

1 **Antarctic bdelloid rotifers: diversity, endemism and evolution**

2

3 **Introduction**

4

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

22 The continent of Antarctica is ice-bound, and surrounded and isolated from the other
23 Southern Hemisphere landmasses by the vastness of the Southern Ocean. The 1000 km Drake
24 Passage separates it from South America, and distances of 4–5000 km from Australia/New
25 Zealand and South Africa. Terrestrial ecosystems reach their greatest development in the

26 coastal regions, where most of the continent's biodiversity is found, most evidently along the
27 Antarctic Peninsula and parts of the coastline of East Antarctica. Terrestrial communities are
28 also present on isolated nunataks and the major mountain ranges inland, as well as in the 'dry
29 valleys' of southern Victoria Land, which are the single largest ice-free areas of the continent
30 (Convey, 2013). However, most ice-free areas are small, and isolated by tens to hundreds of
31 kilometres from neighbouring areas.

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

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

55

56 **Current state of knowledge of bdelloid diversity and biology in Antarctica**

57

58 *Early studies*

59

60 The history of bdelloid research in Antarctica and the sub-Antarctic dates back more than a
61 hundred years. Early records of Bdelloidea date to the start of 20th Century, obtained from
62 material collected by the First German Antarctic (1901-03), Swedish (1901-1904), British
63 (1907-09) and Second French (1908-10) Antarctic Expeditions. Richters (1907, 1908) was
64 the first to record bdelloids from terrestrial mosses. However, the only two species
65 unequivocally recognizable from his records, *Callidina angusticollis* (= *Habrotrocha*
66 *angusticollis* Murray, 1905) and *C. longirostris* (= *Rotaria sordida* (Western, 1893)), were
67 found further north, between 35° and 40° S (St. Paul and Amsterdam islands). The remaining
68 13 bdelloids, also attributed to the genus *Callidina*, are now unidentifiable to species. The
69 illustrations available, depicting contracted bodies, jaws and foot appendages, suggest that
70 these rotifers are most likely correctly referred to the genera *Habrotrocha* and/or
71 *Macrotrachela*.

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

76 Stranded Moraines of McMurdo Sound. Five species of the 12 found were previously
77 unknown: *Philodina gregaria* Murray, 1910, *Ph. antarctica* Murray, 1910, *Ph. alata* Murray,
78 1910, *Habrotrocha* (as *Callidina*) *angularis* (Murray, 1910), and *Adineta grandis* Murray,
79 1910. Four species that Murray identified as cosmopolitan, *A. barbata* Janson, 1893, *A.*
80 *longicornis* Murray, 1906, *Callidina constricta* (= *Habrotrocha constricta* (Dujardin, 1841))
81 and *C. habita* (= *Macrotrachela habita* (Bryce, 1894)), were noted to have morphological
82 differences from the original descriptions of these species as found in Europe. One further
83 species, *Philodina* sp., while apparently new to science, was not further described.

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

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

106

107 *Further taxonomic studies*

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

125 *Rotaria rotatoria* (Pallas, 1766) showed morphological inconsistencies with the original
126 descriptions of non-Antarctic material. *Macrotrachela* (= *Callidina*) *papillosa* (Thompson,
127 1892) was erroneously listed as *Habrotrocha papillosa*, and *M. insolita* de Koning, 1947 as
128 *M. insolata*.

129

130 *Life cycle*

131

132 Several studies have described the life cycles of endemic Antarctic bdelloids. Dougherty
133 (1964) investigated reproductive features of *Ph. gregaria* cultivated in the laboratory, and
134 found its maturation time to be 28-110 days (in a laboratory refrigerator). This suggests a life
135 span considerably longer than in any other cultivated bdelloid, including another Antarctic
136 endemic, *A. grandis* (Dartnall, 1992; Ricci, 2001). The fecundity of the viviparous *Ph.*
137 *gregaria* (up to 24 offspring; Dougherty, 1964), was also much lower than those of various
138 oviparous bdelloid species in cultures maintained at room temperature (Ricci & Caprioli,
139 1995). Dartnall & Hollowday (1985) recorded that *Ph. gregaria* could produce up to 32
140 young per female, a number close to that of many oviparous bdelloids but still lower than
141 others. Dougherty (1964) stated that most *Ph. gregaria* offspring started to reproduce 27-90
142 days after birth. Dartnall (1992) confirmed the unusually long life span of *Ph. gregaria* – up
143 to 89 days at 4°C, and twice that of *A. grandis* at the same temperature (40-50 days). Ruttner-
144 Kolisko & Kronsteiner (1979, cited in Dartnall, 1992) reported that at 6°C *Ph. gregaria* lived
145 longer than at 10°C (60 days vs 26) and produced more offspring (15 vs 7). Also, Dartnall
146 (1992) found the age at the first reproduction to be 36-37 days for *Ph. gregaria*, about 10
147 times more than typical oviparous non-Antarctic bdelloids cultivated at room temperature
148 (Ricci & Caprioli, 1995).

149

152 A number of studies have described the interactions of Antarctic bdelloids with their substrata
153 (moss, algal mats, and soil) comparing these with other terrestrial micro-invertebrates, while
154 others have addressed seasonal changes in their populations. Davis (1981) evaluated the role
155 of bdelloids in bryophyte communities of Signy Island, by estimating their dry biomass from
156 data presented by Jennings (1976b, 1979) on density and species composition. Both average
157 and maximum biomass of Bdelloidea were comparable with or higher than those of
158 Nematoda, though considerably lower than those of Tardigrada. The biomass of bdelloids in
159 mosses could reach up to 29.5 mg dry mass m⁻² – fourfold greater than that of monogonont
160 rotifers, with about a half of the bdelloid biomass being attributed to *Adineta* species. Davis'
161 (1981) data on feeding preferences suggested that the diet of bdelloids consisted entirely of
162 dead organic matter, contrasting with Dougherty (1964) who stressed the importance of
163 unicellular algae in the diet of *Ph. gregaria*. However, members of the genus *Adineta* are also
164 known to feed predominantly on dead organic matter elsewhere (Örstan, 1992). Everitt (1981)
165 observed cyclical changes in abundance throughout the year in the bdelloid population of a
166 saline continental lake in the Vestfold Hills. Rotifers overwintered in a cryptobiotic state, and
167 during the summer reproduced with abundance peaks occurring at three week intervals.
168 Dougherty (1964) and Dartnall (1992) reported that the time between recovery from
169 cryptobiosis, or birth, and the first reproduction in *Ph. gregaria* was at least 1 month in the
170 laboratory, but that it could be shorter in the natural environment. In the relatively stable lake
171 environment, the abundance peaks observed could be successive new generations, especially
172 as Bdelloidea, unlike other rotifer group, Monogononta, do not possess specific larval or
173 programmed dormant stages. However, the largest abundance peak described by Everitt
174 (1981), corresponded to a massive inflow of N and P compounds into the lake, indicating that

175 environmental influences are also important. In the more unstable (in terms of water
176 availability) terrestrial habitats environmental factors seem to be the major driver of bdelloid
177 abundance dynamics (Iakovenko, 2004). Priddle & Dartnall (1978), investigating the
178 microflora and microfauna of aquatic moss and algal communities in lakes of Signy Island,
179 observed three to seven-fold decreases in the abundance of *Philodina* sp. during winter
180 compared to summer. They also reported that two non-sessile bdelloid species showed
181 distinctive space distribution pattern inside moss cushions, dominating in different zones of
182 stems and leaves. Cathey et al. (1981) found *Ph. gregaria* and *Ph. alata* to be able to colonize
183 artificial substrata (polyurethane foam) in eight lakes of southern Victoria Land, the former
184 being present in all the lakes and the latter in only three lakes.

185 Based on recent studies, most or all of rotifer species present in Antarctic soil
186 communities are bdelloids (Smykla et al., 2010). In soils of the McMurdo Dry Valleys, one of
187 the driest places in the Antarctica, rotifers were present in all the sampled localities
188 (Courtright et al., 2001). Confirming Murray's (1910) speculations of almost a century ago,
189 Nkem et al. (2006) found the wind to play an important role in the dispersal of soil rotifers,
190 and this has been proposed as the mechanism allowing them to colonize remote nunataks,
191 where they can reach abundances of up to 135 ind g⁻¹ dry substrate (Sohlenius et al, 1996). In
192 certain types of soil at Edmondson Point, Victoria Land, Smykla et al. (2010, 2012) found
193 bdelloid rotifers to be the dominant group of micro-invertebrates, reaching over 8000 ind 100
194 g⁻¹ raw soil. Smykla et al. (2010, 2012) reported that bdelloids reached high abundances in
195 wet soils under moss and algal and cyanobacterial mats, while being absent in both barren
196 fellfields and heavily nutrient-enriched penguin colonies. The latter observation contradicts
197 that of Porazinska et al. (2002), who reported rotifers to be present and even dominating in
198 terms of abundance (> 4000 ind kg⁻¹ dry soil) in ornithogenic soils collected on Ross Island.
199 Sohlenius et al. (2008) noted that rotifers were the most frequently-encountered and abundant

200 group of invertebrates in the ornithogenic soils and fellfields of Dronning Maud Land, in
201 contradiction with the data of Smykla et al. (2010, 2012). Velasco-Castrillón et al (2014b)
202 reported bdelloid rotifers to be the most widespread and abundant taxon in the soils of East
203 Antarctica, being present in 87% of sampled sites and reaching 44 ind g⁻¹ dry soil. They found
204 bdelloids to be present in soils with various particle size (from fine to coarse), both with and
205 without vegetation, and with a broad variety of abiotic and geochemical parameters,
206 consistent with the high tolerance of this group towards extreme conditions.

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

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

221

222 *Diversity and endemism*

223

224 Studies that have included or provided compilations of the rotifer fauna of Antarctica and the
225 sub-Antarctic, in particular terrestrial Bdelloidea, have been published by Dartnall (1983),
226 Dartnall & Hollowday (1985), Sudzuki (1988), Adams et al. (2006), Segers (2007), Velasco-
227 Castrillón et al. (2014a-c), and Fontaneto et al. (2015). These sources should be referred to for
228 details on species diversity of particular regions. A few studies have been carried out at the
229 same location over time. For example, Dougherty & Harris (1963), investigating Ross Island
230 and the McMurdo Dry Valleys, found virtually the same species as recorded by Murray
231 (1910). A number of previously unrecorded bdelloid species have been reported from
232 Antarctica and sub-Antarctic by Jennings (1976a), Sudzuki (1979), Everitt (1981), and
233 Sohlenius et al. (1996, 2005). These studies reported, along with the indigenous Antarctic
234 bdelloids, some 20 morphospecies similar to species first described from Europe, thus
235 considering Antarctic bdelloid fauna to include many cosmopolitan species. No Antarctic
236 endemic bdelloid families or genera have been reported.

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

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

249 In contrast, the application of contemporary molecular approaches (Velasco-Castrillón et al.,
250 2014a) suggests that the bdelloid fauna of Antarctica comprises mostly endemic species, or at
251 least species not yet recorded from any other continent.

252

253 *Molecular approaches*

254

255 A number of genomic and molecular phylogeographic studies have been performed during the
256 last decade on various groups of Antarctic microfauna, mainly microarthropods and
257 nematodes (Stevens et al., 2006; McGaughan et al., 2008, 2010; Stevens & Hogg, 2006;
258 Velasco-Castrillón & Stevens, 2014), as well as various microbial groups (see Vyverman et
259 al., 2010) and mosses (Pisa et al., 2014). However, the application of such studies to bdelloid
260 rotifers in Antarctica remains at an early stage. Fragmentary sequence data on Antarctic
261 bdelloids have been published in studies of the evolution and global biogeography of
262 Bdelloidea (Barraclough et al., 2007; Fontaneto et al., 2008, 2012). Velasco-Castrillón et al.
263 (2014a) recently evaluated molecular diversity of Antarctic and sub-Antarctic bdelloids across
264 a wide area. Their study identified 47 putative species, counting both sequence clusters, and
265 singletons (entities with only one sequence obtained). All of the putative species were
266 designated as Antarctic or Tierra del Fuego endemics based on percentage sequence
267 similarities in comparison with representatives of eight bdelloid genera from other continents.
268 The study also indicated that the true number of taxa in the genera *Adineta* and *Philodina*
269 determined from the sequence data analysed using the Poisson Tree Processes (PTP) model
270 (Zhang et al., 2013), must be considerably higher than can apparently be determined by
271 morphological approaches alone.

272 The current study shows that when appropriately analysed, the morphological
273 diversity of Antarctic bdelloids is sufficient to reveal most diversity detected by contemporary

274 molecular markers. We also re-evaluate previously published data on Antarctic bdelloid
275 rotifer endemism, and determine the ratio of endemic to cosmopolitan bdelloid species in
276 comparison with such from other continents.

277

278

279 **Materials and Methods**

280

281 *Sampling and extraction of rotifers*

282

283 New samples included in this study were obtained from both maritime and continental regions
284 at sites between 63°60' S and 77°55' S (Fig. 1, Table 1). In the continental Antarctica 11
285 sampling locations were visited in the Ross Sea area, including the Victoria Land coast,
286 Beaufort and Ross Islands (Fig. 1a, Supplementary file 1). The fieldwork and sampling in the
287 Ross Sea area were conducted during five austral summer seasons between 2003/04 and
288 2011/12 within the project of J. Smykla (Smykla et al., 2010, 2011, 2012). Soil and moss
289 were collected at all localities. The soil samples were obtained from barren fellfields,
290 bryophyte communities, wetlands with algal and cyanobacterial mats, and from the vicinity of
291 active and relict penguin colonies. Algal mats were collected in coastal areas and on Ross
292 Island. Most of the collected material was stored frozen (−20°C), but some terrestrial mosses
293 were dried and stored at room temperature; details of collection methods and primary sample
294 processing are given in Smykla et al. (2010, 2012, 2015).

295 In the maritime Antarctic 237 samples were obtained during the summers of 2004/05,
296 2006/07 and 2009/10 from the Argentine Islands archipelago, King George Island, and coastal
297 areas of the Antarctic Peninsula under the projects of K. Janko, I. Kozeretska and V.
298 Trokhymets. These included 50 soil and 183 moss samples, one sediment sample from a pool

299 on King George Island, and three lichen samples from the Argentine Islands archipelago (Fig.
300 1b, Table 1, Supplementary file I). Methods of collection, storage and rotifer extraction were
301 as used for the continental samples, except for mosses which were washed directly along with
302 wet sieving and sugar gradient centrifugation as described by Freckman & Virginia (1993).

303

304 *Alpha taxonomy procedures*

305

306 Detailed procedures of rotifer sorting, identification, digital imaging, and the preparation of
307 type material (glycerin jelly slides and SEM mounts) are described in Iakovenko et al. (2013).
308 We used the keys of Donner (1965) and Kutikova (2005) as a primary guide for identification,
309 but detailed taxonomic analysis was based on the first descriptions (cited in Donner (1965),
310 and further specific studies (Donner, 1972a,b, 1980; Haigh, 1965, 1966; Koste, 1996a; Örstan,
311 1995; Ricci et al., 2001, 2003; Birky et al., 2011).

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

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

324 the voucher specimens from which the morphometric data were taken, were used for these
325 analyses.

326

327 *Morphometric analyses*

328

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

334 To distinguish some Antarctic species from morphologically similar European ones,
335 we measured specimens from clonal cultures, from which we subsequently obtained some
336 COX1 sequences: 113 specimens of Antarctic *Adineta*, 69 specimens of European *Adineta*,
337 and 16 specimens of Antarctic *Habrotrocha*. We used the Linear Mixed Effects Model (LME)
338 and Principal Components Analysis (PCA) to compare body and trophi measurements. The
339 results of PCA were visualized as the two first principle components of variation plotted
340 against each other. All statistical analyses were performed in R 2.15.1 following Crawley
341 (2007).

342

343 *DNA taxonomy procedures*

344

345 The DNA extraction protocol followed Fontaneto et al. (2007) and Iakovenko et al. (2013).
346 The target locus of the mitochondrial COX1 gene (355 bp in length) was amplified and
347 sequenced from 192 bdelloid specimens (Supplementary file III) using universal primers
348 LCO1490 and HCO2198 (Folmer et al., 1994) with the subsequent re-amplification to

349 increase the outcome of the product, using bdelloid-specific primers Bdel_CO1_FW (5'-
350 CGTACWGAGTTAGGAATRGTA-3') and Bdel_CO1_Rev (5'-
351 CCAAAATTWCGATCTAAYA-3') (Robeson et al., 2011).

352 To construct phylogenies, we downloaded available sequences of the taxonomically
353 assigned bdelloid species from GenBank, available from all continents except South America
354 (976 COX1 sequences, their detailed descriptions are given in the Supplementary file II). We
355 used EMBL online version of MAFFT software (Kato et al., 2002) to construct one total
356 alignment of both newly obtained sequences and those downloaded from GenBank, and four
357 separate alignments for four genus-specific datasets (*Adineta* Hudson and Gosse, 1886,
358 *Habrotrocha* Bryce, 1910, *Macrotrachela* Milne, 1886, *Philodina* Ehrenberg, 1830). The
359 monogonont rotifer *Brachionus calyciflorus* Pallas, 1755 was used as outgroup in each of
360 these alignments, and each genus-specific dataset also contained a member of another
361 bdelloid genus as an additional outgroup: *Bradyscela clauda* (Bryce, 1893) for *Adineta*, *M.*
362 *ehrenbergii* (Janson, 1893) for *Habrotrocha*, and *H. constricta* (Dujardin, 1841) for
363 *Macrotrachela* and *Philodina*.

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

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

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

395 Unlike GMYC, the bPTP method does not require a time-calibrated and unrooted tree
396 as input. In this method, the number of substitutions κ between intra- and interspecific events
397 is used instead of time as a tree-calibrating parameter. Assuming that each substitution (which

398 is independent of other substitutions) has a probability ρ of generating a speciation event, κ
399 substitutions generate η speciations in a continuous process, and in a population of the size η
400 the number of substitutions is sufficient, the process proceeds at rate $\rho \times \eta$ and follows a
401 Poisson distribution. The number of substitutions is calculated from the branch lengths of the
402 input tree. We used online implementation of bPTP (<http://species.h-its.org/ptp/>) and the trees
403 produced in MrBayes as the input.

404

405

406 **Results**

407

408 In total, we identified 60 morphospecies, including 20 taxa currently identified to generic
409 level only and still under investigation, and 10 listed as “conformis” that show minor
410 morphological differences from known species. Only 13 of the morphospecies found occurred
411 both in maritime and continental Antarctica. The material examined included six of the seven
412 known Antarctic endemics: *A. grandis*, *H. angularis*, *Mn. ostensa*, *Ph. alata*, *Ph. jeanelli*, *Ph.*
413 *gregaria*. We have identified 10 morphospecies reported by other researchers from Antarctica
414 as *A. barbata* Janson, 1893, *A. vaga* (Davis, 1873), *H. gulosa* Milne, 1916, *H. vicina* Donner,
415 1980, *Macr. ambigua* Donner, 1965, *Macr. concinna* (Bryce, 1912), *Macr. habita* (Bryce,
416 1894), *Macr. musculosa* (Milne, 1886), *Macr. nixa* Donner, 1962, and *Rotaria rotatoria*
417 (Pallas, 1766). These species are considered cosmopolitan, or at least are known from
418 locations other than Antarctica. However, of these 10 species, those resembling *A. barbata*
419 and *A. vaga* s. str. are shown to be distinct new taxa and therefore currently endemic to
420 Antarctica, based on both minor but consistent morphological differences and molecular
421 analyses.

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

432 Phylogenetic trees constructed using 192 original COX1 sequences, and 976
433 sequences downloaded from GenBank, gave similar results on the delimitation of
434 independently evolving entities (IEE) according to the 4x rule, GMYC and bPTP models.
435 These results are discussed in detail below (*DNA taxonomy* sub-section). The 4x rule gave
436 140 IEEs: 44 of *Adineta*, 22 of *Habrotrocha*, 26 of *Macrotrachela*, and 48 of *Philodina*. In
437 total, 132 IEEs were identified by GMYC: 44 of *Adineta*, 20 of *Habrotrocha*, 18 of
438 *Macrotrachela* and 50 of *Philodina*. Finally, bPTP generated a somewhat higher number of
439 IEEs (160): 47 of *Adineta*, 26 of *Habrotrocha*, 29 of *Macrotrachela* and 58 of *Philodina*.
440 Most of the IEEs identified by GMYC and bPTP were confirmed by the 4x rule. Delimitation
441 according to the GMYC approach gave the best correspondence with rotifer morphology,
442 considering both major and minor external features, and morphometric data. Geographical
443 distribution of the identified IEEs is discussed in *Biogeography*.

444 The integrity of most species identified by morphology, with the exception of *A.*
445 *grandis*, *Ph. gregaria* and two new species of *Habrotrocha*, was confirmed by molecular
446 analyses (*DNA taxonomy*). According to the molecular data, *A. grandis* consists of at least two

447 cryptic species, one of which is described below as new for science. Ten putative species
448 (IEEs) were identified from molecular data only, obtained both from the new material
449 examined in this study and COI sequences downloaded from GenBank.

450

451 *Alpha taxonomy*

452

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

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

464 Phylum Rotifera Cuvier, 1817

465 Class Eurotatoria De Ridder, 1957

466 Subclass Bdelloidea Hudson, 1884

467 Order Philodinida Melone & Ricci, 1995

468 Family Adinetidae Hudson & Gosse, 1889

469 Genus *Adineta* Hudson and Gosse, 1886

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

471 Figs 3a, 4

472 Murray, 1910 (*A. barbata?*): 53-54, Pl. XII figs 9a-9c. Dartnall & Hollowday, 1985 (*A.*
473 *barbata*): 30, Fig. 24a-b. Velasco-Castrillón et al., 2014a (*A. sp.* Bd24): 8 (main text), 2, Fig.
474 6 (Annex S1).

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

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

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

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

483 **Barcodes:** GenBank ID KJ543629-30.

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

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

497 Neck of moderate length and width, NL is 12-17% of TL, antenna about 1/4-1/5 of bearing
498 pseudosegment. Trunk oval, BW 17-28% of TL. Rump conical, first pseudosegment slightly
499 swollen, RL is 12-17% of TL, RW is 75-92% of RL. Slim foot of 5 pseudosegments, of
500 moderate length, FL is 28-35% of TL, FW is 40-62% of FL. Spurs sword-like, long, gradually
501 tapering from the base to the points; SL is 115-181% of SSW. Three short unsegmented toes.
502 No eyespots. Throat and straight oesophagus of moderate size. Trophi small, round, 11-12 µm
503 long and 13-15 µm wide; 2/2 major teeth and 26/26 minor teeth in unci. Oviparous; egg oval,
504 101 x 46 µm, smooth, 1-6 round knobs on both poles and the sides.

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

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

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

510 *Adineta editae* sp. nov. Iakovenko

511 Figs 3b, 5

512 Dartnall & Hollowday, 1985 (*A. gracilis*): 31, Fig. 24c. Fontaneto et. al., 2008 (*A. gracilis*):
513 3139. Velasco-Castrillón et al., 2014a (*A. cf gracilis* Bd8): 8 (main text); 1, Figs 2-5 (Annex
514 S1).

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

516 **Type habitat.** Soil.

517 **Type material.** Holotype: SIZ 53.1 (MRockaAED1a, 15.02.2010, Leg. K. Janko), mounted in
518 glycerin jelly. Paratypes: SIZ 53.2-7 (MRockaAED2-7), in glycerin jelly on a separate slide;
519 SIZ 53.8-21 (MRockaAED1b-e, MRockaAED8-18), trophi mounted for SEM. **Additional**
520 **material.** SIZ 53.22-25 (870_1AED1, V12AED1-3), trophi mounted for SEM; SIZ 53.26-33
521 (870_1AED2, VRA01AED2-3, VS03AED1-3), digital photos and videos.

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

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

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

539 **Description.** Body of moderate size, dorsoventrally flattened, transparent, colorless except
540 the yellow-brown stomach. Integument smooth, thin, without knobs, spines or bolsters.
541 Rostrum very short, of moderate width; its lamella wide, semicircular, not divided into lobes.
542 Two claw-like sharp teeth pointing toward each other in each thin rod-like rake. Head wide
543 oval, of regular shape or slightly narrowed towards rostrum, HL is 13-19% of TL. Neck rather
544 short and wide, NL 34-62% of TL, antenna about 1/3 of the bearing pseudosegment width.
545 Trunk wide, oval. Rump somewhat swollen in the middle part, RL is 11-16% of TL. Foot of
546 moderate length, 5 pseudosegments, FL is 10-16% of TL. Spurs short, conical, pointed,

547 divergent, with tiny interspace, SL is 60-83% of SSW. Three short unsegmented toes. No
548 eyespots. Trophi round, 15-19 μm long and 16-20 μm wide; 2/2 major and 28-34 minor teeth
549 in unci. Oviparous. Eggs oval, smooth without knobs or spines. Egg size 71-89 x 45-61 μm by
550 our data and 70 x 50 μm as reported by Dartnall & Hollowday (1985).

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

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

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

559 The head of the new species is 45 ± 4 μm long and 34 ± 4 μm wide, HW/HL is 69-90%.
560 According to our data, *A. gracilis* s. str. has the head 40 ± 7 μm long and 29 ± 4 μm wide
561 (N=42), HW/HL is 53-70%. By our data, *A. gracilis* s. str. has TL 247 ± 45 μm , BW/TL 13-
562 23%, RW/RL 54-82%, FW/FL 27-40% (N=42). The new species TL is 286 ± 41 μm , BW/TL
563 is 13-23%, RW/RL is 67-99%, FW/FL is 38-58%. By our data, the trophi of *A. gracilis*
564 11.1 ± 0.4 μm long, 13.7 ± 0.9 μm wide (N=14), 20-24 minor teeth in each unci. The new
565 species has trophi of 16.6 ± 1 μm long and 18.4 ± 0.7 μm wide, with 28-34 minor teeth in each
566 unci.

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

568 Figs 6a, b, d, e

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

570 **Type locality.** Cape Royds (Ross Island), 27 m asl, S77° 32.500', E166° 8.933'. **Type**

571 **habitat.** Cyanobacterial mats in wetlands.

572 **Type material.** Holotype SIZ 52.1 (CR23matAE1a, 14.1.2010, Leg. J. Smykla) mounted in
573 glycerin jelly. Paratypes: SIZ 52.2-52.16 (CR23matAE2-16) in glycerin jelly on a separate
574 slide; SIZ 52.16-17 (CR23matAE1b-c), trophi mounted for SEM. **Additional material.** SIZ
575 52.18-52.22 (CR23matAE1e-j, CBM2AE1), trophi mounted for SEM; SIZ 52.23-31
576 (CR23matAE17-21, CBM2matAE1-4), digital photos and videos.

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

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

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

588 **Description.** Body of moderate size, wide, flattened, of bright orange color. Integument
589 smooth, thin, transparent, without knobs, spines, bolsters or other appendages. Rostrum short,
590 sickle-like, distal rostral pseudosegment not plate-like flattened. Two short semicircular
591 rostrum lobes, no stiff sensory bristles, only short cilia under the lobes. Wide-oval head of a
592 moderate size, HL is 13-18% of TL, HW is 71-94% of HL. Six thin peg-like teeth in each
593 massive scoop-like rake. Neck of moderate length and width, slightly contracted behind the
594 head, NL is 14-21% of TL, antenna about 1/3 of the bearing pseudosegment width. Trunk
595 oval, wide, BW is 19-27% of TL. Rump conical, somewhat swollen in the middle, RL is 11-
596 16% of TL, RW is 74-98% of RL. Relatively short slim foot of 5 pseudosegments, FL is 10-

597 16% of TL, FW is 29-45% of FL. Spurs short (SL 60-94% of SSW), pointed, needle-shaped
598 with bulb-like swollen bases, divided by straight interspace of ~2 spur widths. Three short
599 unsegmented toes. No eyespots. Trophi ramate, round, 15-18 μm long and 14-18 μm wide.
600 Rami massive, the region of articulation is straight, protruding backwards, without incisure.
601 Interior margins of rami with long numerous peg-like scleropili. Manubria thin, sickle-like.
602 Two major teeth and 29-33 minor teeth in each uncus. Throat small, oesophagus short,
603 straight. Stomach glands of moderate size. Eight nuclei (3-7 according to Murray) in each
604 germovitellarium. Oviparous. Eggs oval, 60-70 x 39-44 μm , shell smooth, without knobs or
605 spines.

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

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

609 **Comments.** According to our data, the new species has TL 294 \pm 44 μm , while TL is 414 \pm 61
610 μm in *A. grandis* (N=20) and 274 \pm 14 μm in *A. vaga* s. str. (N=15). The new species has
611 trophi 15.7 \pm 1.1 μm long with 29-32 minor teeth in each uncus, while *A. grandis* has trophi
612 25.4 \pm 1.4 μm long with 36-44 minor teeth (N=53), and *A. vaga* s. str. has it 13 \pm 0.7 μm long
613 with 25-27 minor teeth (N=14).

614 *Adineta grandis* Murray, 1910

615 Figs 3c, 6g, 7a

616 Murray, 1910: 51-53, Pl. XII fig. 10. Voigt, 1956-57: 71, Taf. 5 Abb. 24, Taf. 8 Abb. 19, Taf.
617 14 Abb. 16. Donner, 1965: 273, Fig. 200a. Donner, 1972a: 252, Abb.1. Koste, 1996b (as *A.*
618 *grandis*, but most likely sibling species): 243, Abb.5. Dartnall & Hollowday, 1985: 31, Fig.
619 24d-f. Kutikova, 2005: 275, Ris. 299. Velasco-Castrillón et al., 2014a (*A.* sp. Bd2): 8 (main
620 text); 2, Fig. 8 (Annex S1).

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

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

623 **Material examined.** BI11, 1 ind.; BI23, 10 ind.; CBM1CYmat, 6 ind.; CBM2mat, 4 ind.;

624 CBC1mat, 5 ind.; CBPc2mat, 1 ind.; CRL21, 2 ind.; CR24, 2 ind.; CRL24, 1 ind.;

625 CzM2Cymat, 8 ind.; CzM3CYmat, 9 ind.; EPL23, 11 ind.; MPM3, 1 ind.; MPM5, 34 ind.;

626 MPM5CYmat, 10 ind.

627 **Description.** The largest species of the genus, and the only known viviparous one. Reported
628 TL is 306-750 μm (Murray, 1910; Donner, 1965; Dartnall, 1985), and 304-505 μm according
629 to our data. Its foot is shorter than in other species of *Adineta*. Trophi length 23-29 μm (our
630 data). Body pale orange or brownish yellow, sometimes reddish (“light brown or yellowish,
631 darker in the alimentary tract” according to Murray). Integument smooth, thin, transparent,
632 without knobs, spines or other appendages. Rostrum short, of moderate width, distal rostral
633 pseudosegment not strongly widened or flattened. Rostral lamella divided into two small
634 semicircular lobes. No stiff sensory bristles under rostrum lobes, only short soft cilia. Head
635 not large (HL is 13-19% of TL), wide-oval (“ovate” by Murray), tapering towards rostrum,
636 HW is 66-97% of HL. 6-10 teeth in each massive scoop-like rake. Neck massive, long (NL is
637 11-27% of TL). Dorsal antenna thick, about 1/4 of width of the antennal pseudosegment.
638 Trunk wide (its width depends on the number of embryos inside), BW is 16-31% of TL.
639 Rump conical, with both pseudosegments somewhat swollen laterally (in some specimens the
640 lateral swellings look like knobs), gradually tapering into a very short narrow foot. RL is 7-
641 16% of TL, RW is 74-103% of RL. Foot short, of 5 pseudosegments, FL is 6-15% of TL, FW
642 is 40-56% of FL. Spurs conical, widened at the base (according to Murray, “short broad
643 cones”, “stout and subacute”), pointed, narrow, divergent, divided by the straight interspace
644 equal to 1-2 spur widths, SL is 60-98% of SSW. Three short unsegmented toes. No eyespots.
645 Trophi ramate, large, round or elongate. Rami massive, interior margin with numerous peg-
646 like scleropili. Articulation protruding to the ventral part, straight and without incisure.

647 Manubria wide, flat, crescent-shaped. Major uncinial teeth thick, dental formula 2/2; 38-41
648 minor teeth. Trophi unusually large for *Adineta*: 30 µm long according to Donner (1965), 24-
649 31 µm long and width is equal to the length, according to our data. Throat voluminous,
650 oesophagus short, straight. Stomach glands large. Eight nuclei in each of germovitellaria.
651 Viviparous, up to 4 embryos with developed trophi can be seen inside trunk.

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

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

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

659 *Adineta fontanetoi* sp. nov.

660 Figure 7b-d

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

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

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

667 **Barcodes.** GenBank ID EF173184-85, KP869896.

668 **Diagnosis.** By external morphology the new species does not differ from *A. grandis* (see the
669 description above) and may be easily confused with the latter under the light microscope.

670 However, it has somewhat larger trophi (ramus length mean±SD 26.8±1.2 µm in *A. fontanetoi*
671 sp. nov. and 25.4±1.4 µm in *A. grandis*).

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

674 **Measurements.** See Table 2.

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

676 Order Philodinida Melone & Ricci, 1995

677 Family Habrotrochidae Bryce, 1910

678 Genus *Habrotrocha* Bryce, 1910

679 ***Habrotrocha antarctica* sp. nov.**

680 Figs 3d, 8

681 Murray, 1910 (*Callidina constricta*): 48-49, Pl. XII figs 13a,b; Dartnall & Hollowday, 1985

682 (*H. constricta*): 32; Fig. 25a-c. Velasco-Castrillón et al., 2014c (Bd12): 8.

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

684 **Type material.** Holotype: SIZ 56.1 (CRL23HE1a, 14.01.2010, Leg. J. Smykla), mounted in
685 glycerine jelly, encircled in green ink. Paratypes: SIZ 56.2-4 (CRL23HE1b-d), on the same

686 slide as holotype, encircled in black ink; SIZ 56. 5-11 (CRL23HE1e-k), trophi mounted for

687 SEM. **Additional material.** SIZ 56.12-23 (EPL24M51-5, CzL4CYmatHE1-7), digital videos

688 and photos; SIZ 56.24 (CzL4CYmatHE8), trophi mounted for SEM.

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

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

691 **Diagnosis.** Very similar to *H. elusa* s. lat. Milne, 1916, except of the rump shape and trophi
692 structure. The foot is much wider and the spurs longer than in *H. elusa vegeta* Milne, 1916.

693 Differently from *H. elusa* s. str. Milne, 1916, it has no lateral knobs on the first rump

694 pseudosegment. The integument on the trunk and rump is not granulated or dotted, unlike

695 reported for *H. elusa* s. str. by Donner (1965). Similar to *H. constricta* by the size and body

696 shape, however distinguished by the upper lip with a notch in the middle (so that the tip is

697 divided into two small lobes), while in *H. constricta* the tip is whole. Dental formula 7/7
698 major uncinal teeth (the last 2-3 thinner than the rest), while in *H. elusa* s. str. it is reported to
699 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
700 teeth in unci and rarely 7/7 or 8/8 (Donner, 1965).

701 **Description.** Body of moderate size, transparent, spindle-shaped, colorless but usually with
702 yellow-brown or bright orange stomach. Integument smooth, thin, without knobs, ribs or
703 spines. No knob on the 1st foot pseudosegment. Rostrum short, lamella divided into two small
704 semicircular lobes. Corona narrower than the oval head base, CW/HW 79-91%, HW is 96-
705 100% of HL. Pedicels short, straight, divided by a narrow sulcus without membrane or ligula.
706 Trochal discs kidney-shaped in apical view. No papillae or sensory bristles on trochi. Upper
707 lip triangular, reaching plane of trochal discs, upper rim thickened by cuticular bolster, tip
708 divided by a notch into two small rounded lobes. Lower lip not wide, not projecting laterally.
709 Cingulum bolster very narrow. Neck of moderate length and width, NL is 15-35% of TL.
710 Trunk slim, BW is 15-20% of TL. Rump conical, 1st pseudosegment swollen, RL is 11-15%
711 of TL, RW is 80-110% of RL. Foot very short, 4 pseudosegments, FL is 10-12% of TL, FW is
712 49-74% of FL. Spurs short, triangular with elongated narrow tips and slightly swollen middle
713 part, divergent, without interspace, SL is 55-74% of SSW. Three short unsegmented toes. No
714 eyespots. Throat narrow, oesophagus short, straight. Stomach glands small, round. Food
715 pellets rounded, small. Trophi ramate, heart-shaped, 15-19 µm long and 17-20 µm wide.
716 Rami thick, with numerous short scleropili along the inner rims. Articulation straight, wide,
717 without incisure. Manubria narrow, sickle-like. Dental formula 7/7, with 24-25 minor teeth in
718 each unci. Oviparous. Eggs oval, 65-70 x 33-41 µm, shell smooth, without knobs or spines.
719 **Measurements.** See Table 2. TL 250 µm by Murray (1910) or 375 µm (Dartnall &
720 Hollowday, 1985).

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

722 **Habitat.** Soil, algal mats, sediment in pools.

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

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

730 Figs 9a-d

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

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

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

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

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

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

747 *H. thienemanni* s. lat. has 2+1/2+1 major teeth (Fig. 9d, e). It differs from *H. crassa* Donner,
748 1949, another species with two-lobed upper lip, by the corona wider than the head base, the
749 smooth integument, body outline, and dental formula (4/4 in *H. crassa*). It differs from *H.*
750 *tranquilla* Milne, 1916 by its smaller size. TL of the new species is 209-282 μm , while for *H.*
751 *tranquilla* it is 340-402 μm . The lower lobes of the upper lip are divided by an interspace,
752 while in *H. tranquilla* they are higher and divided by a notch. Dental formula is not 7/7 - 9/9
753 as in *H. tranquilla*.

754 **Description.** Body of moderate size, spindle-shaped, colorless, transparent. Integument thin,
755 smooth, without knobs, spines or bolsters. Rostrum short, lamella with two small semicircular
756 lobes. Corona wider than the oval head base, CW is 103-118% of HW, HL is 22-31% of TL.
757 Pedicels short, straight. Sulcus very narrow, half-covered with membrane. Trochal discs with
758 papillae and sensory bristles. Upper lip goes up to a half of the pedicels, it is arcuate with two
759 small semicircular lobes divided by an interspace. Lower lip slightly protruding laterally.
760 Cingulum narrow. Neck of moderate length, NL is 15-26% of TL. The length of antenna is
761 about 1/3 of the bearing pseudosegment width. Trunk plump, BW is 17-30% of TL. Rump
762 conical, RL is 12-17% of TL, RW is 81-98% of RL. Foot short, slim, 4 pseudosegments, FL
763 is 8-14% of TL, FW is 51-88% of TL. Spurs short, triangular, divided by interspace as broad
764 as one spur width, SL is 44-76% of SSW. Three short unsegmented toes. No eyespots. Throat
765 and oesophagus short, food pellets round, of moderate size. Stomach glands of medium size.
766 Trophi ramate, heart-shaped, 16-19 μm long and 16-19 μm wide. Rami thin, with numerous
767 short scleropili along the inner rim, articulation straight and without incisure. Manubria thin,
768 sickle-like. 2+2/2+2 major teeth, 30-33 minor teeth in each uncus. Oviparous, egg oval, 67 x
769 39 μm , shell smooth, without knobs or spines.

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

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

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***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

797 absent in the new species. Dental formula of *H. crenata* is 7/7 or 8/8 (Donner, 1965). Differs
798 from *H. antarctica* sp. nov. by the granulated integument, lateral knobs on rump, narrower
799 corona, and slightly longer spurs. Also, it has a larger number of major and minor teeth in the
800 unci (9/9) than *H. antarctica* sp. nov. (7/7).

801 **Description.** Body slim, spindle-shaped, yellowish. Integument granulated on the last neck
802 pseudosegment, trunk, rump and the 1st foot pseudosegment, and smooth on the rest of the
803 body. Four small pointed lateral knobs on rump (2 on the distal rim of the 1st and 2 on the 2nd
804 pseudosegment). No knobs on foot. Rostrum very short, lamella with two small semicircular
805 lobes. Corona narrower than rectangular head base, HL is 11-15% of TL. Upper lip triangular,
806 reaches plane of trochal discs, tip divided by a notch into two small semicircular lobes. Trochi
807 without papillae and sensory bristles. Pedicels short, straight. Sulcus very narrow, partly
808 covered by prominent retractors of trochi. Lower lip not protruding laterally. Neck rather
809 long, of moderate width, NL is 38-61% of TL. Antenna is 1/3-1/4 of the bearing
810 pseudosegment's width. Trunk narrow, BW is 29-62% of TL depending on the amount of
811 eggs in a female. The first rump pseudosegment swollen, RL is 10-15% of TL, RW is 71-99%
812 of RL. Foot short, 4 pseudosegments, FL is 8-11% of TL, FW is 54-85% of FL. Spurs of
813 moderate length, narrow triangular, bases merged but seem to form short interspace, SL is 56-
814 84% of SSW. Three short unsegmented toes. No eyespots. Throat small, oesophagus short,
815 straight. Food pellets small, of irregular shape. Oviparous, eggs oval, shell smooth, without
816 knobs or spines. Trophi ramate, heart shaped, 15-17 μm long and 14-16 μm wide.
817 Articulation straight, without incisure. Numerous short scleropili on inner rims of rami.
818 Manubria narrow, sickle-like. Unci with 9/9 major teeth, gradually diminishing in thickness,
819 the last ones hardly distinguishable from minor teeth (26-27 in each uncus).

820 **Measurements.** See Table 2.

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

822 terrestrial moss.

823 Family Philodinidae Ehrenberg, 1838

824 Genus *Macrotrachela* Milne, 1886

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

826 Figure 11

827 Murray, 1910 (*Callidina habita*): Pl. IX fig. 3, Pl. XI fig. 8a. Donner, 1965 (*Macr. insolita*

828 var. 3): 132, Fig. 96l, m. Donner, 1972a (*Macr. insolita* var.): 252, Abb.2. Iakovenko &

829 Tyshenko, 2006 (*Macr. hewitti*): 2, Ris. 2.

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

831 **habitat.** Soil.

832 **Type material.** Holotype: SIZ 59.1 (MPM5MD1a, 25.01.2010, Leg. J. Smykla), on slide in

833 glycerine jelly, encircled with green ink. Paratypes: SIZ 59.2-6 (MPM5MD2-6), in glycerine

834 jelly on the same slide as the holotype, encircled with black ink; SIZ 59.7-9 (MPM5MD1b-d),

835 trophi mounted for SEM. **Additional material.** SIZ 59.10-15 (KG1MD1, EPL25MD1,

836 MPL1MD1, MPL3MD1-3), digital photos and videos; SIZ 59.15-18 (MPL3MD1-4), trophi

837 mounted for SEM.

838 **Barcodes.** GenBank ID KP869998.

839 **Etymology.** Named after Austrian rotiferologist Dr J. Donner who first depicted this species

840 as *M. insolita* var.

841 **Diagnosis.** Similar to *Macr. habita* (Bryce, 1894) and *Macr. insolita* de Koning, 1947 by the

842 smooth integument, the upper lip with two rounded lobes, and the longitudinal knob on the 1st

843 foot pseudosegment. However, ligula in the sulcus and knobs on rump are absent in the new

844 species. The foot is rather stout (what distinguishes all three species from *Macr. plicata* s.

845 lat.). Similarly to *Macr. insolita*, the dental formula of the new species is 2/2, while in *Macr.*

846 *habita* it is 2+1/1+2 (with an additional thinner teeth). It is easily distinguished by the peg-like

847 or narrow conical spurs – in *Macr. habita* and *Macr. insolita* the spurs are triangular, wide,
848 and flat, with shorter interspace. Larger than *Macr. insolita* (TL 325±44 µm, TL of *Macr.*
849 *insolita* is about 250 µm). CW/HW ratio (107-119%) is intermediate between *Macr. habita*
850 (120%) and *Macr. insolita* (100-103%), and the head seems to be shorter and wider than in
851 *Macr. insolita*. The upper lip is shorter, and the lobes are more separated from each other. The
852 head base is rectangular, while it seems to be trapezoid in *Macr. habita*, and wide-oval in
853 *Macr. insolita*.

854 **Description.** Body large, transparent, colorless, but often with bright-orange stomach.
855 Integument smooth, without spines or bolsters, no knobs except a large longitudinal knob on
856 the 1st foot pseudosegment. Rostrum short, thick, lamella with two semicircular lobes. Head
857 wide, corona wider than head base, CW is 107-119% of HW, HL is 10-15% of TL. Pedicels
858 short, straight. Sulcus as wide as ½ of a trochus, covered with protruding trochi retractors. No
859 papillae or sensory bristles on trochi. Head base rectangular, shorter than its width. Upper lip
860 arcuate with two large semicircular lobes not divided by interspace, reaching ½ of the
861 pedicels' height. Lower lip protrudes laterally. Neck of moderate length and width, NL is 15-
862 21% of TL. Length of antenna is about ¼ of bearing pseudosegment width. Trunk thick, BW
863 is 15-25% of TL. Rump large, swollen, RL is 12-15% of TL, RW is 65-97% of RL. Foot
864 short, of 4 pseudosegments, 1st pseudosegment with dorsal elongated knob. Spurs rather long,
865 rod-like, pointed, gradually tapering from the base to tips, interspace equal to 2 spur widths,
866 SL is 86-111% of SSW. Three thick unsegmented toes. No eyespots. Throat voluminous,
867 oesophagus short, straight. Lumen long, thick, often with a loop. Stomach glands large, round.
868 Trophi ramate, large, heart-shaped, 21-25 µm long and 24-28 µm wide. Rami massive, inner
869 rim with numerous scleropili. Articulation long, flat, straight, without incisure. Manubria
870 long, wide, crescent-like. Dental formula 2/2, with 42-43 minor teeth in each uncus.
871 Oviparous. Egg lemon-shaped, shell smooth with two round knobs on each pole.

872 **Measurements.** See Table 2. TL up to 570 µm, CW 95 µm in Murray (1910). According to
873 Donner (1965), TL 410 µm, CW 66 µm, SL 29 µm, trophi 30 µm long.
874 **Distribution.** Maritime Antarctica: AI*, KG*, SI. Continental Antarctica: VL (CB*, CC*,
875 CR, Cz*, BI*, EP*, MP*). **Habitat.** Soil, algal mats in seepages, terrestrial moss.
876 **Comments.** Murray (1910) first depicted this rotifer from Cape Royds, though he apparently
877 described two different species under the name *C. habita*.

878 ***Macrotrachela ioannae* sp. nov. Iakovenko**

879 Figs 3h, 12

880 **Type locality.** Rocka Islands (Argentine Islands Archipelago), 15 m asl, S65° 10.738', W64°
881 29.522'. **Type habitat.** Soil.

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

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

888 **Barcodes:** GenBank ID KP869995-97.

889 **Diagnosis.** Similar to *Macr. ehrenbergii* (Janson, 1893), *Macr. timida* s. lat., *Macr. induta*
890 Donner, 1951, and *Macr. allani* (Murray, 1911). Differs from all these species by the shape of
891 spurs with papillae-like tips, and dental formula (additional thinner tooth in each uncus). The
892 head base is shorter and wider than in *Macr. ehrenbergii*. The corona is almost equal to the
893 head base, while in *Macr. timida* and *Macr. allani* it is substantially wider. Spurs shorter
894 than the bearing pseudosegment width, while in these species they are, on the contrary,
895 longer. Unlike in *Macr. timida* s. lat., no knobs on foot or rump. Differs from *Macr. induta*
896 also by the shape of the upper lip, which is in *Macr. induta* is wide arcuate, with low rounded

897 lobe in the middle. The new species has narrow arcuate upper lip with a high trapezoid lobe
898 with rounded tip in the middle, bearing thin bolster along the upper rim.

899 **Description.** Body large, colorless, transparent. Integument smooth, without knobs, spines or
900 bolsters. Rostrum thick, of moderate length, lamella with two semicircular lobes. Corona not
901 wide, CW is 100-108% of HW, HL is 11-21% of TL. Pedicels short, straight. Sulcus of
902 moderate width, without ligula, not covered with membrane or trochi retractors. Head base
903 rectangular, its width larger than height. Upper lip arcuate with single trapezoid lobe, its
904 rounded tip has a bolster along the upper rim. Lower lip not protruding laterally. Neck of
905 moderate length and width, NL is 12-18% of TL. Length of antenna is 1/3 of the bearing
906 pseudosegment's width. Trunk cylindrical, BW is 13-17% of TL. First rump pseudosegment
907 swollen, RL is 12-15% of TL, RW is 78-96% of RL. Foot short, 4 pseudosegments, FL is 8-
908 11% of TL, FW is 52-77 of FL. Spurs short, flat, triangular, divergent, with bases merged and
909 tips separated as small papillae. Three unsegmented toes. No eyespots. Throat voluminous,
910 oesophagus short, straight. Lumen wide, with a loop. Stomach glands round, not large. Trophi
911 ramate, heart-shaped, 26 µm long and 20-21 µm wide. Dental formula 2+1/1+2 major teeth
912 (with an additional thinner teeth) and about 30 minor teeth in each uncus. Oviparous. Eggs
913 oval, 124 x 66 µm. Egg shell smooth, without knobs or spines.

914 **Measurements.** See Table 2.

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

916 *Macrotrachela jankoi* sp. nov. Iakovenko

917 Figs 3f-g, 13

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

919 **Type locality.** Squa Island (Argentine Islands archipelago), 20 m asl, S65° 25.117', W64°
920 26.583'. **Type habitat.** Soil.

921 **Type material.** Holotype: SIZ 61.1 (VS02MJ1a, 15.03.2010, Leg. K. Janko), mounted in
922 glycerine jelly. Paratypes: SIZ 61.2-3 (VS02MJ2, VS02MJ3), mounted in glycerine jelly; SIZ
923 61.4 (VS02MJ4), trophi mounted for SEM. **Additional material.** SIZ 61.5-17 (V08MJ1,
924 V11MJ1, V12MJ1-2, VRA01MJ1-7, VRA02MJ1), digital photos and videos; SIZ 61.18-26
925 (V12MJ3, MRockaMJ1-8), trophi mounted for SEM.

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

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

929 **Diagnosis.** Resembles *Macr. insulana* Donner, 1962 by the shape of corona and upper lip, the
930 characteristic sulcus with two denticles on the dorsal side, and the absence of a knob on the 1st
931 foot pseudosegment. Differs by the flat and short triangular spurs, which are longer and peg-
932 like in *Macr. insulana*. Dental formula of the new species is 2/2, while in *Macr. insulana* it is
933 1+2/2+1.

934 **Description.** Body large, transparent, stomach of bright red or orange colour. Integument
935 smooth, thin, without knobs, spines or bolsters. Rostrum stout, of moderate length, lamella
936 with two large rounded lobes. Corona wider than oval head base, CW is 100-133% of HW,
937 HL is 35-54% of TL. Pedicels short, straight. Sulcus wide, not covered with membrane. On
938 dorsal side of head two short denticles divided by interspace visible in sulcus. Trochi large,
939 with papillae and sensory bristles. Upper lip with two triangular lobes reaching about a half of
940 the sulcus, and divided by interspace. Lower lip not protruding laterally. Neck stout, of
941 moderate length, NL is 16-21% of TL. Antenna about 1/3 of the bearing pseudosegment.
942 Trunk plump, BW is 15-21% of TL. Both pseudosegments of the rump swollen, RL is 12-
943 21% of TL, RW is 73-93% of RL. Foot short, stout, 4 pseudosegments, without a dorsal knob,
944 FL is 10-12% of TL, FW is 50-82% of FL. Spurs small, short, isosceles triangular, divided by
945 an interspace almost equal to spur width, SL is 44-79% of SSW. Three short unsegmented

946 toes. No eyespots. Throat voluminous, oesophagus short, straight. Stomach glands round.
947 Trophi ramate, 18-22 µm long and wide. Rami massive, with numerous short scleropili along
948 the inner rim. Articulation somewhat concaved in the middle, but without incisure. Manubria
949 thin, sickle-like. Dental formula 2/2, 39-41 minor teeth in each uncus. Oviparous. Eggs oval,
950 shell smooth, without knobs or spines.

951 **Measurements.** See Table 2.

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

953 **Habitat.** Soil, terrestrial moss.

954 Genus *Philodina* Ehrenberg, 1830

955 *Philodina dartneris* sp. nov.

956 Figs 3i-j, 14

957 Priddle & Dartnall, 1978 (? *Philodina*): 475. Dartnall & Hollowday, 1985 (*Philodina* sp. 'A'):
958 24, figs 27a-e. Velasco-Castrillón et al., 2014a (*Ph.* sp. Bd46): 8.

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

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

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

965 **Etymology.** Named after Antarctic researcher Dr H. Dartnall who first depicted this species
966 as *Ph.* sp. 'A'.

967 **Diagnosis.** Similar to *Ph. flaviceps*, *Ph. australis* Murray, 1911, and some specimens of *Ph.*
968 *brevipes* Murray, 1902. Resembles *Ph. flaviceps* by the shape of corona and spurs; however
969 the spurs of the new species are longer and with broader interspace. Differs from *Ph. australis*
970 by the spur shape, which in the new species are peg-like with a broad interspace, but narrow

971 triangular without interspace in *Ph. australis*. Differs from *Ph. brevipes* by the shape of the
972 upper lip (rounded lobes in the new species, pointed in *Ph. brevipes*), and by the absence of
973 long sensory cilia in the rostrum. The foot seems to be shorter than in *Ph. brevipes*.

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

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

991 *Philodina shackletoni* sp. nov.

992 Figure 15

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

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

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

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

999 **Etymology.** Named in honour of the leading Antarctic explorer, Sir Ernest Henry Shackleton
1000 who in 1909 established his base on Cape Royds where the species was discovered.

1001 **Diagnosis.** Most closely resembles *Ph. flaviceps* Murray, 1906 by the shape of spurs and
1002 corona, however lacking eyespots. Spurs are longer than in *Ph. flaviceps*, and divided by
1003 broader interspace.

1004 **Description.** Body large, spindle-shaped, colorless with yellow-brown stomach. Integument
1005 smooth, thin, without knobs, spines or bolsters. Rostrum of moderate size, with crescent-like
1006 lamella. Corona wider than trapezoid head base, CW is 109-117% of HW, HL is 13-17% of
1007 TL. Pedicels short, slightly bent inwards. Sulcus wider than diameter of a trochus, not covered
1008 with membrane. Trochi with papillae and sensory bristles. Upper lip very low, arcuate, with
1009 two small rounded lobes divided by interspace. Lower lip not protruding laterally. Neck of
1010 moderate length and width, NL is 18-23% of TL. Antenna long, almost equal to the bearing
1011 pseudosegment width. Trunk slim, BW is 15-20% of TL. Rump large, swollen, RL is 15-17%
1012 of TL, RW is 11-13 of RL. Foot long, slim, 5 pseudosegments, FL is 10-15% of TL, FW is
1013 27-32% of FL. Spurs long, needle-like, SL is 115-164% of SSW. Four unsegmented toes. No
1014 eyespots. Throat small, oesophagus short. Lumen wide. Stomach glands small, round. Trophi
1015 ramate, 24 µm long and wide, dental formula 2/2. Oviparous. Eggs oval, shell smooth,
1016 without knobs or spines.

1017 **Measurements.** See Table 2. TL 400 µm (Velasco-Castrillón et al., 2014a).

1018 **Distribution.** Maritime Antarctica: SI. Continental Antarctica: EB, VL (CR*), WK. **Habitat.**
1019 Soil, terrestrial moss, pools.

1020

1021

1022 *Morphometric analyses*

1023

1024 *Adineta editae* sp. nov differed from the similar European species *A. gracilis* by larger size of
1025 body and mastax, and longer spurs. LME demonstrates that the variation of body
1026 measurements between species represented over 60% of total variation for the parameters
1027 describing the width along the rotifer body (HW, MinNW, MxNW, FW, SSW) and for the
1028 spur length. The variation between localities and individuals was not significant for FW, SSW
1029 and SL (ANOVA on LME output: LR=11.1 to 13.1, $p>0.1$). This was in correspondence with
1030 our visual observation that *A. editae* sp. nov. had a distinctively stouter body than *A. gracilis*
1031 s. str. In the PCA plot (Fig. 16a) external measurements of the two species did not overlap
1032 along PC1 (correlating with all measurements) and PC2 (correlating with HW and SL).

1033 The difference in trophi measurements between *A. editae* sp. nov. and *A. gracilis*
1034 represented over 90% of total variation in the number of minor teeth and trophi length, and
1035 over 80% in the case of trophi width. The variation between localities and individuals was
1036 insignificant for all measurements (LR =0.2 to 2.1, $p>0.5$). The trophi measurements of these
1037 species were completely separated on the PCA plot (Fig. 16b) and did not overlap along PC1
1038 (correlates with all measurements) and PC2 (correlates with the number of minor teeth and
1039 the unci width).

1040 The body and trophi of the Antarctic species *A. grandis* and *A. fontanetoi* sp. nov. are
1041 indistinguishable by external morphology, and did not differ significantly in any of the
1042 measured parameters. The Antarctic *A. emsliei* sp. nov. and the European *A. vaga* s. str.
1043 noticeably differed by at least one trophi measurement (the number of minor teeth in unci).
1044 The difference by this parameter consisted over 80% of total variation, with the variation

1045 between localities and individuals being insignificant (LR=0.7 to 1.5, $p>0.5$). The antarctic
1046 species *A. grandis* and *A. emsliei* sp. nov. were distinguished by all trophi measurements, the
1047 difference between species being over 90% of total variation. The variation between localities
1048 and individuals was not significant except for rami length (LR=0.3 to 4.5, $p>0.1$). In the PCA
1049 plot (Fig. 16c) the samples of trophi of *A. grandis* and *A. fontanetoi* sp. nov. overlapped
1050 completely on both PC1 (correlating with all trophi measurements) and PC2 (correlating with
1051 the number of minor teeth and trophi width), but the samples of *A. emsliei* sp. nov. did not
1052 overlap with any of the other species.

1053 The Antarctic *H. antarctica* sp. nov. and *H. vernadskii* sp. nov. did not show any
1054 significant difference in trophi length and width, but could be distinguished by the number of
1055 minor teeth of the unci. Variation between the species on the latter measurement represented
1056 over 70%, the variation between localities and individuals being insignificant (LR=3.6,
1057 $p>0.1$). *Habrotrocha* sp. 4 is indistinguishable from *H. antarctica* sp. nov. by external
1058 morphology, but has an intermediate trophi size between *H. antarctica* sp. nov. and *H.*
1059 *vernadskii* sp. nov, with the variation between species by all trophi parameters representing
1060 50% or less of total variation. On the PCA plot of the samples of trophi measurements (Fig.
1061 16d) *H. antarctica* sp. nov. and *H. vernadskii* sp. nov., did not overlap with each other on any
1062 either axis. *Habrotrocha* sp. 4 did not overlap with either of the two other species.

1063

1064 *DNA taxonomy*

1065

1066 On the phylogenetic tree of *Adineta* (Fig. 17a) the sequences of Antarctic rotifers grouped into
1067 1 singleton and 8 independently evolving entities (IEE) identified by the GMYC and 4x rule
1068 approaches (32 singletons and 6 IEEs according to bPTP). None of the IEEs contained rotifers
1069 originating from any other continents. The phylogeny shows that the Antarctic endemic *A.*

1070 *grandis* consists of at least two morphologically indistinguishable entities (one of which has
1071 been described above as *A. fontanetoi* sp. nov.). For three IEEs the morphology has not been
1072 described, and therefore they are listed under *A.* sp. 1-3. Finally, three IEEs that proved to be
1073 both genetically separate and morphologically distinguishable, are described above as *A.*
1074 *editae* sp. nov., *A. emsliei* sp. nov. and *A. coatsae* sp. nov. Molecular analysis confirmed that
1075 these species occur only in Antarctica, in spite of being previously confused with the
1076 cosmopolitan species *A. gracilis*, *A. vaga* s. str. and *A. barbata*. The integrity of *A. coatsae* sp.
1077 nov. as a single IEE was confirmed by the GMYC model, but not by the bPTP and 4x rule
1078 models. Both the GMYC and 4x rule, but not the bPTP model, confirmed the integrity *A.*
1079 *fontanetoi* sp. nov. as a separate IEE.

1080 On the tree of *Philodina* 19 singletons and 10 IEEs containing sequences of Antarctic
1081 bdelloids were identified by GMYC, mostly confirmed as IEEs by the 4x rule and bPTP (Fig.
1082 17b). *Philodina* is the only one of the four investigated genera with cosmopolitan IEEs
1083 occurring in the Antarctic, those being *Ph.* sp. 4 (two sequences, from the Antarctic and the
1084 USA) and *Ph.* sp. 7 (25 sequences from the USA and one from Antarctica). Unfortunately, for
1085 both of these IEEs no data on morphology are available, and none of the sequences were
1086 obtained from vouchers of already known species. The remaining IEEs did not contain
1087 individuals from continents other than Antarctica. For six of them, listed here as *Ph.* sp. 1-6,
1088 there are no morphological data available. *Philodina gregaria* appeared as one large pan-
1089 Antarctic IEE, 3 singletons and one IEE with atypical morphology, containing only two
1090 sequences. Two IEEs proved to be well distinguishable both morphologically and by the
1091 means of DNA taxonomy, both from *Ph. gregaria* and the morphologically similar non-
1092 Antarctic *Ph. acuticornis* Murray 1902, *Ph. flaviceps* Murray 1906, and *Ph. roseola*
1093 Ehrenberg, 1832. These two species are described above as new Antarctic endemics: *Ph.*

1094 *shackletoni* sp. nov. and *Ph. dartnallis* sp. nov. The integrity of *Ph. dartnallis* sp. nov. clade
1095 was confirmed by two of three delimitation methods.

1096 Putative species delimitation of *Habrotrocha* according to GMYC gave 11 IEEs and 4
1097 singletons with strictly Antarctic distribution (Fig. 18). For three IEEs (*H.* sp. 1, 2 and 3) the
1098 morphology has not been reported. Three IEEs are described above as *H. antarctica* sp. nov.,
1099 *H. devetteri* sp. nov., and *H. vernadskii* sp. nov. based on both morphological and molecular
1100 differences from similar non-Antarctic *H. constricta*, *H. crenata* s. lat., *H. elusa* s. lat., and *H.*
1101 *thienemanni* s. lat. *Habrotrocha* sp. 4 was identified as an IEE by the GMYC model, but not
1102 confirmed as a single entity by the 4x rule, being less than four times separated from both *H.*
1103 *antarctica* sp. nov. and *H. vernadskii* sp. nov. (which are genetically and morphologically
1104 sufficiently separate to be good species). *H.* sp. 4 and *H.* sp. 5 are morphologically identical
1105 to *H. devetteri* sp. nov., but were delimited as separate IEEs by all three molecular
1106 delimitation methods. *H. angularis* has been confirmed as a separate species with a strictly
1107 Antarctic distribution.

1108 Four IEEs and three singletons distributed exclusively in Antarctica were identified in
1109 *Macrotrachela* using GMYC, and this delimitation was in most cases confirmed by the two
1110 other approaches (Fig. 19). For one IEE, *M.* sp. 2, no information is yet available on
1111 morphology. *Macrotrachela donneri* sp. nov., previously reported in Antarctica as *Macr.* cf
1112 *insolita* or *M.* cf *hewitti* (Donner, 1972), was represented by a single sequence which did not
1113 cluster with any non-Antarctic rotifers including the morphologically similar *M. habita*. For
1114 *M.* sp. 1 more morphological data are required. *Macrotrachela ioannae* sp. nov. and *M. jankoi*
1115 sp. nov. were confirmed by GMYC as good species occurring in Antarctic, but the bPTP
1116 approach revealed *M. jankoi* sp. nov. as two IEEs. The integrity of *M.* sp. 2, identified using
1117 GMYC, was not confirmed by the other two approaches.

1118 The identification of IEEs based on our combined set of sequences, including both
1119 new sequences and the previously published data, was the same as in Velasco-Castrillón et al.
1120 (2014a) with the exception of three IEEs. According to our results, the putative species Bd15
1121 and Bd16 were identified as a single entity *H. sp.1* by all three models. Bd31 and Bd32 were
1122 identified as *H. sp. 2* by the 4x rule, but as separate entities by the GMYC and bPTP models.
1123 Bd5 and Bd6 were identified as *M. sp. 2* according to GMYC, but this was not confirmed by
1124 the two other models. All the sequences published by Barraclough et al. (2007) and Fontaneto
1125 et al. (2008) were identified as *Adineta* (*A. editae* sp. nov., *A. sp. 2*, *A. sp. 3*). All 16 sequences
1126 published by Fontaneto et al. (2012) were confirmed as *Philodina* (*Ph. sp. 3-13*).

1127

1128 *Biogeography*

1129

1130 Of the 60 morphospecies found in the new material examined in this study, at least 17 can be
1131 considered true Antarctic and/or sub-Antarctic endemics, with six of these being already
1132 known and 11 newly recognized. A further 10 morphospecies that closely resemble known
1133 and presumed cosmopolitan bdelloids, possessing only minor but consistent morphological
1134 differences from the original descriptions, require further detailed analysis. No molecular data
1135 are available for 13 morphospecies showing no discernible differences from known
1136 cosmopolitan species, as well as 20 identified only to generic level, and these cannot currently
1137 be attributed with confidence to any classification. That gives at least 28 and possibly up to
1138 45% endemism (17 or up to 27 morphospecies out of 60) on the basis of classical taxonomy
1139 alone.

1140 Based on molecular data obtained both from our new samples and GenBank, we
1141 identified 33 IEEs and 37 singletons from Antarctica and the sub-Antarctic using GMYC (this
1142 largely confirmed by the two other approaches used), belonging to the genera *Adineta*,

1143 *Habrotrocha*, *Philodina* and *Macrotrachela*. Including a further five IEEs and eight
1144 singletons apparently representing other genera, a total of 38 IEEs and 45 singletons of
1145 Bdelloidea are now identified from this region. Only two of the IEEs occurring in Antarctica
1146 have been confirmed as having wider non-Antarctic distributions by molecular methods. At
1147 least 13 of the strictly Antarctic IEEs are clearly distinguishable by morphology, while at least
1148 four IEEs appear to represent cryptic species.

1149 The molecular data obtained suggest there is a very high level of endemism among
1150 Antarctic bdelloids – 36 out of 38 IEEs (95%). GMYC also identified considerable apparent
1151 endemism in the rotifer faunas (IEEs) of other major global regions: 55 of 59 found in Eurasia
1152 (93%), 65 of 70 (93%) in North America, and three of four (75%) in both Australia and New
1153 Zealand, and Africa.

1154

1155

1156 **Discussion**

1157

1158 After a 100-year period of research, the Antarctic Bdelloidea still remain obscure and
1159 knowledge sparse, in terms of their diversity, distribution and origin. The current study is only
1160 the fourth to report previously undescribed Antarctic bdelloid rotifers, with all 12 new taxa
1161 being currently known only from the continent. The study is the first to provide a robust
1162 combination of detailed, morphological, morphometric and molecular approaches, which are
1163 being utilized in the description of these new Antarctic taxa. This study is also the second to
1164 apply molecular approaches in describing diversity and biogeography of Antarctic Bdelloidea,
1165 as with that of Velasco-Castrillón et al. (2014a) carried out through COX1 sequencing. To
1166 date, 15 morphospecies of 60 (25%) found in this study have been barcoded successfully. Of
1167 these, we have (1) described 12 new for science morphospecies using both classical and DNA

1168 taxonomy approaches, (2) revealed the presence of a number of cryptic species that are
1169 apparently morphologically identical to *A. grandis*, *H. antarctica* sp. nov. and *H. devetteri* sp.
1170 nov., (3) linked the molecular data provided by Velasco-Castrillón et al. (2014a) with
1171 morphospecies, and (4) provided evidence of generally very high levels of endemism of
1172 bdelloid rotifers in Antarctica, with the exception of the finding of two cosmopolitan species
1173 of *Philodina*, not identified in previous studies.

1174 The difficulty of distinguishing bdelloid species based on morphology alone, due to
1175 their generally highly conserved body morphology and structure, and ambiguity in defining
1176 specific characters, has led to a prevailing misconception that the contemporary Antarctic
1177 fauna includes a large proportion of cosmopolitan bdelloids. Thus, Donner (1965, 1972b)
1178 considered minor difference in size and shape of the bdelloid body and its appendages as only
1179 representing intraspecific variability. Previous studies have often attributed Antarctic
1180 specimens to species already known from elsewhere (mostly Europe) if most of the external
1181 morphological characters matched the original descriptions, or they were identified through
1182 keys based on the latter. Our morphometric and molecular data support the conclusions of
1183 Fontaneto et al. (2007), who proved that careful morphometric measurement of hard parts of
1184 the mastax (trophi) can differentiate several morphologically distinct entities within one
1185 „classical“ species, corresponding with IEEs determined by molecular analysis.

1186 All three models applied here for the delimitation of IEEs based on molecular
1187 sequence data showed good correspondence with rotifer morphology. GMYC gave the best
1188 correspondence with the species-specific morphology and in most cases the results were
1189 supported by two other approaches. However, the bPTP model, used similarly by Velasco-
1190 Castrillón et al. (2014a), tended to give finer subdivision of IEEs, creating an excessive
1191 number of entities unidentifiable at the morphological level. Our data confirmed the integrity
1192 of all but three of the IEEs identified by Velasco-Castrillón et al. (2014a), even after the

1193 inclusion of our new sequence data. Our data also confirm the attribution of some sequences
1194 to *Adineta* and *Philodina* as proposed by Barraclough et al. (2007) and Fontaneto et al. (2008,
1195 2012).

1196

1197

1198 **Conclusions**

1199

1200 A striking feature of the data obtained in the current study is that of the extremely high levels
1201 of endemism to the Antarctic and sub-Antarctic region apparent in the bdelloid fauna. Clearly,
1202 consideration of the concept of endemism is itself limited by the quality and extent of the data
1203 available, both from the Antarctic and from other regions. However, both the current study
1204 and that of Velasco-Castrillón et al. (2014a) are consistent in identifying (1) that considerably
1205 greater diversity in terms of divergence to ‘species level’ is apparent in analyses of molecular
1206 (COX1) data than was the case in previous classical taxonomic studies of the group, and (2)
1207 that Antarctic lineages are distinct from those of bdelloids from other continents available
1208 today in GenBank.

1209 Implicit in the assessment of considerable levels of endemism at continental level is
1210 the conclusion that it is indicative of an extended history (long term presence) allowing
1211 evolutionary divergence in situ in the Antarctic. This is consistent with a range of studies over
1212 the last one to two decades that have used both classical and molecular approaches to confirm
1213 both high levels of endemism and long evolutionary histories in representatives of all the
1214 main terrestrial invertebrate groups occurring in Antarctica, including Tardigrada (Convey &
1215 McInnes, 2005), Nematoda (Andrássy, 1999; Maslen & Convey, 2006), Collembola
1216 (Greenslade, 1995; McGaughan et al., 2010; Torricelli et al., 2010), Acari (Pugh, 1993;
1217 Stevens & Hogg, 2006) and Diptera (Allegrucci et al., 2012), see also wider reviews of this

1218 subject provided by Convey et al. (2008), Pugh & Convey (2008). Similar conclusions are
1219 increasingly being drawn from studies of some microbial groups (De Wever et al., 2009;
1220 Strunecký et al., 2012) and, most recently, mosses (Pisa et al., 2014).

1221 The outcomes of the current study highlight the need for considerably greater survey
1222 effort being applied to groups of microscopic Antarctic fauna rich in cryptic species such as
1223 rotifers. Data obtained in both the study of Velasco-Castrillón et al. (2014a) and the current
1224 study suggest that at least some species of bdelloid rotifer are limited to particular parts of the
1225 Antarctic or sub-Antarctic. This, again, is consistent with recent findings in other groups of
1226 terrestrial biota (Convey et al. 2008; Pugh & Convey 2008), as well as the recent analysis of
1227 Terauds et al. (2012) that identified no less than 15 ‘Antarctic Conservation Biogeographic
1228 Regions’ across the Antarctic continent alone. Thus, further targeted research amongst the
1229 bdelloid rotifers of Antarctica, integrating classical, morphometric and molecular biological
1230 approaches, should identify considerably greater levels of diversity and both continental and
1231 intra-continental regional endemism than are currently appreciated.

1232

1233

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1235

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1258

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1260

1261 **References**

1262

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1536

1537 **Table captions**

1538

1539 Table 1. Locations sampled in the Antarctic, including altitudes and the number of collected
1540 samples

1541

1542 Table 2. Body dimensions (measured from light microscope photographs) and trophi
1543 dimensions (measured from SEM photographs) of the described bdelloid species

1544 **Figure captions**

1545

1546 **Fig. 1** Map indicating sampling locations in Antarctica. a – Ross Sea area, b – Antarctic
1547 Peninsula and adjacent islands. BI – Beaufort Island. Ross Island: CB – Cape Bird, CC –
1548 Cape Crozier, CR – Cape Royds. Coastal zone of Victoria Land: Cz – Cape Chocolate, GH –
1549 Granite Harbour, MP – Marble Point, NF – Northern Foothills. Maritime Antarctica: AI –
1550 Argentine Islands archipelago, KG – King George Island, AP – Mount Demaria, Kiev
1551 Peninsula, Graham Land

1552

1553 **Fig. 2** Measurements of bdelloids of the families Habrotrochidae and Philodinidae (a, b), and
1554 Adinetidae (c). BW – body width, CW – corona width, FL – foot length, FW – foot width, HL
1555 – head length, HW – head width, MinNW – minimal neck width, MxNW – maximal neck
1556 width, NL – neck length, RaL – ramus length, RaW – ramus width, RL – rump length, RW –
1557 rump width, SL – spur length, SSW – spur pseudosegment width, TL – total length

1558

1559 **Fig. 3** New species of Antarctic bdelloids (photographs M. Plewka): a - *Adineta coatsae* sp.
1560 nov., holotype, habitus, dorsal view; b – *A. editae* sp. nov., habitus, dorsal view; c – *A.*
1561 *grandis*, habitus, dorsal view; d – *H. antarctica* sp. nov., holotype, habitus, feeding, dorsal
1562 view; e - *H. vernadskii* sp. nov., habitus, feeding, ventral view; f – *M. jankoi* sp. nov., habitus,
1563 creeping, ventral view; g – same, head, feeding, ventral view; f – *M. ioannae* sp. nov., habitus,
1564 feeding, dorsal view; i – *Ph. dartnallis* sp. nov., habitus, creeping, dorsl view; j – same, spurs.
1565 Scale bar 50 µm

1566

1567 **Fig. 4** *Adineta coatsae* sp. nov. (Antarctica): a – holotype, habitus, dorsal view. *A. barbata*
1568 (Europe): b – habitus, dorsal view. Scale bar 50 µm

1569

1570 **Fig. 5** *Adineta editae* sp. nov. sp. nov. (Antarctica): a, b - holotype, habitus, dorsal view; d -
1571 paratype, trophi, cephalic view; e - paratype, trophi, caudal view. *A. gracilis* (Europe): c -
1572 habitus, dorsal view; f - trophi, caudal view. Scale bar 50 μm (a-c) or 5 μm (d-f)

1573

1574 **Fig. 6** *Adineta emsliei* sp. nov. (Antarctica): a, b - holotype, habitus, dorsal view; d -
1575 paratype, trophi, cephalic view; e - paratype, trophi, caudal view. *A. vaga* (Europe): c -
1576 habitus, dorsal view; f - trophi, cephalic view. *A. grandis* Murray, 1910 (Antarctica): g -
1577 trophi, caudal view. Scale bar 50 μm (a-c) or 5 μm (d-g)

1578

1579 **Fig. 7** *Adineta grandis*: a - trophi, cephalic view. *A. fontanetoi* sp. nov.: b - paratype, trophi,
1580 cephalic view; c, d - holotype, habitus, dorsal view. Scale bar 50 μm (c, d) or 5 μm (a, b)

1581

1582 **Fig. 8** *Habrotrocha antarctica* sp. nov.: a - holotype, habitus, feeding, dorsal view; b - same,
1583 creeping, dorsal view; c - paratype, trophi, cephalic view; d - paratype, trophi, caudal view.
1584 Scale bar 50 μm (a, b) or 5 μm (c, d)

1585

1586 **Fig. 9** *Habrotrocha devetteri* sp. nov. (Antarctica): a - holotype, head, feeding, dorsal view; b
1587 - same, habitus, feeding, ventral view; c - habitus, creeping, dorsal view; d - paratype, trophi,
1588 caudal view. *H. thienemanni* (Europe): e - trophi, cephalic view. Scale bar 50 μm (a-c) or 5
1589 μm (d, e)

1590

1591 **Fig. 10** *Habrotrocha vernadskii* sp. nov.: a - holotype, head, feeding, dorsal view; b - same,
1592 habitus, creeping, dorsal view; c - paratype, trophi, cephalic view; d - paratype, trophi, caudal
1593 view. Scale bar 50 μm (a, b) or 5 μm (c, d).

1594 **Fig. 11** *Macrotrachela donneri* sp. nov.: a – holotype, head, feeding, dorsal view; b – same,
1595 habitus, creeping, dorsal view; c – paratype, trophi, cephalic view; d – paratype, trophi, caudal
1596 view. Scale bar 50 μm (a, b) or 5 μm (c, d)

1597

1598 **Fig. 12** *Macrotrachela ioannae* sp. nov.: a – holotype, habitus, creeping, lateral view; b –
1599 same, head, feeding, dorsal view, c – same, foot, ventral view. Scale bar 50 μm

1600

1601 **Fig. 13** *Macrotrachela jankoi* sp. nov.: a – holotype, head, feeding, dorsal view; b – same,
1602 habitus, feeding, ventral view; c – same, habitus, creeping, dorsal view; d – paratype, trophi,
1603 cephalic view; e – paratype, trophi, caudal view. Scale bar 50 μm (a-c) or 5 μm (d, e)

1604

1605 **Fig. 14.** *Philodina dartnallis* sp. nov.: a – holotype, habitus, creeping, dorsal view; b – same,
1606 feeding, dorsal view. Scale bar 50 μm

1607

1608 **Fig. 15** *Philodina shackletoni* sp. nov.: a – holotype, habitus, feeding, dorsal view; b – foot,
1609 ventral view; c – holotype, habitus, creeping, dorsal view. Scale bar 50 μm

1610

1611 **Fig. 16** Principal components analysis of rotifer body and trophi measurements: a – *A.*
1612 *gracilis* (circles) and *A. editae* sp. nov. (triangles), body dimensions; b – same, trophi
1613 dimensions; c – *A. grandis* (crosses), *A. fontanetoi* sp. nov. (circles), *A. emsliei* sp. nov.
1614 (squares) and *A. vaga* (triangles), trophi dimensions; d – *H. antarctica* sp. nov. (squares), *H.*
1615 *vernadskii* sp. nov. (circles) and *H.* sp. 4 (triangles), trophi dimensions

1616

1617 **Fig. 17** Phylogenetic relationships in the genera *Adineta* (a) and *Philodina* (b). The consensus
1618 Bayesian trees of COX1 mtDNA data sets are shown on the left. Clades are marked as

1619 putative species delimited according to the 4x rule (triangles), GMYC (circles) and bPTP
1620 (squares). Boxes on the right show the distribution of samples across different continents

1621

1622 **Fig. 18** Phylogenetic relationships in the genus *Habrotracha* (consensus Bayesian tree, COX1
1623 mt DNA dataset). Putative species are delimited according to the 4x rule (triangles), GMYC
1624 (circles) and bPTP (squares). Wider distributions of rotifers are shown as in boxes on the right

1625

1626 **Fig. 19** Phylogenetic relationships in the genus *Macrotrachela* (consensus Bayesian tree,
1627 COX1 mt DNA dataset). Putative species are delimited according to the 4x rule (triangles),
1628 GMYC (circles) and bPTP (squares). Wider distributions of rotifers are shown as in boxes on
1629 the right

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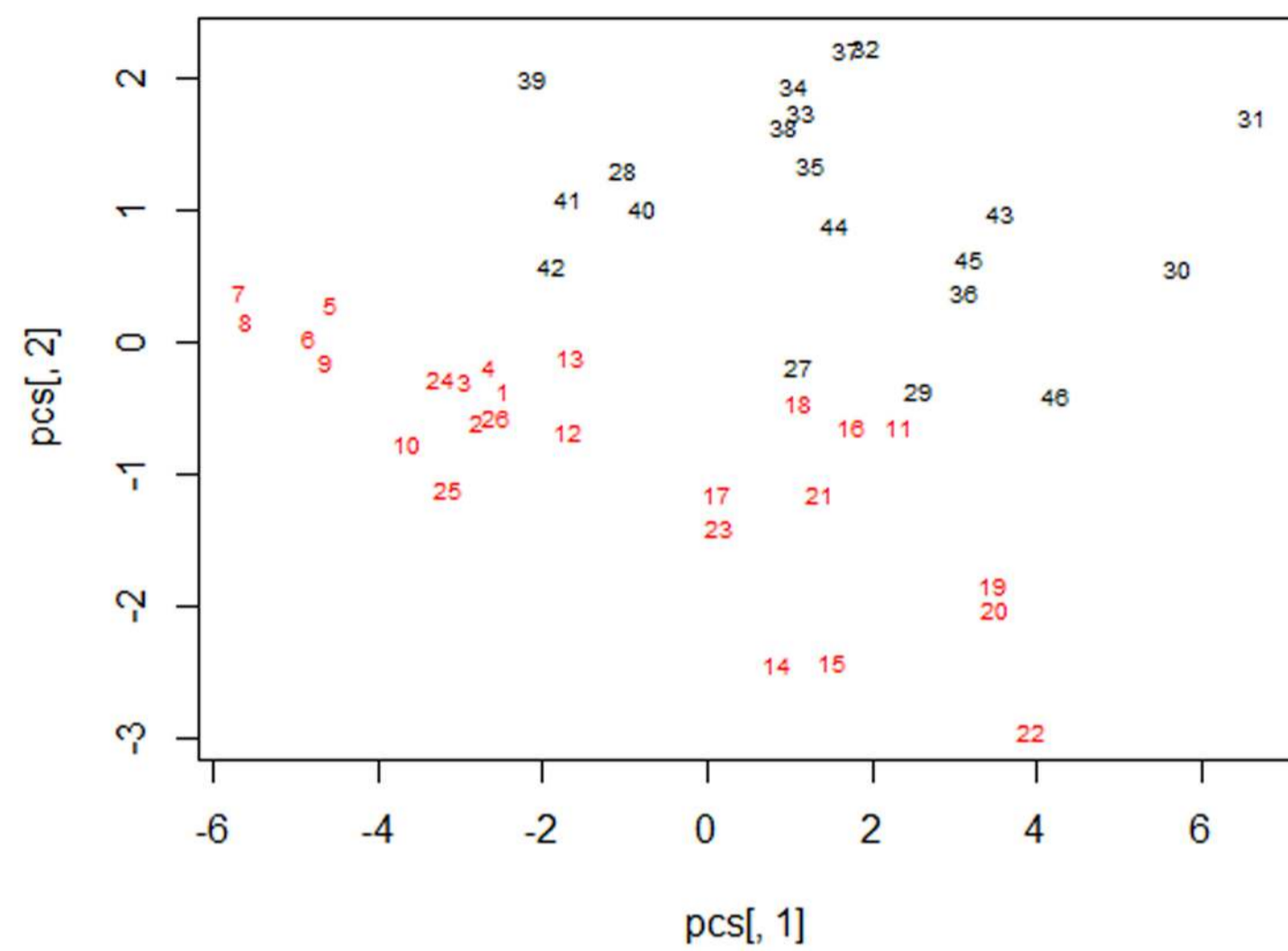
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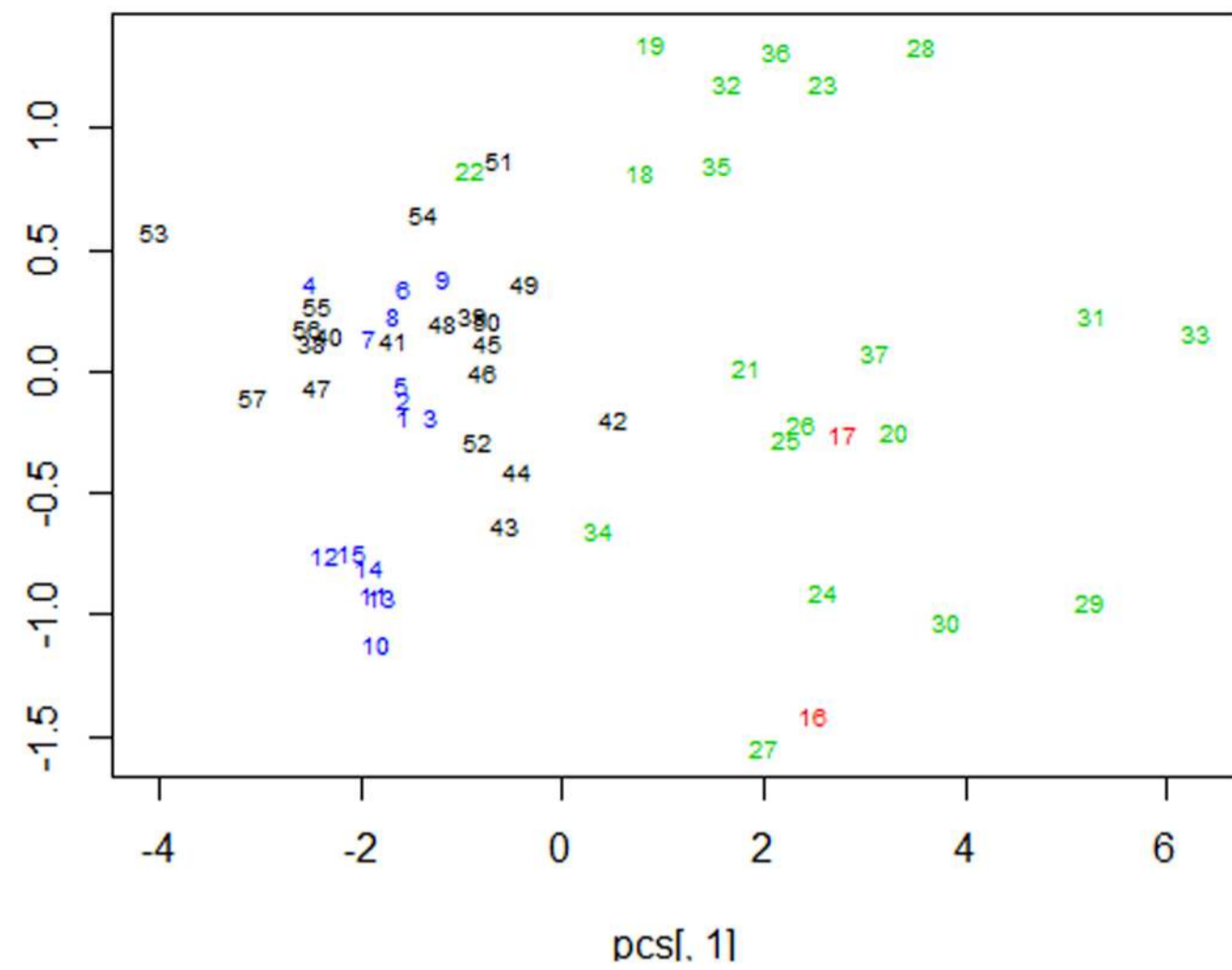
1635

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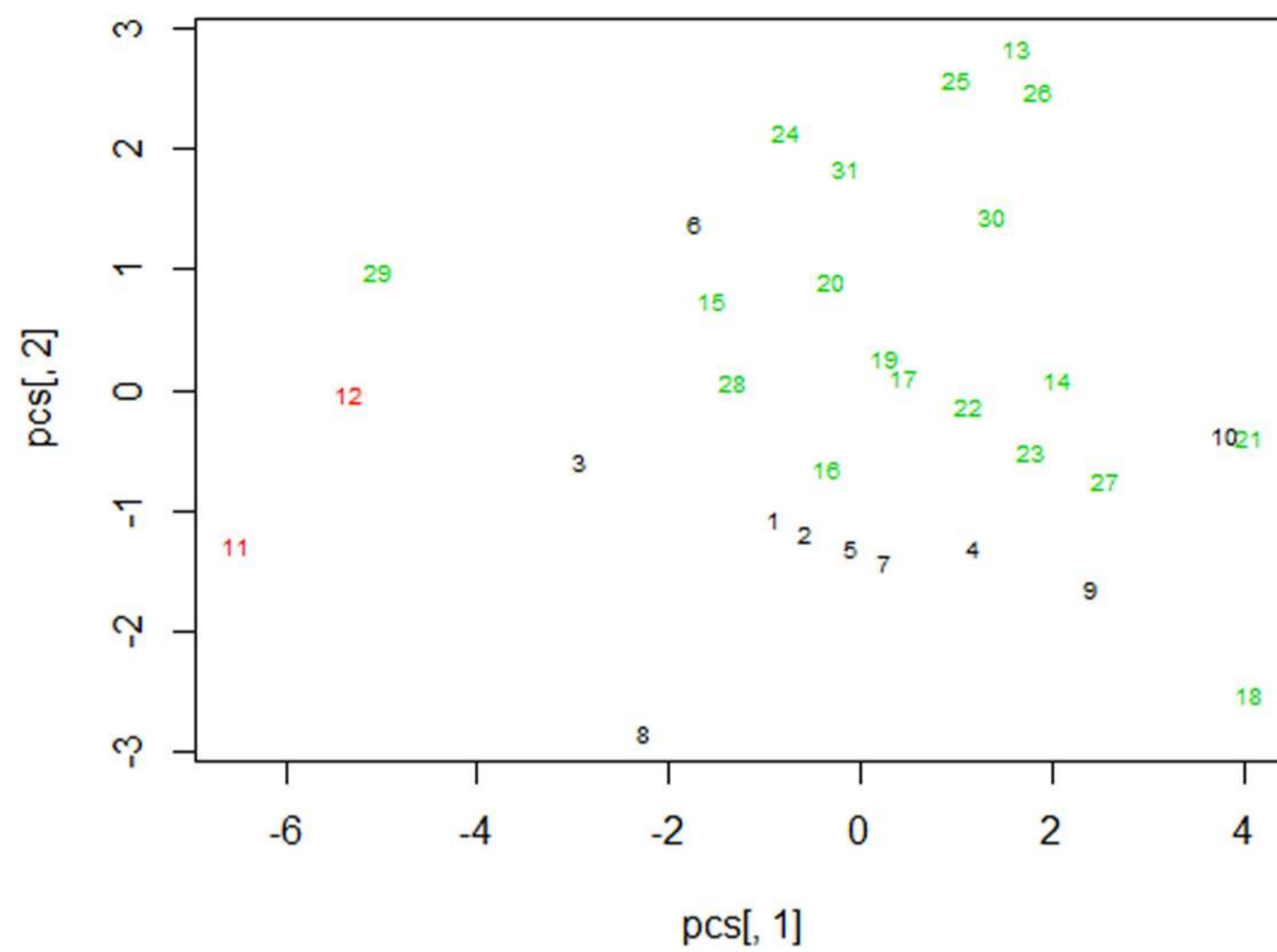
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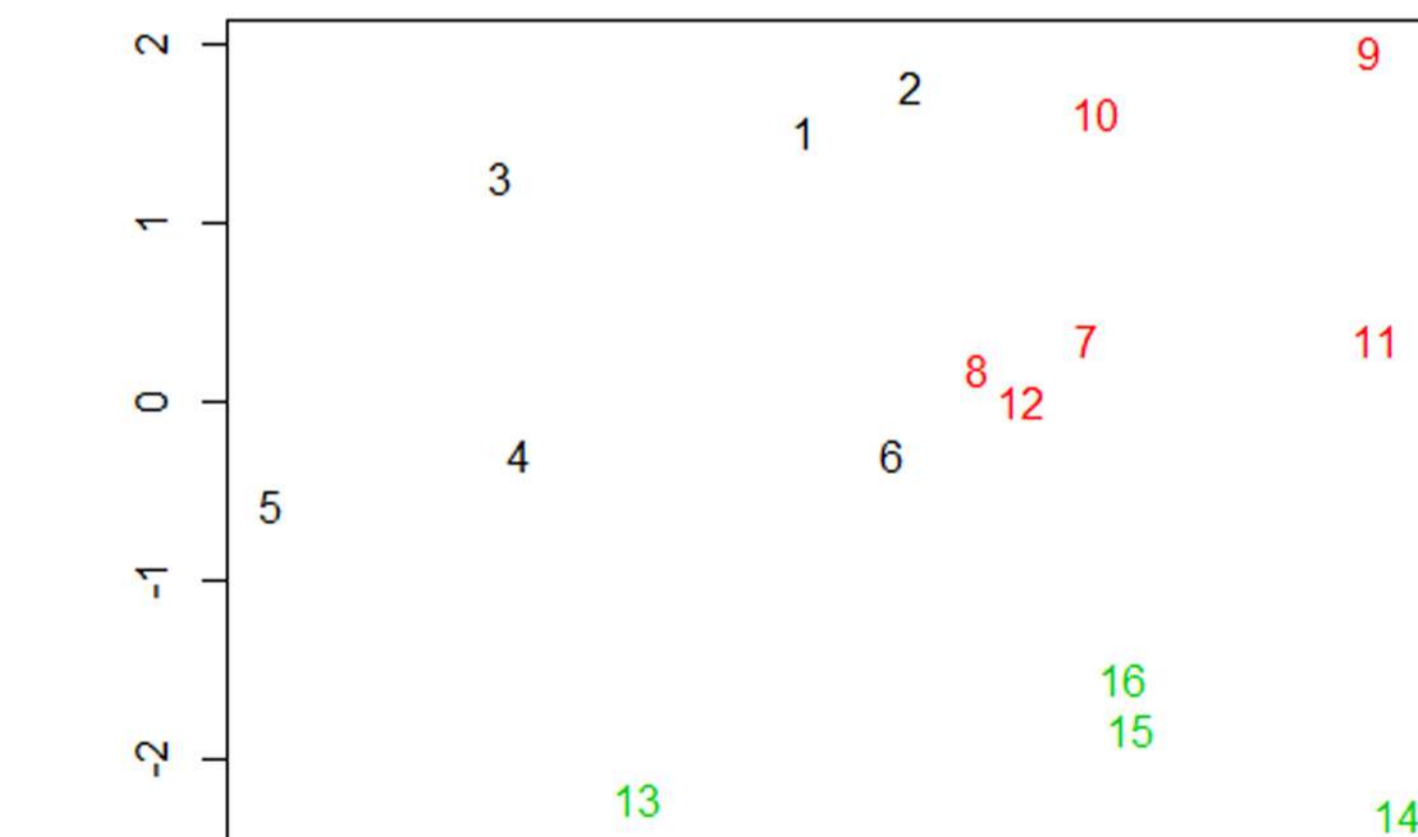
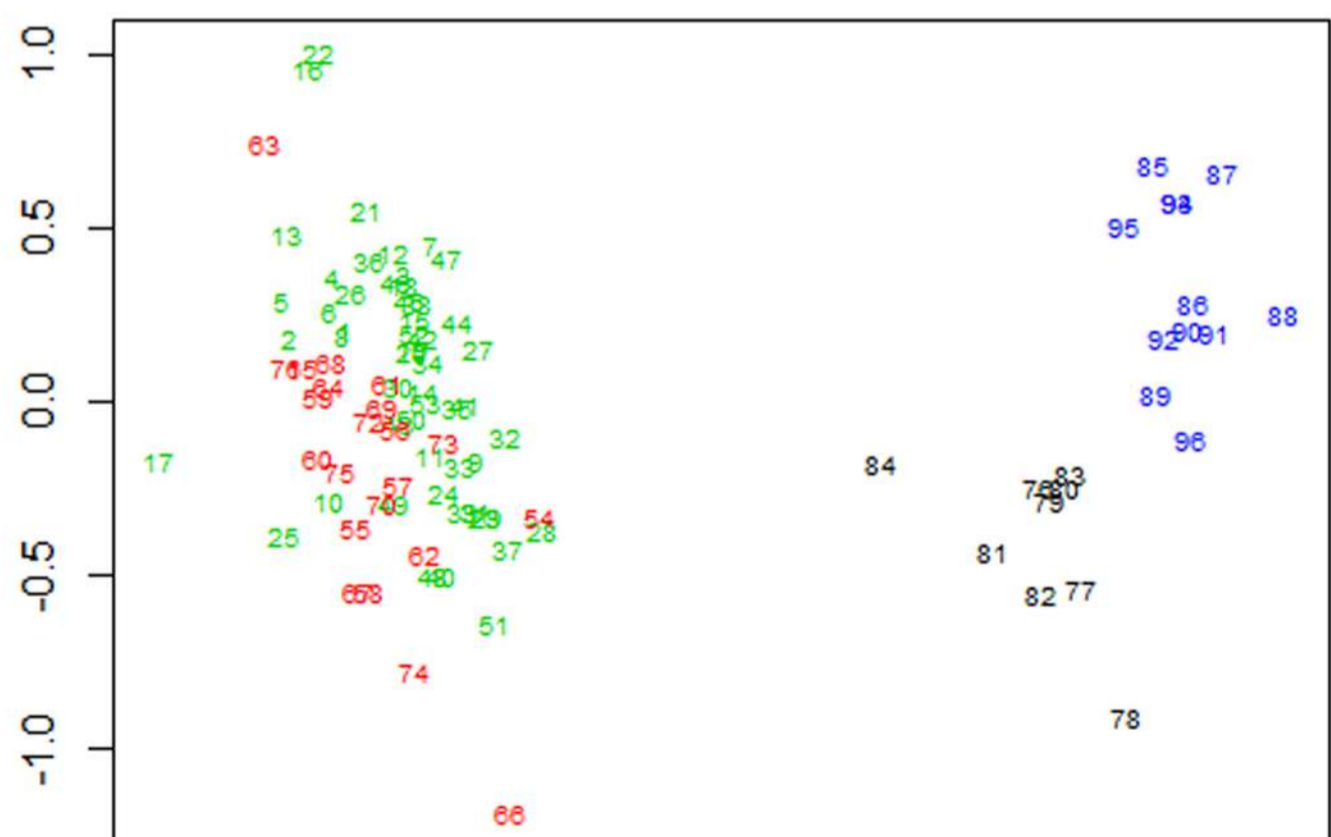
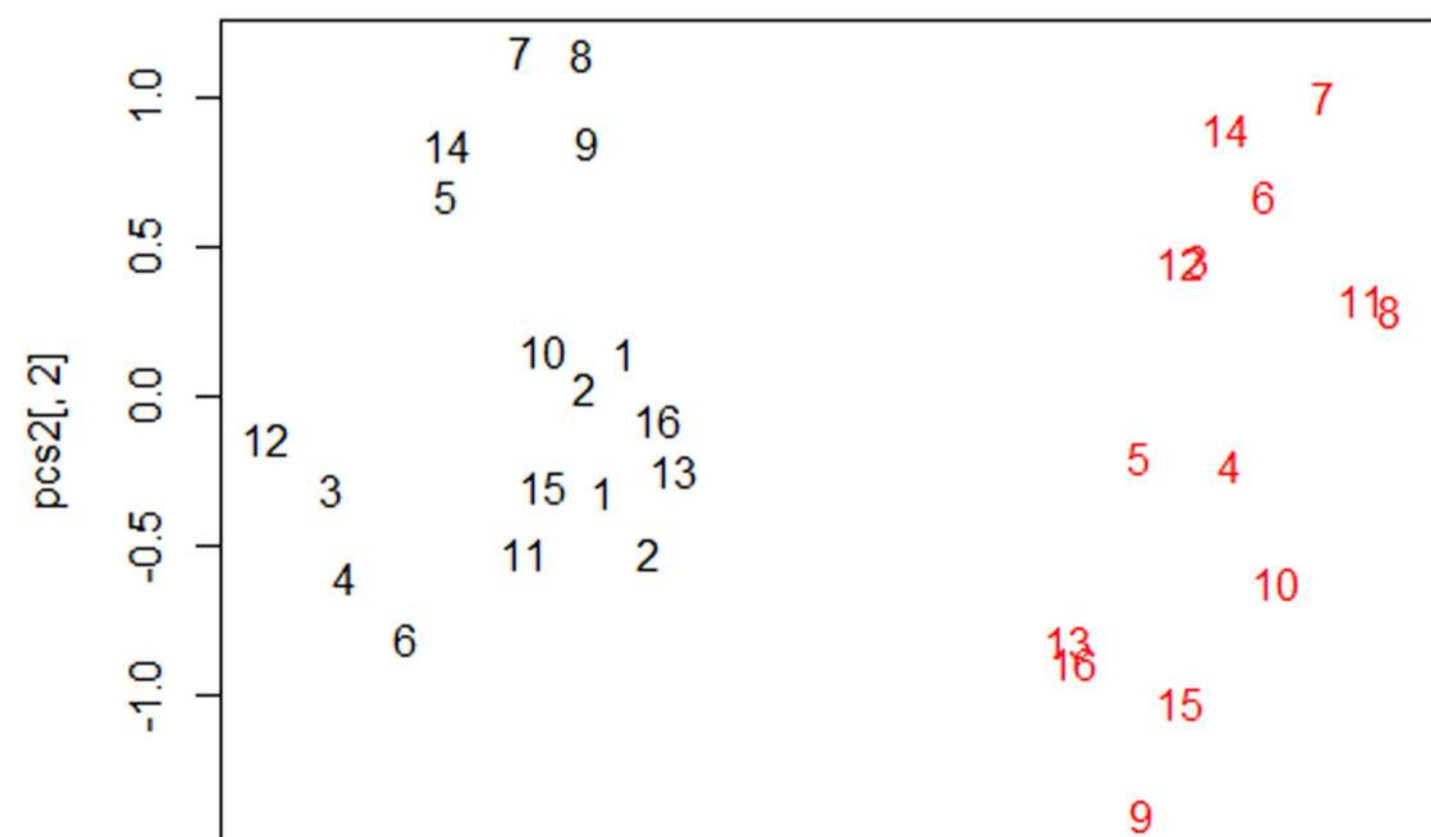
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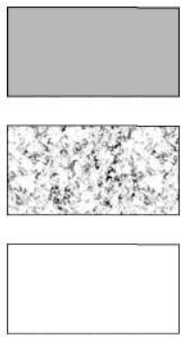


C



E





Europe

North America

Antarctica

H. sp. 2

H. constricta

H. sp. 3

H. angularis

H. sp. 1

H. sp. 5

H. devetteri sp. nov.

H. sp. 6

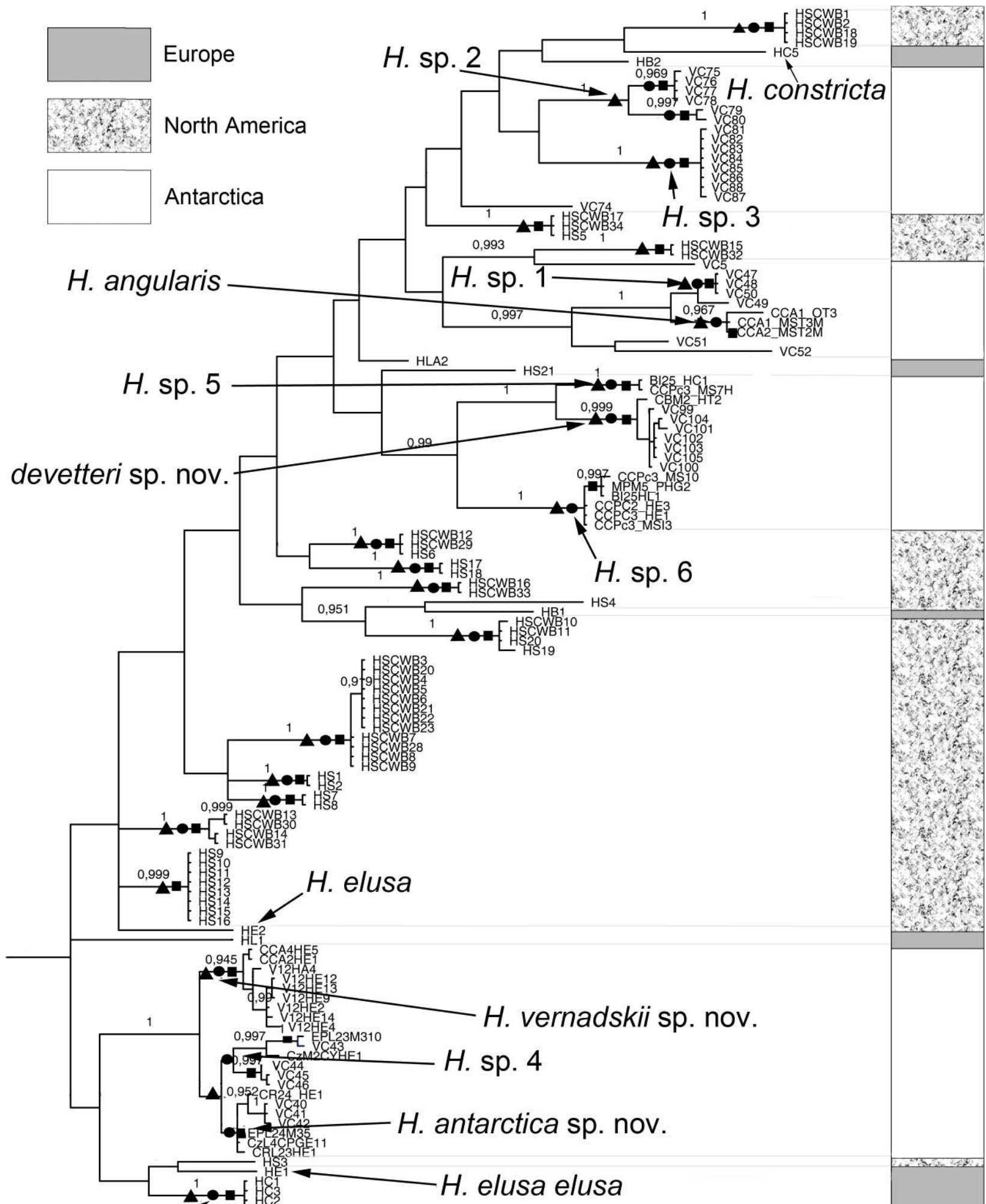
H. elusa

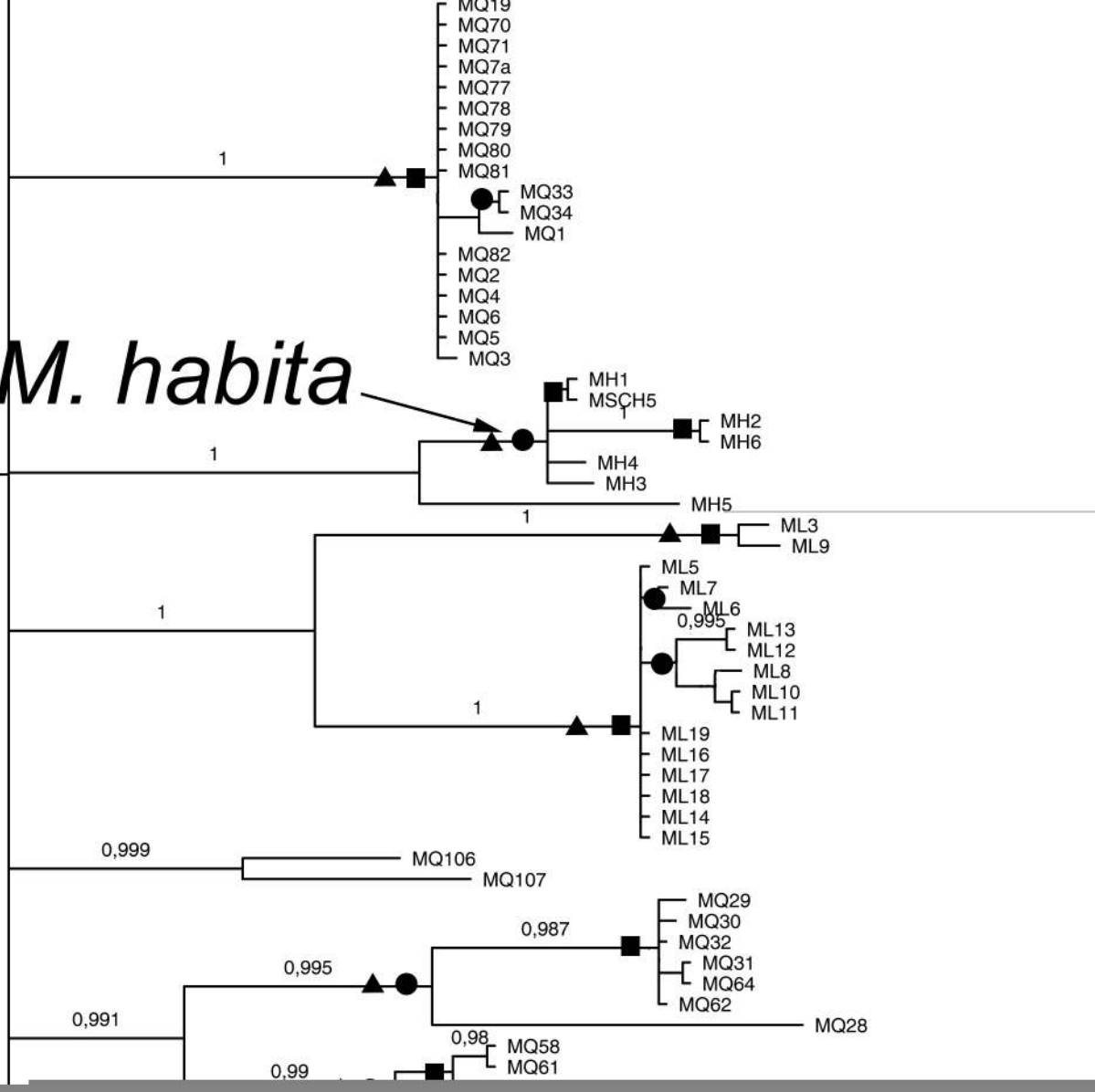
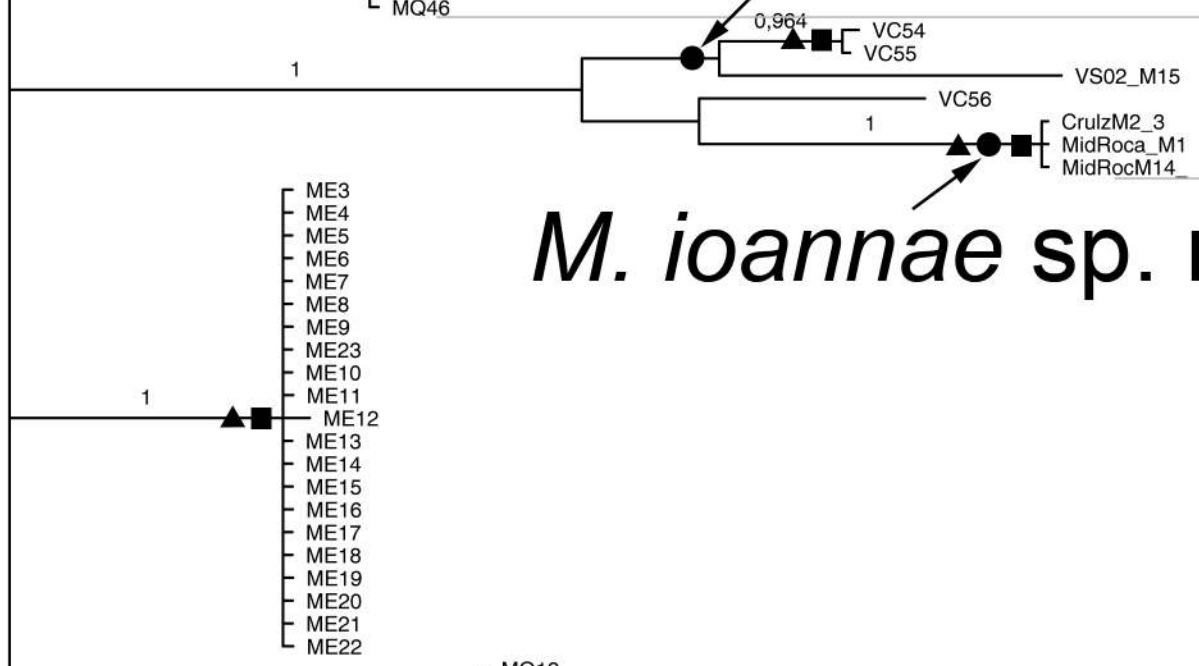
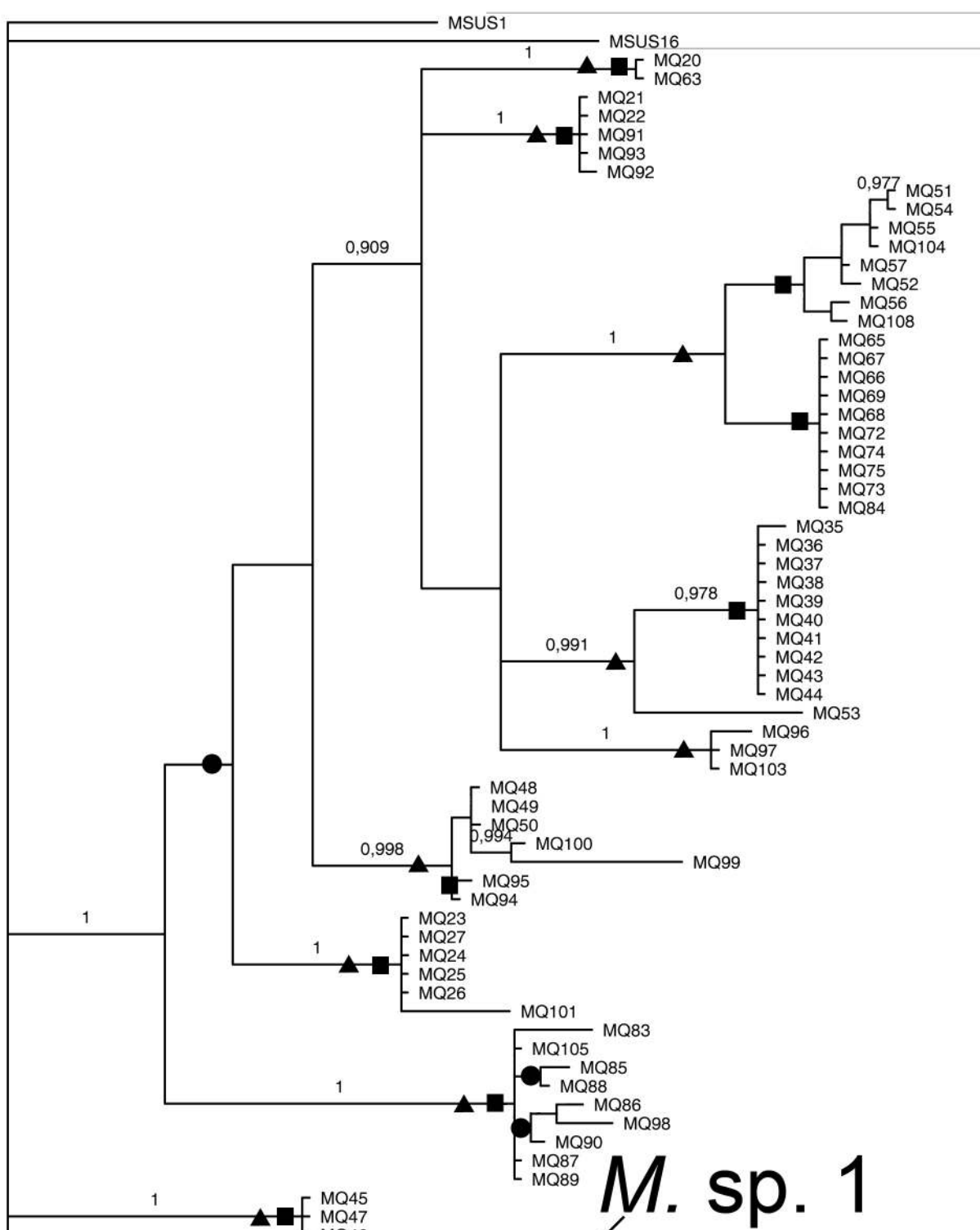
H. vernadskii sp. nov.

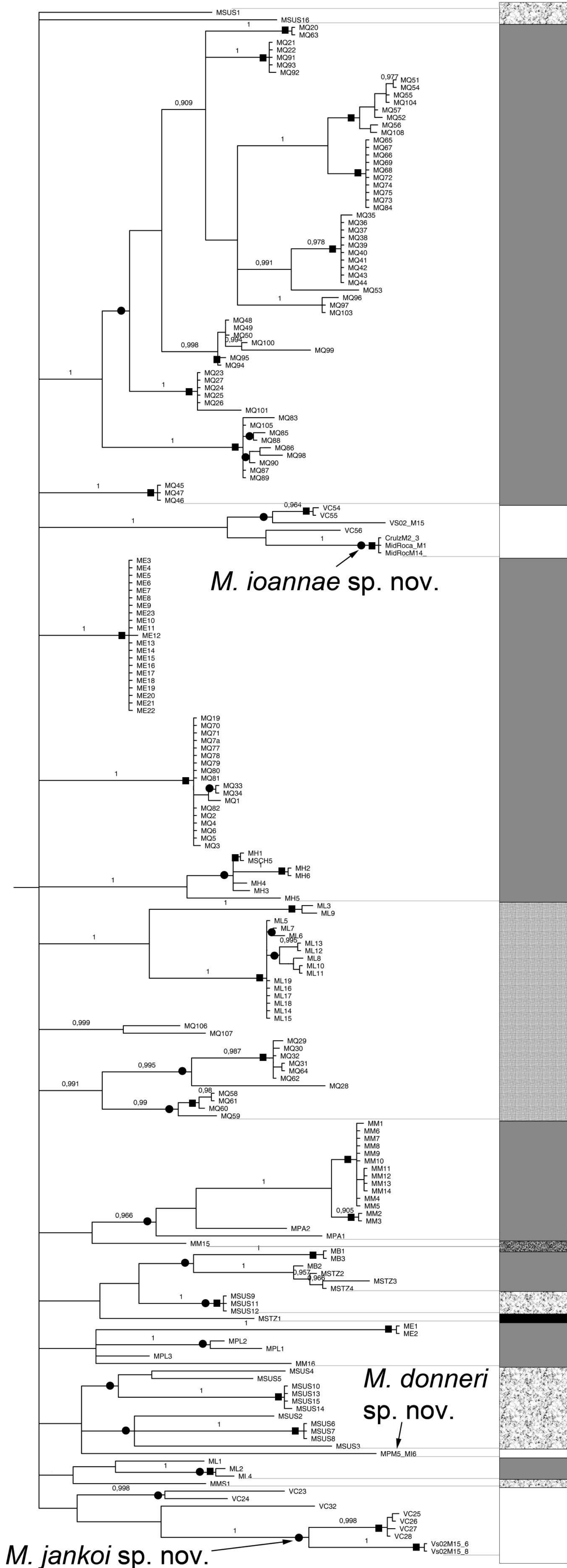
H. sp. 4

H. antarctica sp. nov.

H. elusa elusa







0.04

