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Extreme-tolerance mechanisms in meiofaunal organisms: a case study with tardigrades, rotifers and nematodes

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Abstract:	To persist in extreme environments, some meiofaunal taxa have adopted outstanding resistance strategies. Recent years have seen increased enthusiasm for understanding extreme-resistance mechanisms evolved by tardigrades, nematodes and rotifers, such as the capability to tolerate complete desiccation and freezing by entering a state of reversible suspension of metabolism called anhydrobiosis and cryobiosis, respectively. In contrast, the less common phenomenon of diapause, which includes encystment and cyclomorphosis, is defined by a suspension of growth and development with a reduction in metabolic activity induced by stressful environmental conditions. Because of their unique resistance, tardigrades and rotifers have been proposed as model organisms in the fields of exobiology and space research. They are also increasingly considered in medical research with the hope that their resistance mechanisms could be used to improve the tolerance of human cells to extreme stress. This review will analyse the dormancy strategies in tardigrades, rotifers, and nematodes with emphasis on mechanisms of extreme stress tolerance to identify convergent and unique strategies occurring in these distinct groups. We also examine the ecological and evolutionary consequences of extreme-tolerance by summarizing recent advances in this field.
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1 **Extreme-tolerance mechanisms in meiofaunal organisms: a case study with tardigrades,**
2 **rotifers, and nematodes**

3

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25 **Abstract**

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27 strategies. Recent years have seen increased enthusiasm for understanding extreme-resistance
28 mechanisms evolved by tardigrades, nematodes and rotifers, such as the capability to tolerate
29 complete desiccation and freezing by entering a state of reversible suspension of metabolism called
30 anhydrobiosis and cryobiosis, respectively. In contrast, the less common phenomenon of diapause,
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37 rotifers, and nematodes with emphasis on mechanisms of extreme stress tolerance to identify
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39 and evolutionary consequences of extreme-tolerance by summarizing recent advances in this field.

40

41 **Key words:** anhydrobiosis; cryptobiosis, desiccation, diapause, dormancy, encystment

42

43

44 **Introduction**

45 Tardigrades, rotifers and nematodes are considered permanent and essential members of freshwater
46 and terrestrial meiofaunal communities that can undergo dormancy during their life stages
47 (Bertolani et al., 2019; Guidetti et al., 2018; Hengherr & Schill, 2018; Rundle et al., 2002; Schill
48 & Hengherr, 2018).

49 Tardigrades, commonly called “water bears”, are micrometazoans categorized into two main
50 classes (Eutardigrada and Heterotardigrada) with 1298 species described from marine, freshwater
51 and terrestrial habitats (Degma et al., 2019). The highest number of species belong to the class
52 eutardigrades and to the family Echiniscidae within the heterotardigrades and has been described
53 from terrestrial habitats, where they are inactive unless surrounded by a film of water. The smallest
54 numbers are true limnic species, but several species are limnoterrestrial and can colonize both
55 terrestrial and freshwater habitats (Nelson et al., 2015). Rotifera, also called “wheel animals”, is a
56 phylum of microscopic metazoans, comprising about 2000 species (Segers, 2007) traditionally
57 divided in three main classes: (1) Bdelloidea live in freshwater and terrestrial ephemeral aquatic
58 environments and only reproduce by apomictic parthenogenesis; (2) Monogononta live in
59 freshwater and marine environments and reproduce by cyclical parthenogenesis; and (3) Seisonida,
60 with only a few exclusively marine species (Ricci, 1987; Wallace and Snell, 1991; Melone et al.,
61 1998; Mark Welch and Meselson, 2000; Ricci and Melone, 2000; Segers, 2007). A fourth class,
62 the exclusively parasitic Acanthocephala, has recently been added, although its exact relationship
63 with the other taxa is still debated (e.g. Sørensen et al., 2005; Sielaff et al., 2016). The majority of
64 nematodes, also called “roundworms”, are small free-living animals inhabiting the thin layer of
65 water surrounding soil particles and in aquatic sediments, although some taxa have become
66 endoparasitic and can reach meters in length (Lee, 2002). Some taxa have evolved the ability to

67 resist desiccation during various stages of their life cycles (Ricci and Pagani, 1997; Womersley,
68 1987; Shannon et al., 2005, Erkut et al., 2011).

69 Tardigrades, rotifers and nematodes are meiofaunal aquatic animals common in lakes, rivers,
70 streams, and ponds, but paradoxically they are able to colonize and persist in desiccation-prone
71 environments, such as freshwater (e.g. temporary ponds, Antarctic lakes, cryoconite holes) and
72 terrestrial (e.g. mosses and lichens) habitats where liquid water is not always available (Nelson et
73 al., 2015, 2018; Rundle et al., 2002). In these habitats, water loss can occur via evaporation or
74 freezing, with diel, seasonal, annual, or longer fluctuations in the duration of the wet phase. Since
75 tardigrades, rotifers and nematodes are incapable of active migration to more suitable habitats,
76 occupancy of these unpredictable habitats requires organisms to be versatile, tolerant, or to possess
77 specific and exceptional resistance and adaptive strategies (Fontaneto, 2019). Accordingly, life in
78 these environments is adapted to a dual existence, flourishing when the habitat contains liquid
79 water, and dormant when liquid water is not available and dormant states are linked to a temporary
80 suspension of active life with reduction or interruption of metabolism and/or arrested development.
81 Dormancy includes any form of resting stage, regardless of the cues required for induction or
82 termination (Hand, 1991; Cáceres, 1997). Tardigrades, rotifers, and nematodes exhibit both forms
83 of dormancy: quiescence (cryptobiosis) and diapause (encystment, cyclomorphosis and resting
84 eggs) (e.g. Crowe and Madin, 1975; Ricci, 1987; Guidetti et al., 2011a).

85 Among the various forms of dormancy, cryptobiosis (“hidden life”, Keilin, 1959) is under
86 exogenous control, being directly induced and maintained by adverse environmental conditions,
87 and it is immediately reversed by the removal of the external stimuli. It originated independently
88 several times in the history of life, as it is present in diverse groups of bacteria, metazoans, fungi
89 and plants (Clegg, 2001). Cryptobiosis includes different strategies such as anhydrobiosis,
90 cryobiosis, anoxybiosis and osmobiosis directly induced by desiccation, sub-zero temperatures,

91 low oxygen pressure and osmotic extremes, respectively (Keilin, 1959; Wright et al., 1992).
92 Cryptobiosis allows tardigrades, rotifers and nematodes to survive periods of desiccation, whereas
93 few freshwater and marine species are known to have this adaptive strategy (Ricci and Pagani,
94 1997; Ricci, 1998; Eyres et al., 2005 Guidetti et al., 2011a, b; Clausen et al., 2014). Conversely,
95 encystment and the production of resting eggs are a state of diapause controlled by both exogenous
96 and endogenous stimuli and is more common in freshwater and marine species. Although
97 tardigrades, rotifers, and nematodes exhibit both forms of dormancy, there are differences among
98 taxa. Tardigrades, as well as insects, can undergo both diapause (encystment and cyclomorphosis)
99 (Guidetti and Møbjerg, 2019) and the production of resting eggs (Hansen and Katholm, 2002;
100 Altiero et al., 2010). In comparison, in rotifers the two main types of dormancy are restricted to
101 two separate taxa. The class Bdelloidea can resist adverse environmental conditions *via* quiescence
102 and directly respond to environmental stimuli at any life stage, from eggs to adults, although with
103 age-dependent degrees of resistance (Örstan, 1995, 1998; Ricci, 1987, 1998), while the other main
104 class, the Monogononta, only engage in diapause *via* the production of resting eggs, which tend to
105 stop at a specific and common developmental stage and are generally very resistant to various
106 environmental stresses, including desiccation (e.g. Balompapueng et al., 1997; Cáceres, 1997;
107 Schröder, 2005; Garcia-Roger et al., 2006, Boschetti et al., 2010, Ziv et al., 2017). Within
108 nematodes, dormancy is more scattered across taxa. For example some genera or species can
109 survive desiccation (e.g. Wharton, 1996; Tyson et al., 2012), while other species only have limited
110 resistance at specific life stages (e.g. Erkut et al., 2011; Erkut and Kurzchalia, 2015).

111 This review analyses the dormancy strategies in tardigrades, rotifers, and nematodes with emphasis
112 on mechanisms of stress tolerance in order to identify convergent strategies occurring in these
113 animal taxa. The review also considers the ecological and evolutionary consequences of extreme-
114 tolerance by summarizing recent advances in this field.

115

116 **Diapause: encystment**

117 In terrestrial and freshwater tardigrades, encystment is an adaptive strategy that involves profound
118 morphological changes that occur during the molting process, resulting in the dormant organism
119 lying within retained cuticular exuvia. During this state, the organism also presents a very low or
120 undetectable metabolism, even if the cyst is not desiccated (Patil et al., 2013; Ziv et al., 2017),
121 highlighting possible physiological similarities between diapause and quiescence. Although
122 encystment is rare in moss-dwelling tardigrades, it has been confirmed in grassland and leaf litter
123 habitats but is more common in freshwater sediments (Guidetti et al., 2006). Encystment has been
124 verified in limnic eutardigrades and a few heterotardigrade and eutardigrade limnoterrestrial
125 species, however the phenomenon may be widespread but relatively unstudied (Guidetti and
126 Møbjerg, 2019; Bertolani et al., 2019). In addition, the marine intertidal heterotardigrade
127 *Echiniscoides sigismundi* Plate, 1888, a cryptic species complex, produces two or three new
128 cuticles during cyst formation (Clausen et al., 2014).

129 In response to the gradual onset of adverse environmental conditions (e.g. temperature, oxygen
130 tension, pH), encystment in tardigrades begins with the ejection of the sclerified parts of the buccal-
131 pharyngeal apparatus (“simplex stage”). Instead of undergoing normal ecdysis, however, one to
132 three new cuticles are serially produced in addition to the retained external (old) cuticle (Fig. 1).
133 The animal’s size is reduced by longitudinal contraction, body movements cease completely,
134 metabolism is significantly reduced, and the mouth and cloaca are closed. Modified claws and
135 buccal-pharyngeal apparatus are synthesized, but non-functional. At this stage, the cyst resembles
136 an onion or a Russian doll (“Matryoshka”) (Guidetti et al., 2006), often with one cuticle becoming
137 hardened and pigmented. Encystment ends as environmental conditions improve, and the

138 tardigrade gradually resynthesizes a normal cuticle, claws, and feeding apparatus and leaves the
139 cyst. Unknown endogenous stimuli may also play a role in the process.

140

141 **Ecology of cysts**

142 Limnic eutardigrades that frequently encyst belong to the genera *Dactylobiotus*, *Pseudobiotus*,
143 *Isohypsibius*, *Hypsibius*, *Thulinus*, and *Bertolanius* (See Table 9.1 in Guidetti and Mobjerg, 2019
144 for a list of encysting tardigrade species reported in the literature). Detailed steps in encystment in
145 *Dactylobiotus* and moss-dwelling/freshwater *Bertolanius* were provided by Guidetti et al. (2006).
146 *Dactylobiotus* has only one type of cyst, which is dark-reddish brown (Szymańska, 1995; Guidetti
147 et al. 2006, 2008), whereas *Bertolanius*, which has both limnic and moss-dwelling species, forms
148 two types of cysts (“white/type 1” in cold periods and “red/type 2” in warm periods) that Westh
149 and Kristensen (1992) correlated with seasonal environmental changes in Greenland. Cyst
150 formation in *Bertolanius* is cyclic and be a part of cyclomorphosis, defined as cyclic and reversible
151 morphological modifications within a single species (Kristensen, 1982; Rebecchi and Bertolani,
152 1994; Hansen and Katholm, 2002). The production of extra cuticles isolates and protects the
153 animals from environmental factors. Since the cysts remain viable for several months, encystment
154 enhances tardigrade survival of freezing in winter and desiccation in summer (since limnic
155 tardigrades often disappear in summer). Although encystment is best studied in limnic species,
156 which do not undergo anhydrobiosis (but a few species can withstand cryobiosis), most of the
157 species that produce cysts can also enter anhydrobiosis (Guidetti et al., 2011a). Since diapause
158 (encystment) and cryptobiosis are dormancy states that can be present in a single species, their
159 evolution was not mutually exclusive. Although we are beginning to understand the molecular
160 mediators involved in cryptobiosis, the molecular mechanisms involved in encystment (see
161 Rozema et al., 2019) are unknown.

162

163 **Extreme resistance strategy: anhydrobiosis**

164 The most widespread and best-known form of extreme-stress resistance evolved by tardigrades,
165 rotifers and nematodes is the capability to tolerate complete desiccation (drying to $< 0.1 \text{ g H}_2\text{O g}^{-1}$
166 dry mass) by entering in a state of reversible suspension of metabolism called anhydrobiosis (“life
167 without water”) without the loss of viability. At the end of dehydration process, tardigrades have
168 lost 97% of their body water (Westh and Ramløv, 1991; Horikawa et al., 2008), and similar values
169 have been shown for the anhydrobiotic nematodes *Ditylenchus dipsaci* (Kuhn, 1857), *Aphelenchus*
170 *avenae* Bastian, 1865 and *Panagrolaimus superbus* (Fuchs, 1930) (Crowe and Madin, 1975;
171 Wharton, 1996; Banton and Tunnacliffe, 2012).

172 Anhydrobiosis indicates a fundamental concept about the nature of living systems since an
173 anhydrobiotic organism lacks all dynamic features of living organisms due to the absence of
174 detectable metabolism. In that sense it is not alive, but it is not dead because rehydration produces
175 a living organism and a kind of resuscitation routinely occurs (Clegg, 2001; Tunnacliffe and
176 Lapinski, 2003). Consequently, anhydrobiotic organisms have two distinct living physiological
177 states: active and anhydrobiotic.

178 Despite its clear adaptive potentiality, anhydrobiosis can be found only in a restricted number of
179 metazoans whose sizes generally do not exceed 1 mm, with the exception of a few taxa that can
180 reach 5-7 mm in length, such as the larvae of the African midge *Polypedylum vanderplanki* Hinton
181 1951 (Watanabe et al., 2004). These apparent morphological and ecological characteristics could
182 be linked to limiting factors required for tolerating physical and physiological constraints imposed
183 by complete dehydration (Alpert, 2005). In animals, desiccation tolerance occurs either the whole
184 animal at any stage of their life cycle, from the egg to the adult stage (tardigrades, bdelloid rotifers
185 and nematodes), in which case the animals are defined as holo-anhydrobiotic (Jönsson, 2005;

186 Rebecchi et al., 2007), or at a specific life stage, usually egg/embryo/larval stage (shrimps, the
187 midge *P. vanderplanki*, monogonont rotifers, some nematodes).

188 As described above, anhydrobiosis allows tardigrades, rotifers and nematodes to colonise and
189 persist in various otherwise unavailable environments. A high number of species colonise habitats
190 subjected to periodic desiccation (e.g. lichens, mosses, and ephemeral lakes and ponds) that are
191 prohibitive for most other animals. In these habitats, they perform all activities of routine life only
192 when there is at least a small layer of water around the body of the animals. For example, mosses
193 and lichens provide habitats featuring a myriad of small pockets of water; as their surroundings
194 lose water through evaporation, animals lose water with them. Consequently, their life cycle
195 consists of active periods for growth and reproduction, interrupted by periods of metabolic
196 inactivity (Jönsson, 2005; Glime, 2017). When rehydrated by dew, rain or melting snow, they can
197 return to their active state in a few minutes to a few hours. Therefore, during their life, holo-
198 anhydrobiotic animals can enter anhydrobiosis several times (e.g. Ricci, 1987; Womersley, 1987).
199 An experimental study evidenced that the moss-dwelling eutardigrade *Richtersius coronifer*
200 (Richters, 1903) may survive up to 6 repeated desiccations, with a declining survival rate with an
201 increasing number of desiccation events (Czernekova and Jönsson, 2016). Interestingly, repeated
202 desiccation seems also to improve the long-term survival of rotifer populations. Populations that
203 are regularly subjected to desiccation grow faster than permanently hydrated corresponding
204 cohorts, suggesting that diapause is not only a strategy to survive harsh environmental conditions,
205 but it also has ecological advantages to the organisms that managed to evolve this strategy (Ricci
206 et al., 2007; Sommer et al., 2019).

207 The time for recovery to active life after a period of anhydrobiosis is directly related to the
208 environmental condition during the desiccation phase (e.g. humidity rate during the desiccation
209 process) in which higher stressors lead to longer recovery time, and to the time spent in

210 anhydrobiosis (Rebecchi et al., 2009a). The recovery time is probably function of the metabolic
211 activities linked to the repair of damages caused by desiccation and/or to the restoration of
212 metabolic pathways (see Mattimore and Battista, 1996).

213 Among anhydrobiotic tardigrade and rotifers studied, desiccation tolerance varied from zero to
214 high tolerance (e.g. Ricci, 1987; Wright, 1989a; Bertolani et al., 2004; Rebecchi et al., 2006). These
215 gradients are correlated with the abiotic factors (e.g. humidity) of the substrate inhabited since
216 species living in constantly wet or submerged mosses usually show lower anhydrobiotic
217 performance than those living in mosses growing on trees and rocks (Guidetti et al., 2011b; Eyres
218 et al., 2015). In addition, anhydrobiotic capability is similar among species belonging to distant
219 evolutionary lines, but they can be very different among closely related species. However, species
220 with similar ecological requirements share a close similarity in anhydrobiotic performances
221 (Wright, 1991, 2001; Guidetti et al., 2011b; Ricci, 1998, 2001; Ricci and Caprioli, 2005; Fontaneto
222 et al., 2004; Fontaneto and Ambrosini, 2010; Eyres et al., 2015).

223 Therefore in both rotifers and tardigrades, we hypothesize that anhydrobiosis is more likely linked
224 to local adaptations to habitats than to phylogenetic relationships suggesting that anhydrobiotic
225 capabilities have been evolved once and secondarily lost in some lineages.

226 Some species of nematodes within the genus *Panagrolaimus* Fuchs, 1930 can survive immediate
227 desiccation (e.g. Ricci and Pagani, 1997) and are referred to as fast-desiccation strategists, while
228 others (e.g. *A. avenae*) require a period of slow-drying (pre-conditioning) and are referred to as
229 slow desiccation strategists (e.g. Womersley, 1987; Shannon et al., 2005). Similar patterns were
230 detected in tardigrade and rotifer species when experimentally desiccated under laboratory
231 conditions (e.g. Ricci, 1987, 2001; Wright 1989a; Eyres et al., 2015; Hashimoto et al., 2016;
232 Boothby et al., 2017). Full anhydrobiotic nematodes can undergo desiccation at any stage of their
233 life cycles, but recent studies have suggested that some species, traditionally considered intolerant

234 to desiccation, can actually survive desiccation at least in some stages of their life cycle (e.g. the
235 dauer larvae of the model species *Caenorhabditis elegans* (Maupas, 1900)) (Erkut et al., 2011). As
236 in tardigrades and rotifers, the anhydrobiotic abilities of different taxa of nematodes seem do not
237 appear to be related to their phylogeny, suggesting that the evolutionary processes have affected
238 the loss or maintenance of this remarkable ability. Although traditionally less studied, the recent
239 characterisation of some of the molecular strategies of diapause in nematodes, and especially in the
240 well-known and well-characterised model organism *C. elegans*, allows a better understanding of
241 how diapause is induced, maintained, and what its effects are, as well as common mechanisms to
242 different organisms (e.g. Fielenbach and Antebi, 2008; Hand et al., 2016).

243 In the desiccated state, holo-anhydrobiotic animals are biostable for decades (e.g. tardigrades 20
244 years; Guidetti and Jönsson, 2002; Bertolani et al., 2004; Rebecchi et al., 2006; Jørgensen et al.,
245 2007) even though recently the consistent long-term survival of at least some taxa under desiccation
246 has been debated (Jönsson and Bertolani, 2001; Fontaneto et al., 2012a). For example, a
247 comparative study of the survival rate of different taxa and the statistical model developed from it
248 suggested that recovery of bdelloid rotifers, tardigrades and nematodes found in lichens within
249 collections in museums decreases to almost zero after desiccation periods of up to 10 years; this is
250 significantly longer than the life span of single individuals in the active state, but is not as long as
251 anecdotally suggested by other studies, and not as long as in other taxa like resting eggs of
252 monogonont rotifers (Cáceres, 1997; Fontaneto et al., 2012a). These data confirm that these
253 organisms do survive long periods of desiccation but that the rate and general conditions of
254 desiccation, as well as the substrate and the storage conditions during diapause, influence survival
255 in a significant way (e.g. Ricci and Caprioli, 2001; Fontaneto et al., 2012a).

256 Other than its effect on longevity, anhydrobiosis can have an impact on ageing in meiofauna as
257 illustrated by the “Sleeping Beauty” and “Picture of Dorian Grey” models derived from

258 experimental data on a few species of holo-anhydrobiotic organisms (for a review, see Kaczmarek
259 et al., 2019). The first model predicts that anhydrobiotic organisms do not age during anhydrobiosis
260 in at least some tardigrade and bdelloid rotifer species (Ricci and Covino, 2005; Hengherr et al.,
261 2008a, b). The latter model predicts that anhydrobiotic organisms age, at least in the initial stages
262 of the anhydrobiosis process, as in some species of nematodes (Ricci and Pagani, 1997).
263 Nevertheless, a comprehensive comparative analysis that considers all taxa and strategies is still
264 lacking.

265 Numerous studies have focused on molecular changes during aging in tardigrades, rotifers and
266 nematodes, especially from the molecular approach, and the potential “rejuvenation” of stressed
267 animals, but the full picture is very complex and still poorly understood. Early studies highlighted
268 general changes in protein patterns with age (Carmona et al., 1989), and recent advances have
269 started uncovering specific changes in regulatory molecules (e.g. Snell et al., 2014), protein
270 modifications like carbonylation (Krisko and Radman, 2019), and improved physiological
271 characteristics like fecundity (Ricci and Covino, 2005; Ricci and Perletti, 2006). Based on these
272 and other studies, rotifers can be added to the list of useful model organisms which can be used to
273 study aging (Snell et al., 2015), although the exact links between molecular changes and aging are
274 still not fully characterised. Even more obscure at the moment are the precise links between the
275 ability of some types of dormancy to stop or reverse aging. For example, both desiccation and
276 starvation seem to stop or reverse aging in bdelloid rotifers, allowing dormant bdelloids to “wake
277 up” with similar or higher fitness than animals in the pre-stressed condition (Ricci and Covino,
278 2005; Ricci and Perletti, 2006; Sommer et al., 2019). Some recent advances suggest that some of
279 the mechanisms and molecules involved in the organism’s protection during desiccation, like
280 antioxidants or LEA proteins, can also prevent at least some aspects correlated with aging (e.g.
281 Kaneko et al. 2005; Snare et al., 2013). Aging is generally better characterised in nematodes,

282 although the majority of studies are limited to model species like *C. elegans* (e.g. Schaffitzel and
283 Hertweck, 2006; Hughes et al., 2007; Mack et al., 2018) and therefore lack the more direct link
284 between aging and dormancy in stress-resistant animals from natural habitats. Interestingly, where
285 data are available, they suggest that the rejuvenation effect of desiccation is not present in at least
286 some anhydrobiotic nematodes of the genus *Panagrolaimus* (Ricci and Pagani, 1997), making the
287 understating of the relationship between desiccation resistance and aging even more fascinating
288 and interesting.

289 Even though dehydration can have a major effect on survival, aging and longevity, the
290 anhydrobiotic process *per se* can induce molecular damages that accumulate with time, reducing
291 the viability of desiccated animals (França et al., 2007; Tyson et al., 2007; Neumann et al., 2009;
292 Rebecchi et al., 2009a; Marotta et al., 2010; Hespeels et al., 2014). The amount of these damages
293 is directly impacted by high temperature, high humidity level and high oxygen partial pressure. In
294 tardigrades, the time required to recover active life after a period of desiccation is affected by these
295 abiotic conditions and can be related to the metabolic activities necessary to repair molecular
296 damages and to catabolise damaged molecules (Rebecchi et al., 2009a; Guidetti et al., 2011a).
297 Different strategies and molecules seem to be involved in the reduction and/or repair of molecular
298 damage (see below).

299 Even more striking, in the dry state, anhydrobiotic organisms show extraordinary resistance to
300 physical and chemical extremes (very low sub-zero temperature, high pressure, radiation, extreme
301 pH, toxic chemicals, lack of geomagnetic field) that may far exceed the tolerance ranges of active
302 organisms (Wharton et al., 2003; Jönsson et al., 2005, 2013; Watanabe et al., 2006; Rebecchi et al.,
303 2007; Gladyshev and Meselson, 2008; Rebecchi et al., 2009b; Altiero et al., 2011; Guidetti et al.,
304 2011a; Krisko et al., 2012; Rebecchi, 2013; Hashimoto et al., 2016; Erdmann et al., 2017; Jönsson
305 and Wojcik, 2017; Giovannini et al., 2018).

306 In tardigrades, a strong correlation between the capability to withstand desiccation and the
307 capability to withstand sub-zero temperatures (-20°C, -80°C) was detected, and species that were
308 not able to enter anhydrobiosis showed low or no capability to withstand sub-zero temperatures
309 (Guidetti et al., 2011a, b). This direct relationship could be related to the fact that during both
310 desiccation and freezing stresses, tardigrades are under the same selective pressure induced by a
311 wide variation in body fluid osmolality and in cell volume (Sømme, 1996; Guidetti et al., 2011b).
312 Nevertheless, the freeze resistance of anhydrobiotic tardigrades should be distinguished from
313 cryobiosis, which is the ability of active hydrated animals in contact with water to freeze and
314 survive after thawing (Guidetti et al., 2011b).

315 The aggregate of all these characteristics, especially radiation tolerance, has led to the
316 characterization of tardigrades as the “toughest animals on the Earth” (Copley, 1999) and to make
317 them an emerging model for space biology (Horikawa et al., 2008; Jönsson, 2007; Erdmann and
318 Kaczmarek, 2017), more recently joined by bdelloid rotifers. Tardigrades and rotifers have been
319 exposed to space stressors in Low Earth Orbit several times, on board of the International Space
320 Station and FOTON (Ricci and Boschetti, 2003; Ricci et al., 2005; Leandro et al., 2007; Selch et
321 al., 2008; Jönsson et al., 2008; Rebecchi et al., 2009b, 2011; Persson et al., 2011; Guidetti et al.,
322 2012; Vukich et al., 2012).

323

324 **Morphological, physiological and molecular adaptations enabling anhydrobiosis**

325 The evolution of a series of behavioural, morphological, physiological and molecular/biochemical
326 adaptations provided anhydrobiotic organisms with mechanisms to withstand the deleterious
327 effects caused by the drastic loss of water. The majority of holo-anhydrobiont organisms cannot
328 survive a desiccation rate that is too rapid (as shown in a few hours in laboratory experiments, even
329 though the rate is species-dependent (Wright, 1989a, b, c; Wright et al., 1992; Jönsson and Järemo,

330 2003; Banton and Tunnacliffe, 2012; Boothby et al., 2017), so they have evolved different
331 strategies to slow down the rate of water evaporation.

332 To reduce the rate, the tardigrade shrivels into a barrel-shaped structure (“tun”), about one-third of
333 its original size, by contracting the body anterior-posteriorly and withdrawing the legs and head
334 (Figs. 2-3). Tun formation produces a new spatial organization of some internal organs (such as
335 the pharyngeal bulb), and epidermal cells, storage cells, ovarian cells, and digestive system cells
336 undergo shrinkage, containing electron dense cytoplasm (Czernekova et al., 2016). Lipids and
337 polysaccharides dominate in the reserve material of the storage cells, whereas the amount of protein
338 is small (Czernekova et al., 2016). The tun minimizes the permeability and evaporative surface of
339 the organism by removing the high permeability areas of the cuticle from direct contact with the
340 air, resulting in a slow rate of desiccation (Wright, 1988a, b, 1989a, b, c, 2001). Differences in the
341 reduction of cuticle permeability detected among tardigrade species are related to the level of
342 desiccation tolerance of each species and to the morphology of the cuticle in eutardigrades and
343 heterotardigrades (Wright, 1989a, b). The permeability slump of the cuticle permits animals to lose
344 water slowly, allowing animals to produce bioprotectants. Somewhat similarly to tardigrades,
345 bdelloid rotifers contract their body into a compact shape by withdrawing their cephalic and caudal
346 extremities into the trunk, facilitated by muscle contractions and by a coordinated morphological
347 arrangement of internal structures (Ricci, 2001; Ricci et al., 2003; Marotta et al., 2010; Fig. 4.) A
348 decrease in permeability (the permeability slump) during the early stages of desiccation was
349 detected in the anhydrobiotic plant-parasitic nematode *D. dipsaci* during which the surface of the
350 animal body was coated with an extracuticular layer of lipid (triglyceride) that produced a slow
351 rate of water loss necessary for its survival (Wharton et al., 2008). Nematodes tend to coil their
352 body (Crowe, 1971) and certain nematodes are also reported to congregate into masses of
353 “nematode wool”, with better survival of specimens in the centre of the mass (Ellenby, 1968). The

354 aggregation effect has also been experimentally produced in tardigrades (Ivarsson and Jönsson,
355 2004), but not yet verified in nature.

356 As water evaporates and dry conditions set in, holo-anhydrobiotic organisms start generating a
357 variety of protective agents, collectively termed bioprotectants, which they accumulate in and
358 around the cells of their body. It was initially thought that non-reducing disaccharides, like
359 trehalose, were solely responsible for preventing damage (e.g. Crowe et al., 1984, 1992), but more
360 recent studies point to a complex picture of molecular adaptations. These bioprotectants molecules
361 include: sugars, mostly disaccharides such as trehalose; a unique repertoire of proteins generally
362 lacking persistent tertiary structure classified as intrinsically disordered proteins (IDPs) or proteins
363 with intrinsically disordered regions (IDRs) and represented by Late Embryogenesis Abundant
364 proteins (LEAp), Heat Shock proteins (HSPs), cytoplasmic abundant heat soluble (CAHS)
365 proteins, secretory abundant heat soluble (SAHS) proteins, and mitochondrial abundant heat
366 soluble (MAHS) proteins; antioxidants, and molecules involved in protection from or repair of
367 DNA damage (e.g. Lapinski and Tunnacliffe, 2003; Schill et al., 2004; Altiero et al., 2007; Jönsson
368 and Schill, 2007; Pouchkina-Stantcheva et al., 2007; Förster et al., 2009, 2011; Schokraie et al.,
369 2010; Boschetti et al., 2011; Yamaguchi et al., 2012; Boschetti et al., 2013; Rebecchi 2013; Wang
370 et al., 2014; Tanaka et al., 2015; Hashimoto et al., 2016; Boothby et al., 2017; Schill and Hengherr,
371 2018). A recent study showed that one of the most stress-tolerant tardigrade species (*Ramazzottius*
372 *varieornatus* Bertolani and Kinchin, 1993) has a tardigrade-unique DNA-associating protein,
373 termed Dsup, which is able to suppress the incidence of DNA breaks caused by radiation
374 (Hashimoto et al., 2016). Accumulation of these xeroprotectants is generally slow and gradual,
375 taking place in parallel with the drying process, although a few taxa, like the nematode *P. superbus*,
376 seem to express a full repertoire of protective molecules and have therefore been defined as fast-
377 desiccation strategist (Shannon et al., 2005; Banton and Tunnacliffe, 2012). Even though many

378 organic compounds have been identified in tardigrades, rotifers, and nematodes, the biochemical
379 and molecular mechanisms involved in complete desiccation tolerance are currently little known
380 and constitute an intriguing challenge for biologists. For instance, it is well known that in some
381 species of tardigrades the synthesis of the disaccharide trehalose counteracts the loss of water, as
382 well as other environmental extremes (Westh and Ramløv 1991; Hengherr et al., 2008b, Jönsson
383 and Persson, 2010; Welnicz et al., 2011; Cesari et al., 2012; Schill and Hengherr, 2018). In any
384 case, the absolute trehalose levels detected in tardigrades are much lower than those reported for
385 other anhydrobiotic organisms. This sugar has a double role in desiccation tolerant organisms. As
386 the trehalose replaces water, it protects biomolecules and the integrity of membranes during
387 dehydration, and participates in the formation of a glassy matrix that reduces the rates of chemical
388 reactions and inhibits free radical production (Crowe et al., 1984; Teramoto et al., 2008). The
389 CAHS, SAHS and MAHS proteins have been detected so far only in eutardigrades (Tanaka et al.,
390 2015; Boothby et al., 2017). However, the distribution of the encoding genes of these proteins is
391 scattered among tardigrades, suggesting species- or at least taxon-specific adaptations (Yoshida et
392 al., 2017; Kamilari et al., 2019). The CAHS and SAHS proteins probably form a molecular shield
393 inside and outside cells, respectively, whereas MAHS proteins are defined as potent specific
394 mitochondrial protectants (Boothby et al., 2017). The heterologous expression of some CAHS
395 proteins in both prokaryotic and eukaryotic cells allows an increase in their tolerance to desiccation,
396 and purified CAHS proteins protect desiccation-sensitive proteins *in vitro* (Boothby et al., 2017).
397 The very low or null metabolic activity of anhydrobionts limits the production/accumulation of
398 products of metabolism such as Reactive Oxygen Species (ROS). Even though the origin of ROS
399 in anhydrobiosis is not yet well known, their production can occur both during the dehydration, in
400 a permanent desiccated state as well as during rehydration, so an efficient antioxidant mechanism
401 is necessary (França et al., 2007; Cornette et al., 2010; Rebecchi, 2013). For example, in desiccated

402 specimens of the eutardigrade *Paramacrobiotus spatialis* Guidetti, Cesari, Bertolani, Altiero and
403 Rebecchi, 2019, glutathione peroxidase was the most abundant antioxidant enzyme in hydrated
404 animals, followed by the enzyme superoxide dismutase and glutathione content (Rizzo et al., 2010).
405 With regard to the repair of DNA damages, desiccation enhanced the expression of DNA-repair
406 proteins in tardigrades (Wang et al., 2014; Kamilari et al., 2019).

407 Rotifers possess similar strategies, but there are also marked differences. Neither trehalose nor the
408 metazoan genes for its synthesis have been found in bdelloid rotifers (Lapinski and Tunnacliffe,
409 2003), although trehalose has been found in monogonont rotifers (Caprioli et al., 2004), suggesting
410 that non-reducing disaccharides are not necessary for successful recovery from desiccation.
411 Instead, other molecules are now thought to be essential to protect molecules, cells and tissues and
412 to repair any damage caused by anhydrobiosis. It is becoming clear that no single class of
413 protectant/repair molecules is sufficient, but successful desiccation depends on the co-ordinated
414 action of all of them. These molecules, which are usually upregulated upon desiccation, include
415 different types of LEA proteins which perform different and often still uncharacterised functions,
416 other (non-LEA) protein families, often at least partially unstructured (IDPs and proteins
417 containing IDRs) and with still uncharacterised functions, other types of hydrophilins or
418 chaperones, different types antioxidants, molecules involved in DNA repair as well as, probably,
419 other still unknown molecules and mechanisms (e.g. Browne et al., 2002; Browne et al., 2004;
420 Goyal et al, 2005; Pouchkina-Stantcheva et al., 2007; Denekamp et al., 2010, 2011; Boschetti et
421 al., 2011, 2012; Hanson et al., 2013; Hespels et al., 2014).

422 All these molecules, some of which are taxon-specific while others are common to all analysed
423 taxa (e.g. Denekamp et al., 2010; Mali et al., 2010; Boschetti et al., 2011, 2012; Hanson et al.,
424 2013; Hashimoto et al., 2016; Boothby et al., 2017; Hashimoto and Kunieda, 2017; Kamilari et al.,

425 2019; Kamilari et al, 2019), seem to be necessary for an integrated and effective response to
426 anhydrobiosis.

427 Interestingly, the majority of the previously mentioned studies were based on the analyses of one
428 or a relatively small subset of genes, but recent technological advances have allowed analyses of
429 whole genomes and transcriptomes and are uncovering an even more fascinating story, suggesting
430 that some animals, and bdelloid rotifers in particular, have been acquiring genes, which code for
431 protective/repair molecules, from organisms that are not direct ancestors and can even belong to
432 different taxa, in a process known as Horizontal Gene Transfer (HGT, also called lateral gene
433 transfer, LGT). Horizontal gene transfer was previously known only in bacterial and archaeal
434 organisms and was thought to be absent in eukaryotic organisms, but recent studies suggest that it
435 is more widespread than previously thought (e.g. Dunning Hotopp, 2011; Boto, 2014, Drezen et
436 al., 2017) and that these “foreign” genes can indeed contribute to the resistance to desiccation of
437 some organisms, especially bdelloid rotifers. Initial suggestions that bdelloid rotifers possess a very
438 high percentage of genes acquired *via* HGT (Gladyshev et al., 2008; Boschetti et al., 2012) and
439 “domesticated” (Barbosa et al., 2016) have now been confirmed and expanded (Flot et al., 2003;
440 Eyres et al., 2015; Hespeels et al., 2015; Nowell et al., 2018). This unusual characteristic is made
441 even more interesting by the recent understanding of the role that these foreign genes play in stress
442 resistance: many foreign genes are over-expressed during desiccation or rehydration and might
443 therefore at least partially responsible for their successful anhydrobiotic capabilities (Boschetti et
444 al., 2011, 2012; Eyres et al., 2015), although the precise link between desiccation resistance and
445 levels of HGT is still unclear (e.g. Eyres et al., 2015; Nowell et al., 2018). Indeed, recent studies
446 have found genes involved in trehalose synthesis in bdelloids, but they seem to have been
447 originated by HGT (Hespeels et al., 2015), while other foreign genes can add various biochemical
448 capabilities, some of which might improve the desiccation abilities of bdelloids (Boschetti et al.,

449 2012; Szydlowski et al., 2015). This unusual high level of foreign genes seems to be a characteristic
450 only of bdelloid rotifers: other taxa have been analysed and, although a few foreign genes are
451 present (e.g. Boothby et al., 2015; Bemm et al., 2016; Koutsovoulos et al., 2016; Nowell et al.,
452 2018), they are not so abundant, and the details of their contribution to successful desiccation is
453 still being characterised (e.g. Yoshida et al., 2017; Nowell et al., 2018; Kamilari et al., 2019).

454

455 **Ecological and evolutionary consequences of extreme tolerance of meiofaunal organisms**

456 The evolution of anhydrobiosis is the result of trade-offs between the selective advantages of this
457 adaptive strategy, the energetic costs, and the physical and physiological constraints related to the
458 process (Jönsson, 2005; Guidetti et al., 2011a). Energy is probably necessary to produce and
459 accumulate bioprotectants during the initial phase of anhydrobiosis and to catabolize them during
460 the exit phase (rehydration). There are few data on anhydrobiotic energetic costs, but a substantial
461 energetic cost of anhydrobiosis was shown in the tardigrade *Richtersius coronifer* (Jönsson and
462 Rebecchi, 2002) and in some species of nematodes (Madin and Crowe, 1975; Demeure et al.,
463 1978). Little is known about rotifers, but the presence of lipid droplets (Wurdak et al., 1978) and
464 the differential expression of some genes potentially involved in lipid metabolism or protection
465 (Denekamp et al., 2009) in monogonont resting eggs and in desiccated bdelloids (Marotta et al.,
466 2010) suggest that costs are present in these taxa as well.

467 The anhydrobiotic process requires energy that is withdrawn from other physiological functions
468 such as growth and reproduction. This should have strong effects on the life histories of holo-
469 anhydrobiotic organisms. Even though there is no direct evidence for a trade-off between
470 anhydrobiosis and fitness, the few ecological studies on this topic are consistent with the hypothesis
471 that fitness of desiccation tolerant organisms is lower (Jönsson, 2005; Alpert, 2006; Guidetti et al.,
472 2007). In tardigrades, both positive and negative relationships between body size (as an indication

473 of age) and desiccation performance have been demonstrated at the intraspecific and interspecific
474 levels. In species living in the same moss and with high anhydrobiotic performance, desiccation
475 survival increases in *R. coronifer* with an increase of the body size, whereas it decreases in
476 *Ramazzottius oberhaeuseri* (Doyère, 1840) (Jönsson et al., 2001; Jönsson and Rebecchi, 2002).
477 These contrasting models could be due to genetic differences and/or contingent factors, such as
478 nutritional state, level of molecular protectants and some life history traits, including age,
479 reproductive stage, and phenotypic plasticity. Lastly, differences in anhydrobiotic performances
480 among geographically isolated populations of eutardigrades have been reported, but the literature
481 data are conflicting (Horikawa and Higashi, 2004; Jönsson et al., 2001) probably due to the
482 presence of cryptic species and differences in ecological conditions of the microhabitats.
483 Interestingly, as previously mentioned, bdelloid rotifers seem to be different, i.e. desiccation
484 improves individual and population fitness (Ricci et al., 2007; Sommer et al., 2019), but with still
485 unknown mechanisms.
486 In addition, low fitness associated with a long-lifespan could slow down rates of evolution in
487 comparison to organisms with similar lifespans but without the capability to perform
488 anhydrobiosis. Furthermore, ancestral genetic traits may reappear after a long time in
489 anhydrobiosis, jumping generations, and contributing to the longer existence of unchanged traits
490 (Kaczmarek et al., 2019). Anhydrobiosis represents an “escape in time” from habitat conditions
491 hostile to active life, opposed to an “escape in space” performed by organisms with an ability to
492 migrate away from unfavorable conditions (Jönsson, 2005). In addition, it limits selection and
493 creates a “seedbank” for maintaining haplotypes in time and space (environment) (Guidetti et al.,
494 2011a). These advantages are reinforced by the ageing models (“Sleeping Beauty” and “Picture of
495 Dorian Grey”) allowing organisms to withstand adverse conditions for a long time and the
496 capability to restore active life and reproduction when environmental conditions become suitable

497 (Kaczmarek et al., 2019). Such scenarios are in line with the hypothesis that anhydrobiotic
498 organisms almost avoid environmental selection since they are active only under favourable
499 environmental conditions (Pilato, 1979). Therefore, anhydrobiotic periods could have an impact
500 on generation time, which in turn influences the potential rate of evolution. This could be the cause
501 of the surprising morphological uniformity at the species, genera and family level of terrestrial
502 anhydrobiotic tardigrades in contrast to marine species that, in practice, are not able to enter
503 anhydrobiosis (Kaczmarek et al., 2019). Interestingly, molecular analyses have suggested that
504 bdelloid and monogonont rotifers might have different diversification and mutation rates, although
505 it is still unclear if this is due to the different dormancy patterns (quiescence *vs* dormancy,
506 respectively) or the different reproductive strategies (obligately *vs* cyclical parthenogenesis,
507 respectively) of these taxa or other, still unknown, factors, and if they are indeed common
508 (Barraclough et al., 2007; Swanstrom et al., 2011; Fontaneto et al., 2012b).

509 Further selective advantages of anhydrobiosis can be cited. Anhydrobiosis allows the reduction of
510 predators, competitors, and parasites since stochastic habitats are colonized only by a reduced
511 number of species (Wilson and Sherman, 2013; Guidetti et al., 2011a). Since holo-anhydrobiotic
512 organisms are aquatic animals, desiccation tolerance allows them to colonize and persist in
513 terrestrial habitats other than in stochastic and extreme habitats. Moreover, the capability to
514 withstand extreme conditions by entering anhydrobiosis increases the number of possible
515 “refugia” that can be utilized by the species during long harsh environmental conditions, with a
516 decrease in the rate of extinction and the loss of diversity (Guidetti et al., 2011a). Anhydrobiosis
517 increases passive dispersal capability since dormant anhydrobiotic animals and eggs can act as
518 propagules, be transported over long distances, and cross physical barriers for months without
519 losing viability, which active animals cannot do, and establish new populations in new territories
520 (Guidetti et al., 2011a; Mogle et al., 2018; Fontaneto, 2019). This capability is aided by the fact

521 that mostly holo-anhydrobionts reproduce via telytokous parthenogenesis, a reproductive strategy
522 favourably adapted to colonise new and isolated habitats with a single individual (Bertolani, 2001,
523 Ricci and Fontaneto, 2009; Fontaneto, 2019). This could influence the biogeographical pattern of
524 holo-anhydrobionts that supports the hypothesis that “everything is everywhere”. This hypothesis
525 was confirmed for many bdelloid rotifers (Fontaneto et al., 2008), monogonont rotifers (e.g. Gómez
526 et al. 2002; Mills et al., 2017) and for few tardigrade species (Kaczmarek et al., 2019), although
527 some caution should be exercised, as other variables, including sampling effort or hidden diversity,
528 might influence results and should therefore be carefully considered (Fontaneto et al., 2007, 2008,
529 2009; Mills et al., 2017). Nevertheless, despite that tardigrades are able to disperse by wind as are
530 other terrestrial anhydrobionts (Nkem et al., 2006; Rivas et al., 2019), most tardigrade species have
531 a narrow species range, with a large number of endemic species (e.g. Pilato and Binda, 2001;
532 Guidetti et al., 2019). In contrast, biogeographic patterns were detected in several anhydrobiotic
533 taxa of nematodes (Faurby and Barber, 2015; Zullini, 2018). In any case, the paucity of faunistic
534 data, the presence of cryptic species, and the high level of confounding factors make the distribution
535 patterns more complex with the exigency to collect further experimental and faunistic data. Some
536 of these effects on the life history, like the ability to “escape in time”, aging, “refugia”, generation
537 time, selection, and avoidance of predators, are also valid for other dormant stages, for example
538 resting eggs and cysts, even when they are not desiccated, highlighting common ecological effects
539 of dormancy, irrespective of the physiological adaptations of each taxon or response to stress.
540 These conditions, together with the capability of desiccation-tolerant organisms to repopulate
541 habitats when liquid water returns, affect community dynamics and produce substantial
542 modifications in the structure of biological communities, even leading to modifications in the
543 functional integrity of the ecosystems (Irons et al., 1993; Walsh et al., 2104).

544

545 **Implications/application of extreme tolerance of meiofaunal organisms**

546 A better understanding of the life strategies of anhydrobiotic animals both at the ontogenetic and
547 phylogenetic levels can provide answers to many fundamental questions as well as useful practical
548 outcomes in many branches of applied sciences. Understanding desiccation tolerance in
549 anhydrobiotic organisms will enable us to induce or engineer tolerance in sensitive species and to
550 produce subsequent long-term stabilization and preservation of biological material in a dry state
551 This is a topic of considerable practical importance both in medical and commercial fields since
552 drying is widely used in the food and pharmaceutical industries as a long-term preservation
553 technique (Saragusty and Loi, 2019).

554 Based on knowledge accumulated from anhydrobiotic organisms, much of the research on
555 stabilising cellular membranes and proteins has centered on trehalose, which preserves cell
556 membranes, and proteins, which can allow fluids to solidify without forming crystals through glass
557 transition or vitrification, forming a large number of hydrogen bonds with membranes and proteins,
558 and by replacing water molecules during the drying process (Hengherr et al., 2009), although many
559 other protein families, as well as molecules, perform many functions, some of which still
560 uncharacterised (e.g. Tompa, 2002; Tunnacliffe and Wise, 2007; Tunnacliffe et al., 2010). Some
561 anhydrobiotic organisms naturally possess the molecular mechanisms to produce these sugars and
562 load and unload them to and from the cells of the body or the intracellular spaces. Since the first
563 report that biomolecules, membranes, and organisms can be stabilized in a dry state, due to the
564 presence of trehalose, an array of possible applications for trehalose have been reported, ranging
565 from the stabilization of vaccines, lysosomes, platelets, spermatozoa and oocytes to the
566 hypothermic storage of human organs (Chen et al., 2001; Crowe et al., 2005; Schill et al., 2009;
567 Saragusty and Loi, 2019). The determination of the properties of trehalose and the debate whether
568 trehalose alone is sufficient to preserve biomolecules (e.g. Garcia de Castro and Tunnacliffe, 2000;

569 Ratnakumar and Tunnacliffe, 2006; Pouchkina-Stantcheva et al., 2007; Tapia et al., 2015, Chau et
570 al., 2016) have stimulated the continuation of basic research to discover the secret of life without
571 water. Recently, Boothby and co-workers (2017) indicated that the heterologous expression of
572 some CAHS proteins in both prokaryotic and eukaryotic cells is sufficient to increase desiccation
573 tolerance in these sensitive systems, and purified CAHS proteins protect desiccation-sensitive
574 proteins *in vitro*. Moreover, Hashimoto et al. (2016) found that tardigrade DNA-associating protein
575 (Dsup) suppresses X-ray-induced DNA damage by 40% and improves radiotolerance of human
576 cultured cells. The story of the already known bioprotectants tells us that the tolerant ability of
577 anhydrobiotic animals could be transferred to more sensitive organisms at least partly by
578 transferring the corresponding genes. Recent rapid progress of molecular analyses should
579 accelerate the elucidation of the mechanisms at the basis of extreme stresses, including complete
580 desiccation stress, providing novel clues that open new avenues to confer stress resistance to
581 intolerant species, including humans.

582

583 **Conclusions**

584 Various extreme-tolerance mechanisms have evolved in meiofauna, enabling micrometazoans like
585 tardigrades, rotifers, and nematodes, to reduce or interrupt metabolism and survive stressful
586 environments. In response to the gradual onset of adverse environmental conditions (e.g. water
587 availability, temperature, oxygen tension, pH), these organisms undergo complex molecular,
588 physiological, morphological and behavioural changes, which can share common characteristics
589 but also present some differences. For example, tardigrades undergo encystment, an adaptive
590 strategy that involves profound morphological changes that occur during the molting process,
591 resulting in the dormant organism lying within retained cuticular exuvia. On the other side,
592 cryptobiosis happens at any stage of the life cycle of the organisms and includes different strategies

593 such as anhydrobiosis, cryobiosis, anoxybiosis and osmobiosis directly induced by desiccation,
594 sub-zero temperatures, low oxygen pressure and osmotic extremes, respectively. The most
595 widespread and best-known form of these is anhydrobiosis, the capability evolved by tardigrades,
596 rotifers and nematodes to tolerate complete desiccation by entering in a state of reversible
597 suspension of metabolism without the loss of viability.

598 When dormant, these taxa show extraordinary resistance to physical and chemical extremes that
599 may far exceed the tolerance ranges of active organisms, therefore the two dormancy strategies,
600 quiescence and diapause, allows tardigrades, rotifers and nematodes to colonise and persist in
601 various otherwise unavailable environments. Interestingly, while dormant, some taxa do not age,
602 although the specific effects of dormancy on aging varies with the taxa and is poorly understood
603 but this ability make tardigrades, rotifers, and nematodes very useful model organisms that can be
604 used to study the aging process. Furthermore, the evolution of anhydrobiosis resulted in selective
605 advantages but also in energetic costs with effects on growth, reproduction, life history, and fitness,
606 in turn affecting the rate of evolution, but more studies are needed to fully understand the ecological
607 and evolutionary implications of these resistance strategies on these taxa.

608 Furthermore, novel findings have also contributed to expand other aspects of these taxa, with
609 potential exciting applications in other fields: the evolution of a series of behavioural,
610 morphological, physiological and molecular/biochemical adaptations provided anhydrobiotic
611 organisms with different unusual mechanisms to withstand desiccation. To prevent cell damage
612 during dehydration, bioprotectant molecules that accumulate in and around the cells of their body
613 are generated; the identification of these molecules and their mechanisms are the focus of much
614 current research, including the role of horizontal gene transfer. It is becoming clear that no single
615 class of protectant/repair molecules is sufficient, but successful desiccation depends on the co-
616 ordinated action of all of them. The understating of the detailed mechanisms and consequences of

617 extreme tolerance, these meiofauna taxa are becoming popular model organisms in the fields of
618 exobiology and medical research, with the hope that they might also help to improve the tolerance
619 of human cells to extreme stress in the future.

620

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1433 **FIGURE LEGENDS**

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1435 Fig. 1. A) *In toto* cyst of the freshwater eutardigrade *Hypsibius* sp. (phase contrast). B) *In toto* cyst
1436 of the freshwater eutardigrade *Dactylobiotus parthenogeneticus* (phase contrast). Bar = 100 µm.

1437
1438 Fig. 2. A) *In toto* specimen of the limnoterrestrial eutardigrade *Acutuncus antarcticus* (*in vivo* and
1439 Nomarski contrast). B) *In toto* female of the lichen-dwelling eutardigrade *Ramazzottius* cf.
1440 *oberhaeuseri*; the ovary containing three oocytes (asterisk). (*in vivo* and Nomarski contrast). C)
1441 Tun (desiccated animal) of the eutardigrade *Ramazzottius* cf. *oberhaeuseri* (*in vivo*).
1442 Arrow: buccal-pharyngeal apparatus; arrowhead: midgut; cross: gonad. Bar = 100 µm.

1443
1444 Fig. 3. Scanning electron micrographs of the moss-dwelling heterotardigrade *Echiniscus* sp. A)
1445 Dorsal view of an *in toto* and hydrated specimen. B) Dorsal view of an *in toto* desiccated specimen
1446 (tun). C) Ventral view of an *in toto* desiccated specimen (tun). Bar = 100 µm.

1447
1448 Fig. 4. Scanning electron micrographs of the rotifer *Adineta tuberculosa*. A) *In toto* and hydrated
1449 specimen. B) *In toto* desiccated specimen (tun). Bar = 100 µm. (Courtesy of Giulio Melone and
1450 Diego Fontaneto).

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