous scleropili. Articulation long, flat, straight, without incisure. Manubria long, wide, crescent-like. Dental formula 2/2, with 42-43 minor teeth in each uncus. Oviparous. Egg lemon-shaped, shell smooth with two round knobs on each pole.

Measurements. See Table 2. TL up to 570 µm, CW 95 µm in Murray (1910). According to Donner (1965), TL 410 µm, CW 66 µm, SL 29 µm, trophi 30 µm long.

Distribution. Maritime Antarctica: AI*, KG*, SI. Continental Antarctica: VL (CB*, CC*, CR, Cz*, BI*, EP*, MP*). **Habitat.** Soil, algal mats in seepages, terrestrial moss.

Comments. Murray (1910) first depicted this rotifer from Cape Royds, though he apparently described two different species under the name *C. habita*.

Macrotrachela ioannae sp. nov. Iakovenko

Figs 3h, 12

Type locality. Rocka Islands (Argentine Islands Archipelago), 15 m asl, S65° 10.738', W64° 29.522'. **Type habitat**. Soil.

Type material. Holotype: SIZ 60.1 (MRockaMI1a, 15.02.2010, Leg. K. Janko), mounted in glycerin jelly, encircled with green ink. Paratypes: SIZ 60.2-3 (V12MI1-2), on the same slide with holotype, encircled with black ink. **Additional material.** SIZ 60.4-6 (MRockaMI2-3, CrulsBMI1), digital photos.

Etymology. Named after Mgr. Ioanna Vaňkova, a friend and a specialist in linguistics, who gave much advice on creating Latin names for the new rotifer species.

Barcodes: GenBank ID KP869995-97.

Diagnosis. Similar to *Macr. ehrenbergii* (Janson, 1893), *Macr. timida* s. lat., *Macr. induta* Donner, 1951, and *Macr. allani* (Murray, 1911). Differs from all these species by the shape of spurs with papillae-like tips, and dental formula (additional thinner tooth in each uncus). The head base is shorter and wider than in *Macr. ehrenbergii*. The corona is almost equal to the head base, while in *Macr. timida* and *Macr. allani* it is substantionally wider. Spurs shorter than the bearing pseudosegment width, while in these species they are, on the contrary, longer. Unlike in *Macr. timida* s. lat., no knobs on foot or rump. Differs from *Macr. induta* also by the shape of the upper lip, which is in *Macr. induta* is wide arcuate, with low rounded lobe in the middle. The new species has narrow arcuate upper lip with a high trapezoid lobe with rounded tip in the middle, bearing thin bolster along the upper rim**.**

Description. Body large, colorless, transparent. Integument smooth, without knobs, spines or bolsters. Rostrum thick, of moderate length, lamella with two semicircular lobes. Corona not wide, CW is 100-108% of HW, HL is 11-21% of TL. Pedicels short, straight. Sulcus of moderate width, without ligula, not covered with membrane or trochi retractors. Head base rectangular, its width larger than height. Upper lip arcuate with single trapezoid lobe, its rounded tip has a bolster along the upper rim. Lower lip not protruding laterally. Neck of moderate length and width, NL is 12-18% of TL. Length of antenna is 1/3 of the bearing pseudosegment's width. Trunk cylindrical, BW is 13-17% of TL. First rump pseudosegment swollen, RL is 12-15% of TL, RW is 78-96% of RL. Foot short, 4 pseudosegments, FL is 8-11% of TL, FW is 52-77 of FL. Spurs short, flat, triangular, divergent, with bases merged and tips separated as small papillae. Three unsegmented toes. No eyespots. Throat voluminous, oesophagus short, straight. Lumen wide, with a loop. Stomach glands round, not large. Trophi ramate, heart-shaped, 26 µm long and 20-21 µm wide. Dental formula 2+1/1+2 major teeth (with an additional thinner teeth) and about 30 minor teeth in each uncus. Oviparous. Eggs oval, 124 x 66 µm. Egg shell smooth, without knobs or spines.

Measurements. See Table 2.

Distribution. Maritime Antarctica: AI*. **Habitat.** Soil, terrestrial moss.

Macrotrachela jankoi **sp. nov. Iakovenko**

Figs 3f-g, 13

Velasco-Castrillón et al., 2014a (Bd7): 8.

Type locality. Squa Island (Argentine Islands archipelago), 20 m asl, S65° 25.117', W64° 26.583'. **Type habitat.** Soil.

Type material. Holotype: SIZ 61.1 (VS02MJ1a, 15.03.2010, Leg. K. Janko), mounted in glycerine jelly. Paratypes: SIZ 61.2-3 (VS02MJ2, VS02MJ3), mounted in glycerine jelly; SIZ 61.4 (VS02MJ4), trophi mounted for SEM. **Additional material.** SIZ 61.5-17 (V08MJ1, V11MJ1, V12MJ1-2, VRA01MJ1-7, VRA02MJ1), digital photos and videos; SIZ 61.18-26 (V12MJ3, MRockaMJ1-8), trophi mounted for SEM.

Barcodes. GenBank ID KJ543594-97, KJ543597, KP869999, KP870000.

Etymology. Named after Dr Karel Janko, the leader of the project conducted on Vernadsky Base, and the collector of the material.

Diagnosis. Resembles *Macr. insulana* Donner, 1962 by the shape of corona and upper lip, the characteristic sulcus with two denticles on the dorsal side, and the absence of a knob on the 1st foot pseudosegment. Differs by the flat and short triangular spurs, which are longer and peg-like in *Macr. insulana*. Dental formula of the new species is 2/2, while in *Macr. insulana* it is 1+2/2+1.

Description. Body large, transparent, stomach of bright red or orange colour. Integument smooth, thin, without knobs, spines or bolsters. Rostrum stout, of moderate length, lamella with two large rounded lobes. Corona wider than oval head base, CW is 100-133% of HW, HL is 35-54% of TL. Pedicels short, straight. Sulcus wide, not covered with membrane. On dorsal side of head two short denticles divided by interspace visible in sulcus. Trochi large, with papillae and sensory bristles. Upper lip with two triangular lobes reaching about a half of the sulcus, and divided by interspace. Lower lip not protruding laterally. Neck stout, of moderate length, NL is 16-21% of TL. Antenna about 1/3 of the bearing pseudosegment. Trunk plump, BW is 15-21% of TL. Both pseudosegments of the rump swollen, RL is 12-21% of TL, RW is 73-93% of RL. Foot short, stout, 4 pseudosegments, without a dorsal knob, FL is 10-12% of TL, FW is 50-82% of FL. Spurs small, short, isosceles triangular, divided by an interspace almost equal to spur width, SL is 44-79% of SSW. Three short unsegmented toes. No eyespots. Throat voluminous, oesophagus short, straight. Stomach glands round. Trophi ramate, 18-22 µm long and wide. Rami massive, with numerous short scleropili along the inner rim. Articulation somewhat concaved in the middle, but without incisure. Manubria thin, sickle-like. Dental formula 2/2, 39-41 minor teeth in each uncus. Oviparous. Eggs oval, shell smooth, without knobs or spines.

Measurements. See Table 2.

Distribution. Maritime Antarctica: AI*, AP*, KG*. Continental Antarctica: EB, VL (Cz*).

Habitat. Soil, terrestrial moss.

Genus *Philodina* Ehrenberg, 1830

***Philodina dartnallis* sp. nov.**

Figs 3i-j, 14

Priddle & Dartnall, 1978 (? *Philodina*): 475. Dartnall & Hollowday, 1985 (*Philodina* sp. 'A'): 24, figs 27a-e. Velasco-Castrillón et al., 2014a (*Ph.* sp. Bd46): 8.

Type locality. Cape Bird, 77 m asl, S77° 13.207', E166° 26.568'. **Type habitat.** Soil.

Type material. Holotype: SIZ 63.1 (CBM2PHD1a, 19.01.2010, Leg. J. Smykla), mounted in glycerin jelly, encircled with green ink. Paratypes: SIZ 63.2-4 (CBM2PHD1b-d), on the same slide with holotype, encircled with black ink. **Additional material.** SIZ 63.5-12 (CzM4PHD1-5, CzM4matPHD1, VDM2PHD1-2), digital photos and videos.

Barcodes. GenBank ID KJ543683-86, see also Supplementary file III.

Etymology. Named after Antarctic researcher Dr H. Dartnall who first depicted this species as *Ph.* sp. ‘A’.

Diagnosis. Similar to *Ph. flaviceps*, *Ph. australis* Murray, 1911, and some specimens of *Ph. brevipes* Murray, 1902. Resembles *Ph. flaviceps* by the shape of corona and spurs; however the spurs of the new species are longer and with broader interspace. Differs from *Ph. australis* by the spur shape, which in the new species are peg-like with a broad interspace, but narrow triangular without interspace in *Ph. australis*. Differs from *Ph. brevipes* by the shape of the upper lip (rounded lobes in the new species, pointed in *Ph. brevipes*), and by the absence of long sensory cilia in the rostrum. The foot seems to be shorter than in *Ph. brevipes*.

Description. Body of moderate size, colorless, transparent. Integument smooth, thin, without knobs, spines or bolsters. Rostrum of moderate length, thick, lamella with two very small semicircular lobes. Corona wider than head base, CW is 102-119% of HW, HL is 27-38% of TL. Upper lip wide, arcuate, with two large low rounded lobes not reaching plane of trochal discs, and divided by broad interspace. Pedicels short, trochi large, with papillae and sensory bristles, retractors visible. Sulcus wide, not covered with membrane, without ligula. Lower lip not protruding laterally. Neck wide, of moderate length. Antenna 1/3 of bearing pseudosegment width. Trunk plump, BW is 14-25% of TL. Rump conical, first pseudosegment slightly swollen, without protrusions, RL/TL is 10-18%, RW/RL is 74-96%. Foot of moderate length, stout, 5 pseudosegments, without knobs or protrusions, FL is 8-15% of TL, FW is 13-22% of FL. Spurs peg-like, parallel to each other, divided by very narrow interspace, SL is 51-72% of SSW. Four thick unsegmented toes. Two cerebral orange or bright-red eyespots. Throat and oesophagus of moderate length. Trophi ramate, round, 3/2. Stomach bright red, lumen wide. Egg oval, with rounded knob on one pole, shell without spines or sculpturation, egg size 50-59 x 34-42 µm.

Distribution. Maritime Antarctica: AI*, AP*. Continental Antarctica: EB, VL (CB*, Cz*), WK. **Habitat.** Soil, terrestrial moss, lakes.

***Philodina shackletoni* sp. nov.**

Figure 15

Velasco-Castrillón et al., 2014a (*Ph.* sp. Bd45): 8 (main text); 5, Figs 23-27 (Annex S1).

Type locality. Cape Royds, 18 m asl, S77° 32.532', E166° 08.855'. **Type habitat.** Soil.

Type material. Holotype : SIZ 62.1 (CRL25PHC1a, 14.01.2010, Leg. J. Smykla), mounted in glycerine jelly. Paratypes: SIZ 62.2-3 (CRL25PHC2-3), the same. **Additional material:** SIZ 62.4 (CRL21PHC1), digital photos.

Barcodes. GenBank ID KJ543677-86, see also Supplementary file III.

Etymology. Named in honour of the leading Antarctic explorer, Sir Ernest Henry Shackleton who in 1909 established his base on Cape Royds were the species was discovered.

Diagnosis. Most closely resembles *Ph. flaviceps* Murray, 1906 by the shape of spurs and corona, however lacking eyespots. Spurs are longer than in *Ph. flaviceps*, and divided by broader interspace.

Description. Body large, spindle-shaped, colorless with yellow-brown stomach. Integument smooth, thin, without knobs, spines or bolsters. Rostrum of moderate size, with crescent-like lamella. Corona wider than trapezoid head base, CW is 109-117% of HW, HL is 13-17% of TL. Pedicels short, slightly bent inwards. Sulcus wider than diameter of a trochus, not covered with membrane. Trochi with papillae and sensory bristles. Upper lip very low, arcuate, with two small rounded lobes divided by interspace. Lower lip not protruding laterally. Neck of moderate length and width, NL is 18-23% of TL. Antenna long, almost equal to the bearing pseudosegment width. Trunk slim, BW is 15-20% of TL. Rump large, swollen, RL is 15-17% of TL, RW is 11-13 of RL. Foot long, slim, 5 pseudosegments, FL is 10-15% of TL, FW is 27-32% of FL. Spurs long, needle-like, SL is 115-164% of SSW. Four unsegmented toes. No eyespots. Throat small, oesophagus short. Lumen wide. Stomach glands small, round. Trophi ramate, 24 µm long and wide, dental formula 2/2. Oviparous. Eggs oval, shell smooth, without knobs or spines.

Measurements. See Table 2. TL 400 µm (Velasco-Castrillón et al., 2014a).

Distribution. Maritime Antarctica: SI. Continental Antarctica: EB, VL (CR*), WK. **Habitat.** Soil, terrestrial moss, pools.

Morphometric analyses

Adineta editae sp. nov differed from the similar European species *A. gracilis* by larger size of body and mastax, and longer spurs. LME demonstrates that the variation of body measurements between species represented over 60% of total variation for the parameters describing the width along the rotifer body (HW, MinNW, MxNW, FW, SSW) and for the spur length. The variation between localities and individuals was not significant for FW, SSW and SL (ANOVA on LME output: LR=11.1 to 13.1, p>0.1). This was in correspondence with our visual observation that *A. editae* sp. nov. had a distinctively stouter body than *A. gracilis* s. str. In the PCA plot (Fig. 16a) external measurements of the two species did not overlap along PC1 (correlating with all measurements) and PC2 (correlating with HW and SL).

The difference in trophi measurements between *A. editae* sp. nov. and *A. gracilis* represented over 90% of total variation in the number of minor teeth and trophi length, and over 80% in the case of trophi width. The variation between localities and individuals was insignificant for all measurements (LR =0.2 to 2.1, p>0.5). The trophi measurements of these species were completely separated on the PCA plot (Fig. 16b) and did not overlap along PC1 (correlates with all measurements) and PC2 (correlates with the number of minor teeth and the unci width).

The body and trophi of the Antarctic species *A. grandis* and *A. fontanetoi* sp. nov. are indistinguishable by external morphology, and did not differ significantly in any of the measured parameters. The Antarctic *A. emsliei* sp. nov. and the European *A. vaga* s. str. noticeably differed by at least one trophi measurement (the number of minor teeth in unci). The difference by this parameter consisted over 80% of total variation, with the variation between localities and individuals being insignificant (LR=0.7 to 1.5, *p*>0.5). The antarctic species *A. grandis* and *A. emsliei* sp. nov. were distinguished by all trophi measurements, the difference between species being over 90% of total variation. The variation between localities and individuals was not significant except for rami length (LR=0.3 to 4.5, p>0.1). In the PCA plot (Fig. 16c) the samples of trophi of *A. grandis* and *A. fontanetoi* sp. nov. overlapped completely on both PC1 (correlating with all trophi measurements) and PC2 (correlating with the number of minor teeth and trophi width), but the samples of *A. emsliei* sp. nov. did not overlap with any of the other species.

The Antarctic *H. antarctica* sp. nov. and *H. vernadskii* sp. nov. did not show any significant difference in trophi length and width, but could be distinguished by the number of minor teeth of the unci. Variation between the species on the latter measurement represented over 70%, the variation between localities and individuals being insignificant (LR=3.6, *p*>0.1). *Habrotrocha* sp. 4 is indistinguishable from *H. antarctica* sp. nov. by external morphology, but has an intermediate trophi size between *H. antarctica* sp. nov. and *H. vernadskii* sp. nov, with the variation between species by all trophi parameters representing 50% or less of total variation. On the PCA plot of the samples of trophi meausrements (Fig. 16d) *H. antarctica* sp. nov. and *H. vernadskii* sp. nov., did not overlap with each other on any either axis. *Habrotrocha* sp. 4 did not overlap with either of the two other species.

DNA taxonomy

On the phylogenetic tree of *Adineta* (Fig. 17a) the sequences of Antarctic rotifers grouped into 1 singleton and 8 independently evolving entities (IEE) identified by the GMYC and 4x rule approaches (32 singletons and 6 IEEs according to bPTP). None of the IEEs contained rotifers originating from any other continents. The phylogeny shows that the Antarctic endemic *A.*

grandis consists of at least two morphologically indistinguishable entities (one of which has been described above as *A. fontanetoi* sp. nov.). For three IEEs the morphology has not been described, and therefore they are listed under *A.* sp. 1-3. Finally, three IEEs that proved to be both genetically separate and morphologically distinguishable, are described above as *A. editae* sp. nov., *A. emsliei* sp. nov. and *A. coatsae* sp. nov. Molecular analysis confirmed that these species occur only in Antarctica, in spite of being previously confused with the cosmopolitan species *A. gracilis*, *A. vaga* s. str. and *A. barbata*. The integrity of *A. coatsae* sp. nov. as a single IEE was confirmed by the GMYC model, but not by the bPTP and 4x rule models. Both the GMYC and 4x rule, but not the bPTP model, confirmed the integrity *A. fontanetoi* sp. nov. as a separate IEE.

On the tree of *Philodina* 19 singletons and 10 IEEs containing sequences of Antarctic bdelloids were identified by GMYC, mostly confirmed as IEEs by the 4x rule and bPTP (Fig. 17b). *Philodina* is the only one of the four investigated genera with cosmopolitan IEEs occurring in the Antarctic, those being *Ph.* sp. 4 (two sequences, from the Antarctic and the USA) and *Ph.* sp. 7 (25 sequences from the USA and one from Antarctica). Unfortunately, for both of these IEEs no data on morphology are available, and none of the sequences were obtained from vouchers of already known species. The remaining IEEs did not contain individuals from continents other than Antarctica. For six of them, listed here as *Ph.* sp. 1-6, there are no morphological data available. *Philodina gregaria* appeared as one large pan-Antarctic IEE, 3 singletons and one IEE with atypical morphology, containing only two sequences. Two IEEs proved to be well distinguishable both morphologically and by the means of DNA taxonomy, both from *Ph. gregaria* and the morphologically similar non-Antarctic *Ph. acuticornis* Murray 1902, *Ph. flaviceps* Murray 1906, and *Ph. roseola* Ehrenberg, 1832. These two species are described above as new Antarctic endemics: *Ph.*

shackletoni sp. nov. and *Ph. dartnallis* sp. nov. The integrity of *Ph. dartnallis* sp. nov. clade was confirmed by two of three delimitation methods.

Putative species delimitation of *Habrotrocha* according to GMYC gave 11 IEEs and 4 singletons with strictly Antarctic distribution (Fig. 18). For three IEEs (*H.* sp. 1, 2 and 3) the morphology has not been reported. Three IEEs are described above as *H. antarctica* sp. nov., *H. devetteri* sp. nov., and *H. vernadskii* sp. nov. based on both morphological and molecular differences from similar non-Antarctic *H. constricta*, *H. crenata* s. lat., *H. elusa* s. lat., and *H. thienemanni* s. lat. *Habrotrocha* sp. 4 was identified as an IEE by the GMYC model, but not confirmed as a single entity by the 4x rule, being less than four times separated from both *H. antarctica* sp. nov. and *H. vernadskii* sp. nov. (which are genetically and morphologically sufficiently separate to be good species). *H.* sp. 4 and *H.* sp. 5 are morphologically identical to *H. devetteri* sp. nov., but were delimitated as separate IEEs by all three molecular delimitation methods. *H. angularis* has been confirmed as a separate species with a strictly Antarctic distribution.

Four IEEs and three singletons distributed exclusively in Antarctica were identified in *Macrotrachela* using GMYC, and this delimitation was in most cases confirmed by the two other approaches (Fig. 19). For one IEE, *M.* sp. 2, no information is yet available on morphology. *Macrotrachela donneri* sp. nov., previously reported in Antarctica as *Macr.* cf *insolita* or M. cf *hewitti* (Donner, 1972), was represented by a single sequence which did not cluster with any non-Antarctic rotifers including the morphologically similar *M. habita*. For *M.* sp. 1 more morphological data are required. *Macrotrachela ioannae* sp. nov. and *M. jankoi* sp. nov. were confirmed by GMYC as good species occurring in Antarctic, but the bPTP approach revealed *M. jankoi* sp. nov. as two IEEs. The integrity of *M.* sp. 2, identified using GMYC, was not confirmed by the other two approaches.

The identification of IEEs based on our combined set of sequences, including both new sequences and the previously published data, was the same as in Velasco-Castrillón et al. (2014a) with the exception of three IEEs. According to our results, the putative species Bd15 and Bd16 were identified as a single entity *H.* sp.1 by all three models. Bd31 and Bd32 were identified as *H.* sp. 2 by the 4x rule, but as separate entities by the GMYC and bPTP models. Bd5 and Bd6 were identified as *M.* sp. 2 according to GMYC, but this was not confirmed by the two other models. All the sequences published by Barraclough et al. (2007) and Fontaneto et al. (2008) were identified as *Adineta* (*A. editae* sp. nov., *A.* sp. 2, *A.* sp. 3). All 16 sequences published by Fontaneto et al. (2012) were confirmed as *Philodina* (*Ph*. sp. 3-13).

Biogeography

Of the 60 morphospecies found in the new material examined in this study, at least 17 can be considered true Antarctic and/or sub-Antarctic endemics, with six of these being already known and 11 newly recognized. A further 10 morphospecies that closely resemble known and presumed cosmopolitan bdelloids, possessing only minor but consistent morphological differences from the original descriptions, require further detailed analysis. No molecular data are available for 13 morphospecies showing no discernible differences from known cosmopolitan species, as well as 20 identified only to generic level, and these cannot currently be attributed with confidence to any classification. That gives at least 28 and possibly up to 45% endemicity (17 or up to 27 morphospecies out of 60) on the basis of classical taxonomy alone.

Based on molecular data obtained both from our new samples and GenBank, we identified 33 IEEs and 37 singletons from Antarctica and the sub-Antarctic using GMYC (this largely confirmed by the two other approaches used), belonging to the genera *Adineta*, *Habrotrocha*, *Philodina* and *Macrotrachela*. Including a further five IEEs and eight singletons apparently representing other genera, a total of 38 IEEs and 45 singletons of Bdelloidea are now identified from this region. Only two of the IEEs occurring in Antarctica have been confirmed as having wider non-Antarctic distributions by molecular methods. At least 13 of the strictly Antarctic IEEs are clearly distinguishable by morphology, while at least four IEEs appear to represent cryptic species.

The molecular data obtained suggest there is a very high level of endemism among Antarctic bdelloids – 36 out of 38 IEEs (95%). GMYC also identified considerable apparent endemism in the rotifer faunas (IEEs) of other major global regions: 55 of 59 found in Eurasia (93%), 65 of 70 (93%) in North America, and three of four (75%) in both Australia and New Zealand, and Africa.

Discussion

After a 100-year period of research, the Antarctic Bdelloidea still remain obscure and knowledge sparse, in terms of their diversity, distribution and origin. The current study is only the fourth to report previously undescribed Antarctic bdelloid rotifers, with all 12 new taxa being currently known only from the continent. The study is the first to provide a robust combination of detailed, morphological, morphometric and molecular approaches, which are being utilized in the description of these new Antarctic taxa. This study is also the second to apply molecular approaches in describing diversity and biogeography of Antarctic Bdelloidea, as with that of Velasco-Castrillón et al. (2014a) carried out through COX1 sequencing. To date, 15 morphospecies of 60 (25%) found in this study have been barcoded successfully. Of these, we have (1) described 12 new for science mophospecies using both classical and DNA taxonomy approaches, (2) revealed the presence of a number of cryptic species that are apparently morphologically identical to *A. grandis*, *H. antarctica* sp. nov. and *H. devetteri* sp. nov., (3) linked the molecular data provided by Velasco-Castrillón et al. (2014a) with morphospecies, and (4) provided evidence of generally very high levels of endemism of bdelloid rotifers in Antarctica, with the exception of the finding of two cosmopolitan species of *Philodina*, not identified in previous studies.

The difficulty of distinguishing bdelloid species based on morphology alone, due to their generally highly conserved body morphology and structure, and ambiguity in defining specific characters, has led to a prevailing misconception that the contemporary Antarctic fauna includes a large proportion of cosmopolitan bdelloids. Thus, Donner (1965, 1972b) considered minor difference in size and shape of the bdelloid body and its appendages as only representing intraspecific variability. Previous studies have often attributed Antarctic specimens to species already known from elsewhere (mostly Europe) if most of the external morphological characters matched the original descriptions, or they were identified through keys based on the latter. Our morphometric and molecular data support the conclusions of Fontaneto et al. (2007), who proved that careful morphometric measurement of hard parts of the mastax (trophi) can differentiate several morphologically distinct entities within one „classical“ species, corresponding with IEEs determined by molecular analysis.

All three models applied here for the delimitation of IEEs based on molecular sequence data showed good correspondence with rotifer morphology. GMYC gave the best correspondence with the species-specific morphology and in most cases the results were supported by two other approaches. However, the bPTP model, used similarly by Velasco-Castrillón et al. (2014a), tended to give finer subdivision of IEEs, creating an excessive number of entities unidentifiable at the morphological level. Our data confirmed the integrity of all but three of the IEEs identified by Velasco-Castrillón et al. (2014a), even after the inclusion of our new sequence data. Our data also confirm the attribution of some sequences to *Adineta* and *Philodina* as proposed by Barraclough et al. (2007) and Fontaneto et al. (2008, 2012).

Conclusions

A striking feature of the data obtained in the current study is that of the extremely high levels of endemism to the Antarctic and sub-Antarctic region apparent in the bdelloid fauna. Clearly, consideration of the concept of endemism is itself limited by the quality and extent of the data available, both from the Antarctic and from other regions. However, both the current study and that of Velasco-Castrillón et al. (2014a) are consistent in identifying (1) that considerably greater diversity in terms of divergence to 'species level' is apparent in analyses of molecular (COX1) data than was the case in previous classical taxonomic studies of the group, and (2) that Antarctic lineages are distinct from those of bdelloids from other continents available today in GenBank.

Implicit in the assessment of considerable levels of endemism at continental level is the conclusion that it is indicative of an extended history (long term presence) allowing evolutionary divergence in situ in the Antarctic. This is consistent with a range of studies over the last one to two decades that have used both classical and molecular approaches to confirm both high levels of endemism and long evolutionary histories in representatives of all the main terrestrial invertebrate groups occurring in Antarctica, including Tardigrada (Convey & McInnes, 2005), Nematoda (Andrássy, 1999; Maslen & Convey, 2006), Collembola (Greenslade, 1995; McGaughran et al., 2010; Torricelli et al., 2010), Acari (Pugh, 1993; Stevens & Hogg, 2006) and Diptera (Allegrucci et al., 2012), see also wider reviews of this subject provided by Convey et al. (2008), Pugh & Convey (2008). Similar conclusions are increasingly being drawn from studies of some microbial groups (De Wever et al., 2009; Strunecký et al., 2012) and, most recently, mosses (Pisa et al., 2014).

The outcomes of the current study highlight the need for considerably greater survey effort being applied to groups of microscopic Antarctic fauna rich in cryptic species such as rotifers. Data obtained in both the study of Velasco-Castrillón et al. (2014a) and the current study suggest that at least some species of bdelloid rotifer are limited to particular parts of the Antarctic or sub-Antarctic. This, again, is consistent with recent findings in other groups of terrestrial biota (Convey et al. 2008; Pugh & Convey 2008), as well as the recent analysis of Terauds et al. (2012) that identified no less than 15 'Antarctic Conservation Biogeographic Regions' across the Antarctic continent alone. Thus, further targeted research amongst the bdelloid rotifers of Antarctica, integrating classical, morphometric and molecular biological approaches, should identify considerably greater levels of diversity and both continental and intra-continental regional endemism than are currently appreciated.

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Table captions

Table 1. Locations sampled in the Antarctic, including altitudes and the number of collected samples

Table 2. Body dimensions (measured from light microscope photographs) and trophi dimensions (measured from SEM photographs) of the described bdelloid species

Figure captions

Fig. 1 Map indicating sampling locations in Antarctica. a – Ross Sea area, b – Antarctic Peninsula and adjacent islands. BI – Beaufort Island. Ross Island: CB – Cape Bird, CC – Cape Crozier, CR – Cape Royds. Coastal zone of Victoria Land: Cz – Cape Chocolate, GH – Granite Harbour, MP – Marble Point, NF – Northern Foothills. Maritime Antarctica: AI – Argentine Islands archipelago, KG – King George Island, AP – Mount Demaria, Kiev Peninsula, Graham Land

Fig. 2 Measurements of bdelloids of the families Habrotrochidae and Philodinidae (a, b), and Adinetidae (c). BW – body width, CW – corona width, FL – foot length, FW – foot width, HL – head length, HW – head width, MinNW – minimal neck width, MxNW – maximal neck width, NL – neck length, RaL – ramus length, RaW – ramus width, RL – rump length, RW – rump width, SL – spur length, SSW – spur pseudosegment width, TL – total length

Fig. 3 New species of Antarctic bdelloids (photographs M. Plewka): a - *Adineta coatsae* sp. nov., holotype, habitus, dorsal view; b – *A. editae* sp. nov., habitus, dorsal view; c – *A. grandis*, habitus, dorsal view; d – *H. antarctica* sp. nov., holotype, habitus, feeding, dorsal view; e - *H. vernadskii* sp. nov., habitus, feeding, ventral view; f – *M. jankoi* sp. nov., habitus, creeping, ventral view; g – same, head, feeding, ventral view; f – *M. ioannae* sp. nov., habitus, feeding, dorsal view; i – *Ph. dartnallis* sp. nov., habitus, creeping, dorsl view; j – same, spurs. Scale bar 50 µm

Fig. 4 *Adineta coatsae* sp. nov. (Antarctica): a – holotype, habitus, dorsal view. *A. barbata* (Europe): b – habitus, dorsal view. Scale bar 50 µm

Fig. 5 *Adineta editae* sp. nov. sp. nov. (Antarctica): a, b - holotype, habitus, dorsal view; d – paratype, trophi, cephalic view; e – paratype, trophi, caudal view. *A. gracilis* (Europe): c – habitus, dorsal view; f – trophi, caudal view. Scale bar 50 µm (a-c) or 5 µm (d-f)

Fig. 6 *Adineta emsliei* sp. nov. (Antarctica): a, b – holotype, habitus, dorsal view; d – paratype, trophi, cephalic view; e – paratype, trophi, caudal view. *A. vaga* (Europe): c – habitus, dorsal view; f – trophi, cephalic view. *A. grandis* Murray, 1910 (Antarctica): g – trophi, caudal view. Scale bar 50 µm (a-c) or 5 µm (d-g)

Fig. 7 *Adineta grandis*: a – trophi, cephalic view. *A. fontanetoi* sp. nov.: b – paratype, trophi, cephalic view; c, d – holotype, habitus, dorsal view. Scale bar 50 µm (c, d) or 5 µm (a, b)

Fig. 8 *Habrotrocha antarctica* sp. nov.: a – holotype, habitus, feeding, dorsal view; b – same, creeping, dorsal view; c – paratype, trophi, cephalic view; d – paratype, trophi, caudal view. Scale bar 50 µm (a, b) or 5 µm (c, d)

Fig. 9 *Habrotrocha devetteri* sp. nov. (Antarctica): a – holotype, head, feeding, dorsal view; b – same, habitus, feeding, ventral view; c – habitus, creeping, dorsal view; d – paratype, trophi, caudal view. *H. thienemanni* (Europe): e – trophi, cephalic view. Scale bar 50 µm (a-c) or 5 µm (d, e)

Fig. 10 *Habrotrocha vernadskii* sp. nov.: a – holotype, head, feeding, dorsal view; b – same, habitus, creeping, dorsal view; c – paratype, trophi, cephalic view; d – paratype, trophi, caudal view. Scale bar 50 µm (a, b) or 5 µm (c, d).

Fig. 11 *Macrotrachela donneri* sp. nov.: a – holotype, head, feeding, dorsal view; b – same, habitus, creeping, dorsal view; c – paratype, trophi, cephalic view; d – paratype, trophi, caudal view. Scale bar 50 µm (a, b) or 5 µm (c, d)

Fig. 12 *Macrotrachela ioannae* sp. nov.: a – holotype, habitus, creeping, lateral view; b – same, head, feeding, dorsal view, c – same, foot, ventral view. Scale bar 50 µm

Fig. 13 *Macrotrachela jankoi* sp. nov.: a – holotype, head, feeding, dorsal view; b – same, habitus, feeding, ventral view; c – same, habitus, creeping, dorsal view; d – paratype, trophi, cephalic view; e – paratype, trophi, caudal view. Scale bar 50 µm (a-c) or 5 µm (d, e)

Fig. 14. *Philodina dartnallis* sp. nov.: a – holotype, habitus, creeping, dorsal view; b – same, feeding, dorsal view. Scale bar 50 µm

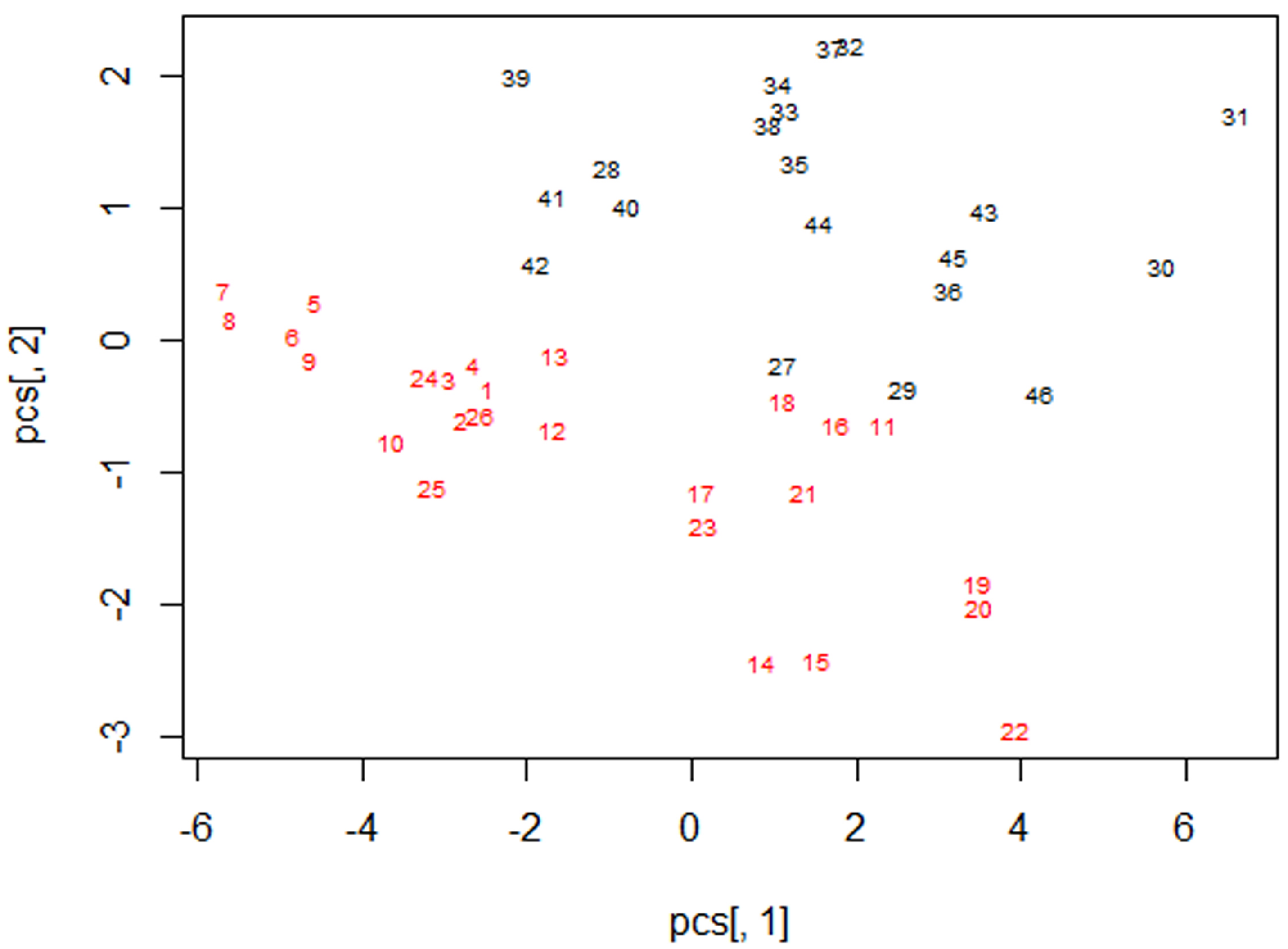
Fig. 15 *Philodina shackletoni* sp. nov.: a – holotype, habitus, feeding, dorsal view; b – foot, ventral view; c – holotype, habitus, creeping, dorsal view. Scale bar 50 µm

Fig. 16 Principal components analysis of rotifer body and trophi measurements: a – *A. gracilis* (circles) and *A. editae* sp. nov. (triangles), body dimensions; b – same, trophi dimensions; c – *A. grandis* (crosses), *A. fontanetoi* sp. nov. (circles), *A. emsliei* sp. nov. (squares) and *A. vaga* (triangles), trophi dimensions; d – *H. antarctica* sp. nov. (squares), *H. vernadskii* sp. nov. (circles) and *H.* sp. 4 (triangles), trophi dimensions

Fig. 17 Phylogenetic relationships in the genera *Adineta* (a) and *Philodina* (b). The consensus Bayesian trees of COX1 mtDNA data sets are shown on the left. Clades are marked as putative species delimited according to the 4x rule (triangles), GMYC (circles) and bPTP (squares). Boxes on the right show the distribution of samples across different continents

Fig. 18 Phylogenetic relationships in the genus *Habrotrocha* (consensus Bayesian tree, COX1 mt DNA dataset). Putative species are delimitated according to the 4x rule (triangles), GMYC (circles) and bPTP (squares). Wider distributions of rotifers are shown as in boxes on the right

Fig. 19 Phylogenetic relationships in the genus *Macrotrachela* (consensus Bayesian tree, COX1 mt DNA dataset). Putative species are delimited according to the 4x rule (triangles), GMYC (circles) and bPTP (squares). Wider distributions of rotifers are shown as in boxes on the right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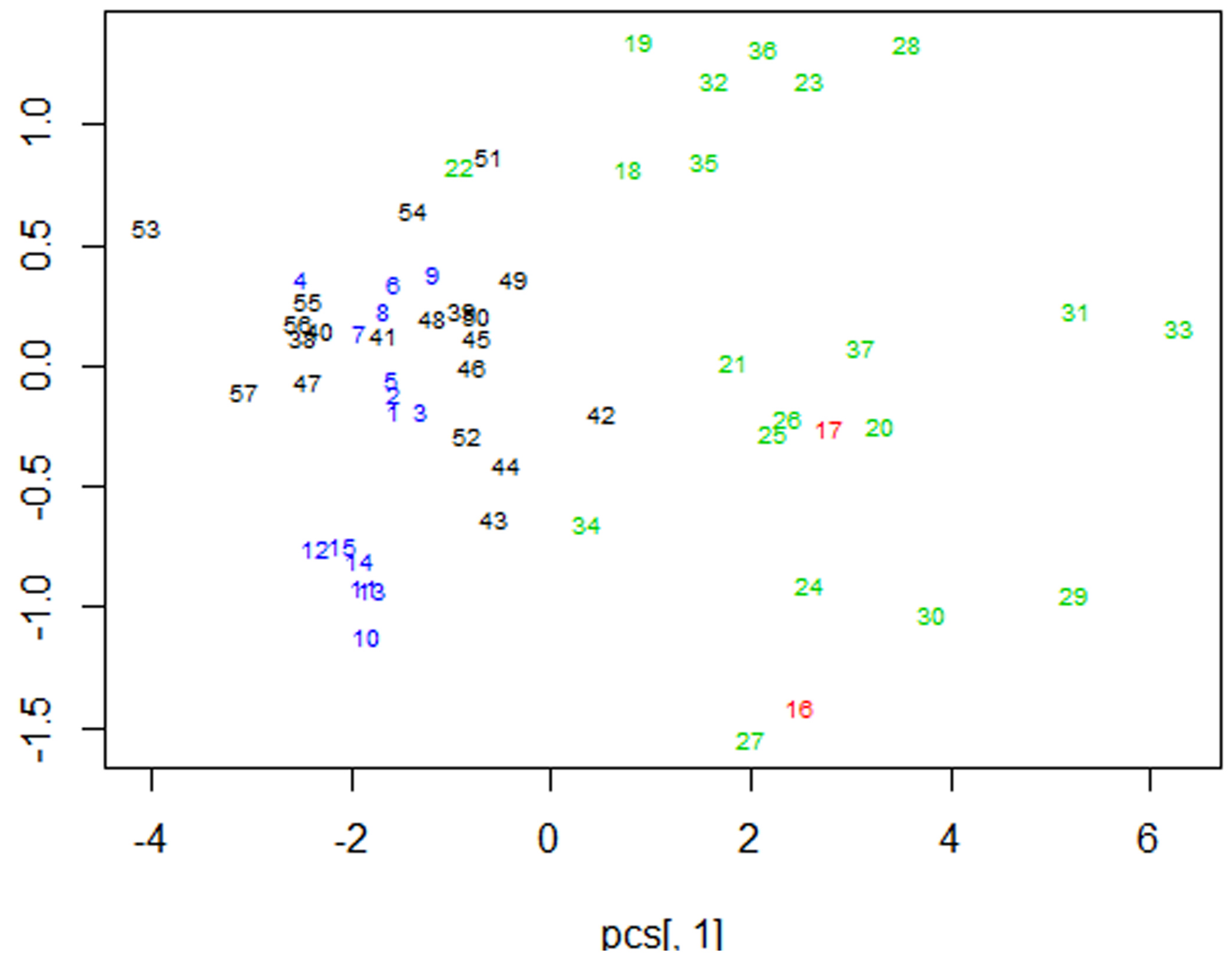
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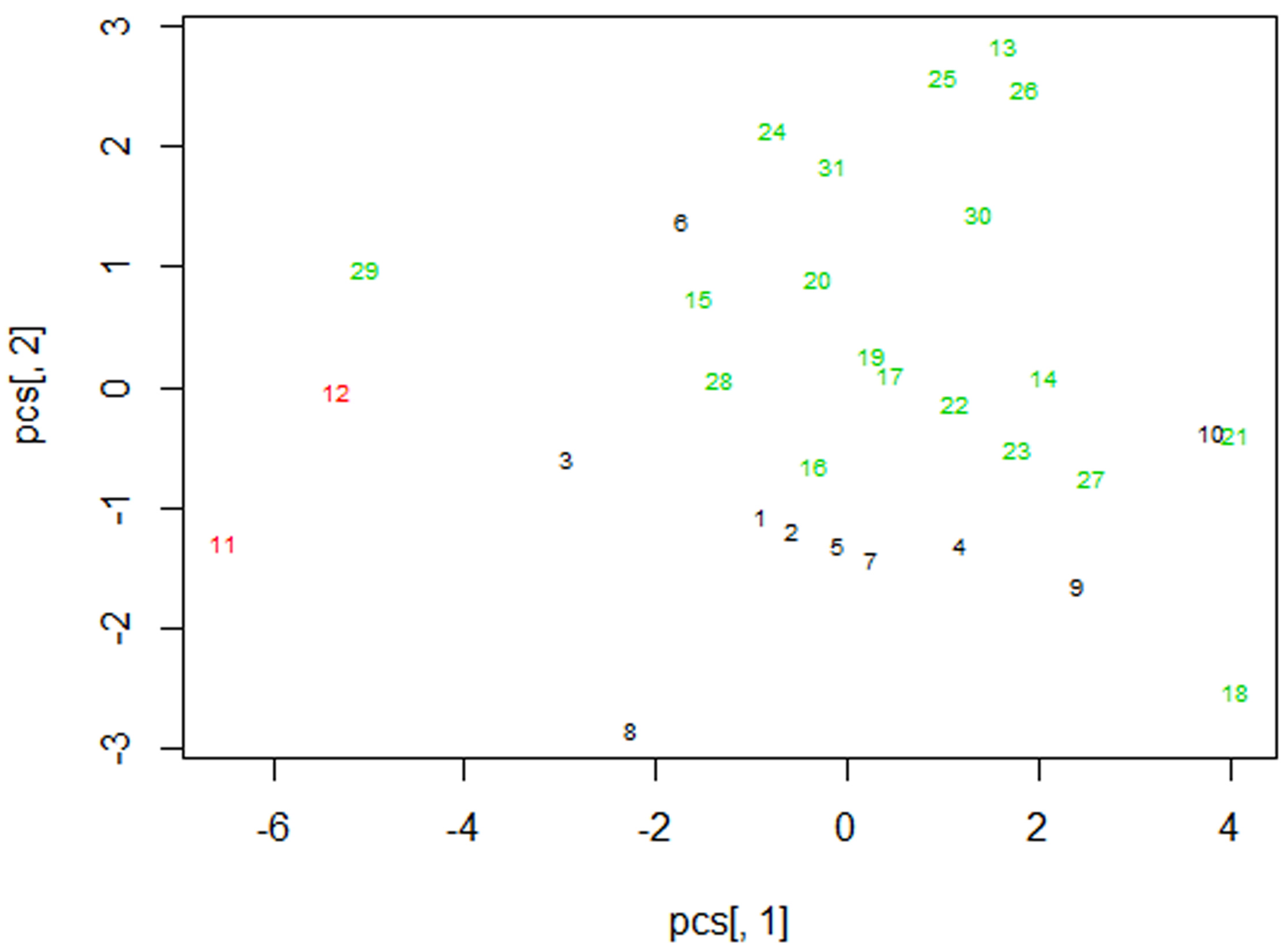
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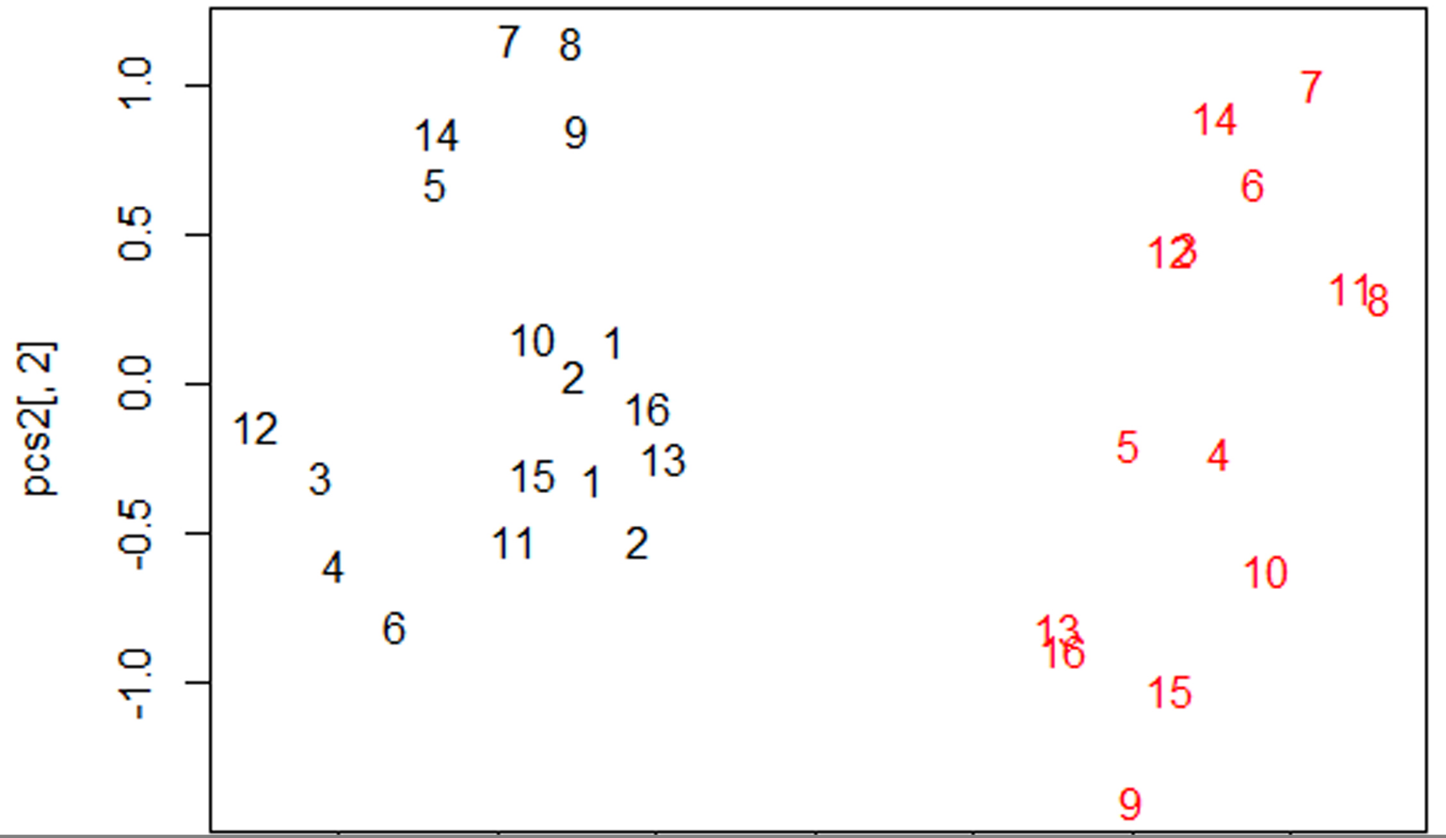
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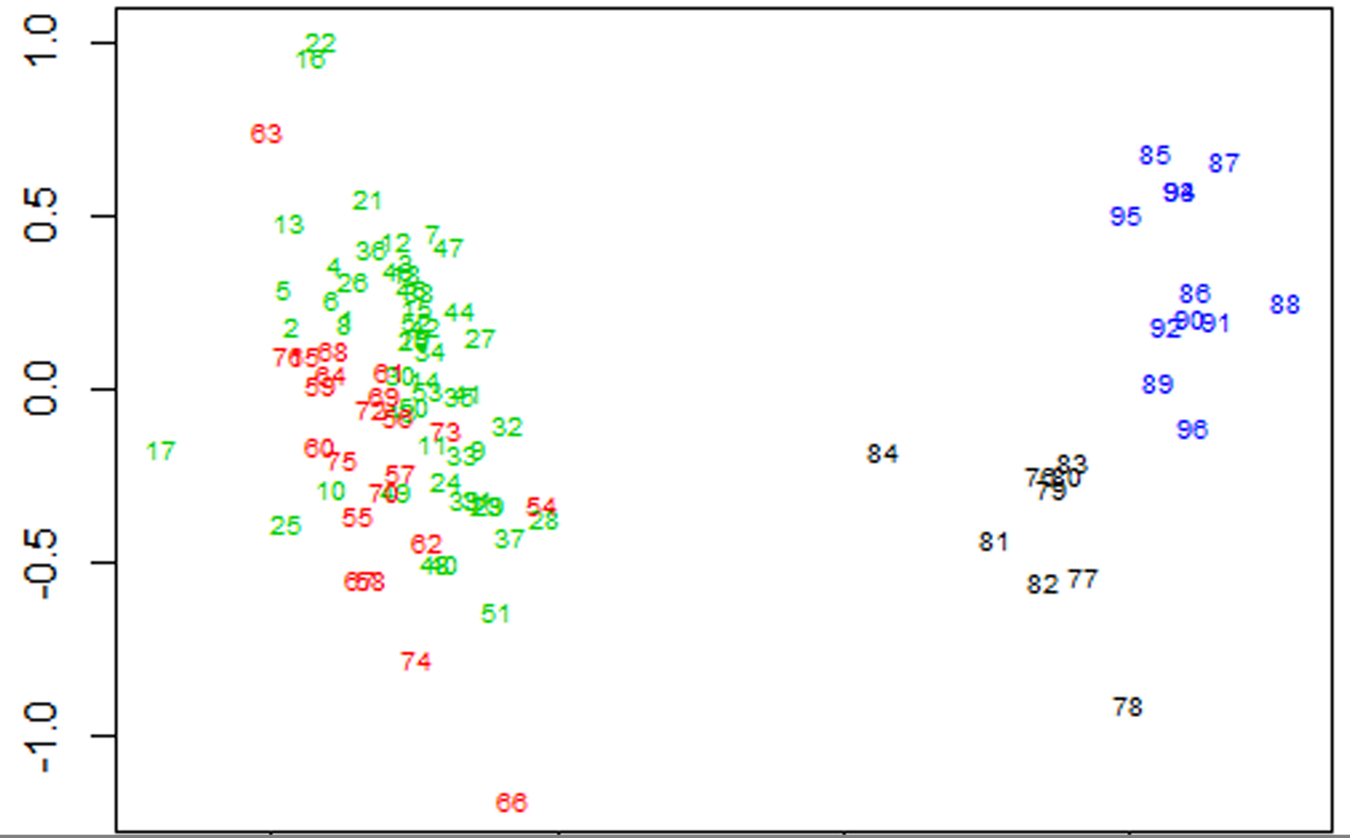
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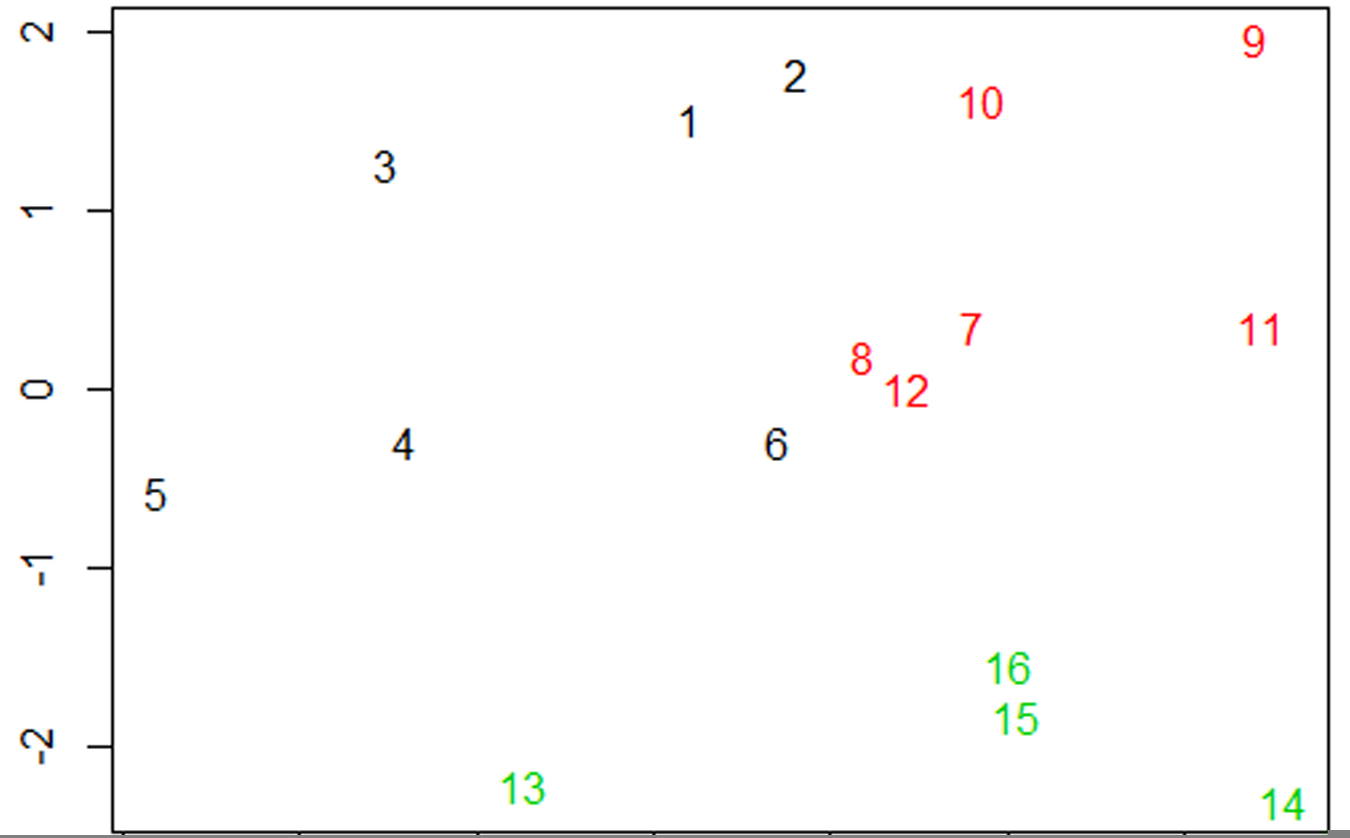
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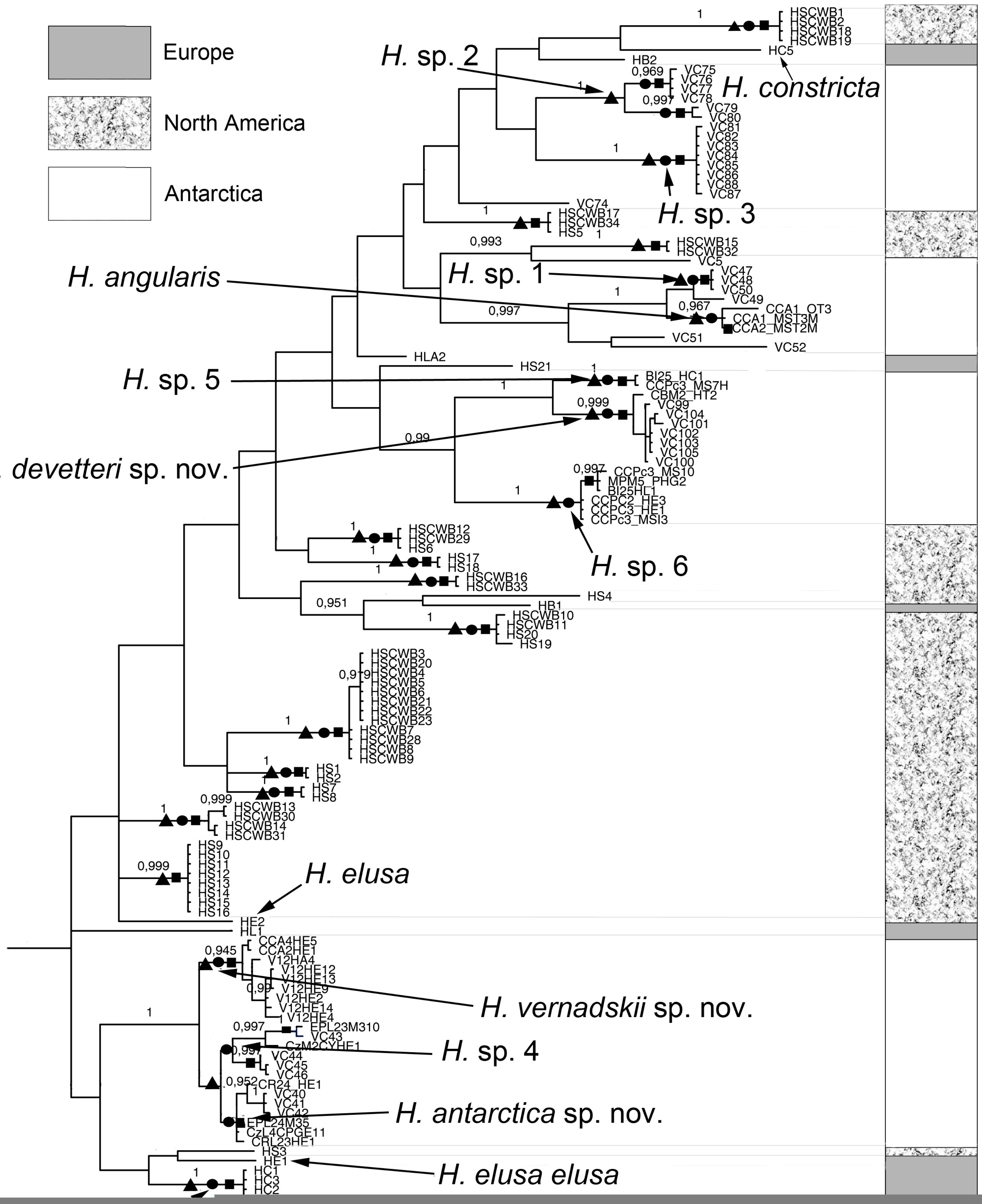
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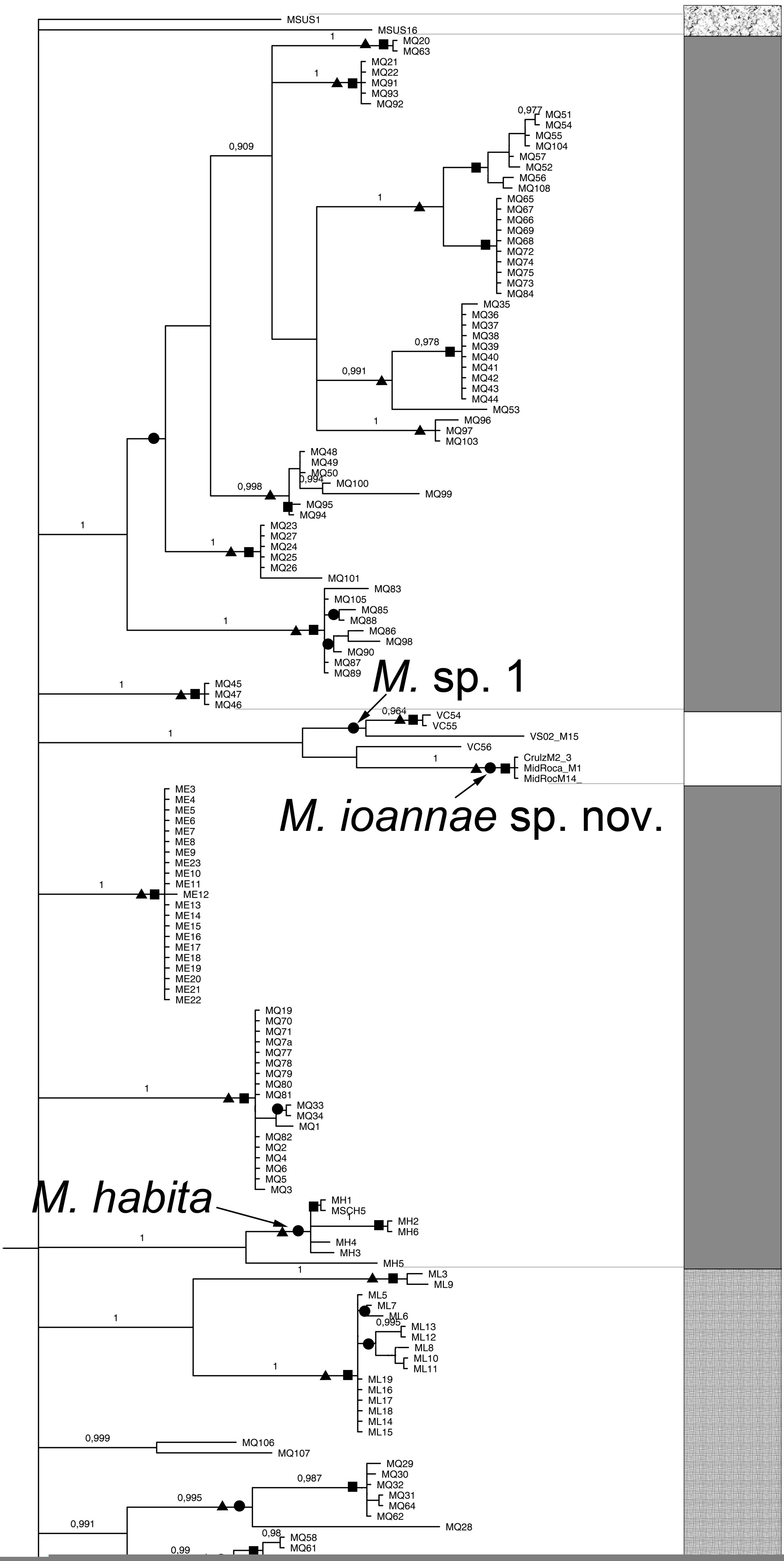
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