Organisms Diversity & Evolution
https://doi.org/10.1007/s13127-024-00642-1

REVIEW

Evolution of egg deposition strategies, exaptations of exuvia, and thanatochresis in tardigrades

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Received: 21 December 2023 / Accepted: 4 April 2024
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Abstract

The cuticle is the tardigrade exoskeleton that, limiting animal growth, needs to be periodically shed. New cuticles must be formed (within the old ones) before getting rid of the obsolete exoskeletons at the end of moulting process. After ecdysis (the release of the old cuticle), the exuvia has different destinies according to tardigrade evolutionary lines. In the marine tardigrades (Heterotardigrada), the exuvia is lost and useless, while in the other taxa, it acquires interesting uses to be considered exaptations, since the cuticle previously shaped by natural selection for a function (i.e. as exoskeleton) is coopted for new adaptive scopes. These are related to egg deposition, parental care, mating, and diapause. Egg deposition within the exuvia is one of the three different egg deposition strategies developed by tardigrades: smooth eggs can be laid freely or within the exuvia, while ornamented eggs are laid freely. A new scenario for the evolution of such egg deposition strategies is characterised by five schematic steps: smooth eggs laid freely (ancestral state), synchronization of egg maturation with moulting (developed in tardigrade ancestor in sea), use of the exuvia for oviposition (for enhanced mechanical and physiological egg protection), acquisition of egg ornamentation, and ornamented eggs laid freely (related to a risk-spreading strategy). An interesting thanatochresis case related to the release of free eggs in crustaceans exuviae, convergently developed in two distant taxa of eu- and heterotardigrades, is presented and discussed.

Keywords Adaptation · Exuvium · Tardigrada · Parental care · Ecdysis · Diapause

Introduction

Ecdysozoa is a clade composed of phyla whose representatives are surrounded by an exoskeleton formed by a cuticle (Aguinaldo et al., 1997). The exoskeleton of these animals defines, supports, and protects the body; it is the site for muscle attachments and acts as a filter for the external environment. The cuticle also limits the growth of animals; therefore, it needs to be periodically shed to allow the enlargement of their body. These animals must build a new cuticle (within the old one) before getting rid of the obsolete exoskeleton. The process of releasing the old cuticle is called ecdysis (from ancient Greek 'to take off, strip off'), and the old, shed cuticle is named 'exuvia' (from Latin 'things stripped from a body').

From an evolutionary point of view, the ecdysis represents 'a waste': animals must produce several times during their life cycle a structure (the cuticle) that will be released and lost, consuming material, energy, and time. Moreover, during the building of the new cuticle and/or during and after the ecdysis process, animals are more fragile and, in several phyla, they are not able to feed (e.g. Rackauskas et al., 2006; Guidetti et al., 2012; Fahrbach, 2019; Bellés, 2020). However, the cuticular exoskeleton is effective, and the trade-off between pros and cons is evidently skewed in favour of the pros, since ecdysozoans include the most successful organisms on Earth in terms of biodiversity and colonised habitats (i.e. nematodes and arthropods).

Not all ecdysozoans waste their exuvia: many arthropods eat their cast cuticle after moulting, as it constitutes a meal with which the animal recycles its nitrogen content (Buřič et al., 2016; Mira, 2000). Even many species of tardigrades do not throw away their old cuticle. Tardigrades belong to ecdysozoans; they are microscopic aquatic animals (from 0.2 to 1 mm in length) that need a film of water surrounding their body to conduct active life and are able

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Published online: 27 April 2024

to colonise terrestrial environments thanks to their ability to perform different forms of cryptobiosis (for reviews, see Guidetti et al., 2011; Schill & Hengherr, 2018; Hengherr & Schill, 2018; Kaczmarek et al., 2019; Rebecchi et al., 2020; Jönsson, 2020; Hvidepil & Møbjerg, 2023). Tardigrades have a metameric body of five segments, a head, and four body segments each one with latero-ventral legs (for detailed morphology, see Nelson et al., 2015; Møbjerg et al., 2018) (Fig. 1A). Tardigrade integument is formed by a monolayered epidermis and a cuticle. The cuticle is not just an exoskeleton, but a surface for the exchange of gases and water with the external environment; the involvement of the cuticle in evaporation and intake of water is little studied (Marcus, 1929; Wright, 1988, 1989), and very probably, it has an impact in the anhydrobiotic process (Crowe, 1972; Crowe et al., 1971). Tardigrade cuticle covers the body and extends into the hind- and foregut; it is multi-layered and made of polysaccharides (e.g. acid mucopolysaccharides and alpha-chitin), proteins (e.g. glycoproteins and lipoproteins), and lipids; and it can be organized in dorsal or ventral plates (for reviews see Greven, 1984; Czerneková & Vinopal, 2021). Tardigrades moult several times during their entire life cycle. Environmental conditions or food shortage can induce moulting as well, and animals can become even smaller than they were prior to shedding their cuticle (Baumann, 1961; Marcus, 1929; Walz, 1982).

The first step of tardigrade moulting process is the discharging of the entire cuticular lining of the foregut (stomodeum) through the mouth opening (see video: https://youtu.be/eASEPJECYXo). Interestingly, this phase underlines that the foregut is released independently from both the body cuticle and the hindgut lining (proctodeum), which are discharged only during ecdysis. Therefore, during evolution, the release of the old foregut (i.e. buccal tube, pharyngeal thickenings, and oesophagus lining) with all the annexed structures of the feeding apparatus (i.e. stylets, stylet coats,

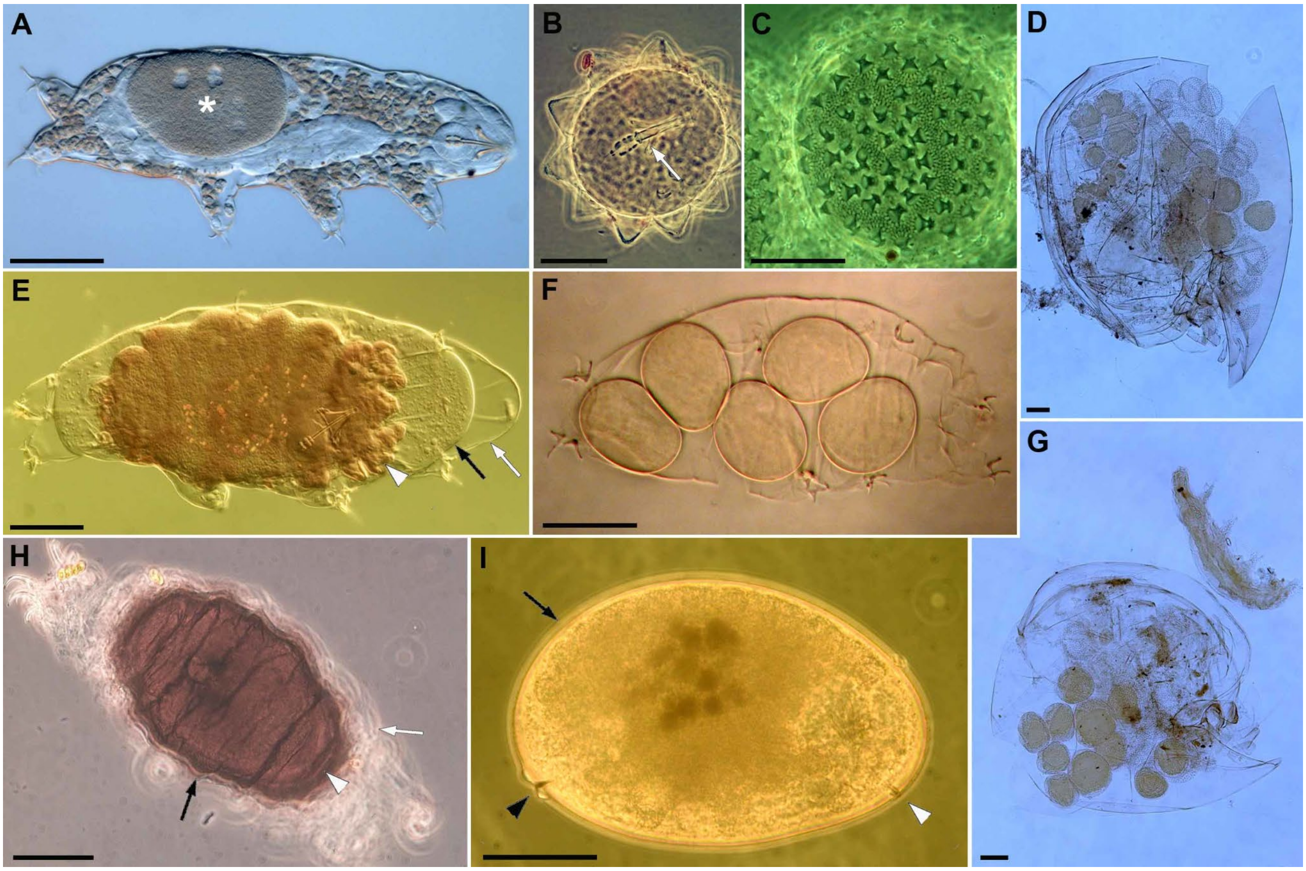

Fig. 1 **A** Tardigrade (*Acutuncus antarcticus*; asterisk = egg in the gonad). **B** Ornamented egg (*Paramacrobiotus* sp., Macrobiotoidea; arrow = feeding apparatus of the embryo). **C** Ornamented egg (*Macrobiotus* sp., Macrobiotoidea). **D** Tardigrades' egg (*Murrayon pullari*) deposited in an ostracod's exuvia. **E** Tardigrade cyst (*Bertolanius* sp., Eohypsibioidea; white arrow = old cuticle; black arrow = sarcophagus cuticle; arrowhead = animal cuticle). **F** Tardigrade exuvia (*Isohypsibius* sp.) with five eggs inside. **G** Tardigrade eggs (*Murrayon pullari*) deposited in an ostracod's exuvia and a *M. pullari* animal. **H** Tardigrade cyst (*Dactylobiotus* sp., Macrobiotoidea; white arrow = old cuticle; black arrow = sarcophagus cuticle; arrowhead = mummy cuticle). **I** Tardigrade cyst (*Dactylobiotus* sp.) from which the external cuticular involucra were removed (white arrowhead = mouth; black arrowhead = cloaca; arrow = mummy cuticle). **A**, **D**–**G** = 50 µm; **B**, **C** = 25 µm

and stylet supports) became asynchronous with respect to that of the body cuticle and hindgut. The reasons of this asynchronicity are unclear, but further underline the peculiar nature of the feeding apparatus (i.e. with a stylet system presenting two piercing stylets able to protrude out of the mouth) that represents the main apomorphy of the phylum (for more details on feeding apparatus see Guidetti et al., 2012, 2013; Massa et al., 2023). After the discharging of the cuticular parts of the feeding apparatus and the $CaCO_3$ stylets, the animal is in the so-called 'simplex stage', which is characterised by cuticular folds closing the mouth opening (Guidetti et al., 2011). Therefore, until the complete resynthesis of a new feeding apparatus, which can take more than 3 days (Guidetti et al., 2012), the animal cannot feed. During the reconstruction of the feeding apparatus, by anterior glands (i.e. stylet glands, a.k.a. salivary glands) and the pharynx, the epidermis synthesises the new cuticle with its annexes (e.g. appendages as protuberances, spines, and filaments), while the glands positioned in the legs synthesise the claws (or digits). The new cuticle within the old one is folded extensively and it expands after ecdysis, while the old cuticle is released without evidence of re-absorption (Greven, 1984). The animal leaves the old cuticle producing a hole in its anterior portion, starting from the former mouth opening (see video: https://youtu.be/4rHUD4r6qeY).

The different uses of the exuvia developed by tardigrades, the evolution of the egg deposition strategies evolved in this phylum, which can also involve the exuvia, and the peculiar cases of thanatochresis, which involve egg deposition, will be presented and discussed in an evolutionary context.

Exaptations related to the different uses of the exuvia

After the ecdysis, the exuvia has different destinies according to the tardigrade evolutionary lines (Figs. 2 and 3). As far as we know, only in the marine tardigrades (Heterotardigrada),

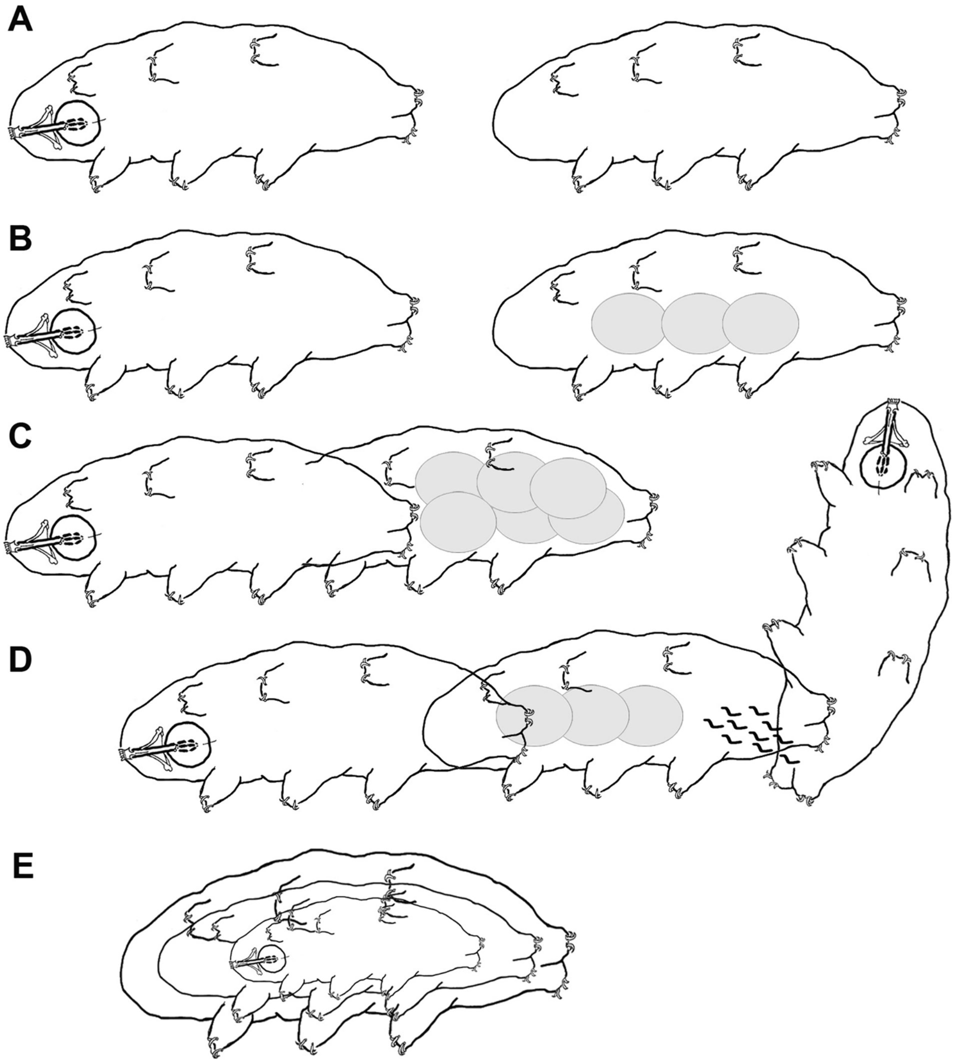

Fig. 2 **A** An animal and its exuvia released after moulting. **B** A female and its exuvia used for egg deposition. **C** Exuvia used for egg deposition that remains attached to the animal to carry the eggs. **D** A female and its exuvia used for egg deposition; the male is releasing the sperms in the exuvia for a protected fecundation. **E** Several exuviae each one within each other to protect the animal in diapause state, forming a cyst

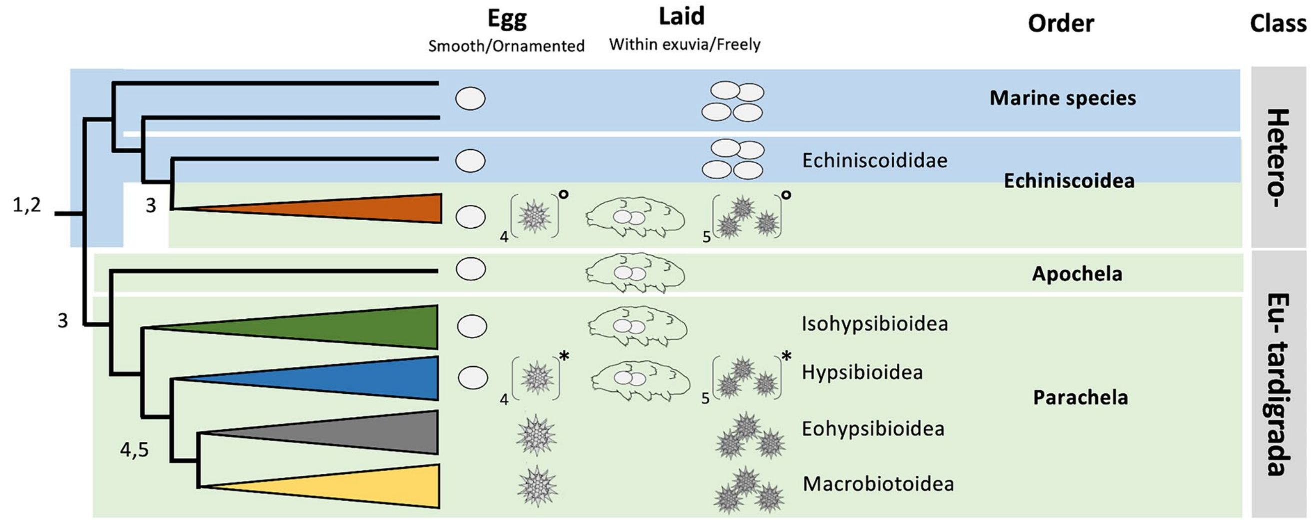

Fig. 3 Phylogenetic tree of the main tardigrade taxa, associated with the type of egg deposition (within exuvia or freely, smooth eggs or egg with ornamentations). In parentheses, type of egg deposition in taxa which show a different egg deposition strategy with respect to their phylogenetic relatives. °*Oreella mollis* Murray, 1910. *Species of the family Ramazzottidae and *Acutuncus antarcticus* (Acutuncidae). Numbers refer to evolutionary steps of the egg deposition strategies: 1. Smooth eggs freely released (ancestral state). 2. Synchronization of egg maturation with moulting. 3. Deposition of the eggs in the exuvia followed by the release of the exuvia. 4. Acquisition of egg ornamentation. 5. Ornamented eggs laid freely. Taxa within sky-blue rectangles are marine, those in the green rectangles are limno-terrestrial

the exuvia is lost and useless, while in the other taxa, it acquires some interesting uses.

As egg keeper

A first and the most widespread use of the exuvia in tardigrades is related to egg deposition, in which the exuvia is used as a fertilized egg ‘keeper’ (Figs. 1F and 2B).

In females and hermaphrodites limno-terrestrial tardigrades (both Eutardigrada and Heterotardigrada; no evidence for marine tardigrades), there is a synchronization between moulting and oocyte maturation (Bertolani et al., 1996). Most terrestrial and freshwater species lay their eggs within the exuvia during the ecdysis process thanks to this synchronization (Fig. 2B). This synchronization is present even in species that lay eggs freely (Altiero et al., 2015; Giovannini et al., 2023). When eggs are laid in the exuvia, all mature gametes present in the gonad are released in it during ecdysis. Within the exuvia, the embryos complete their development, and after hatching, the newborns exit from the exuvia from the anterior opening produced by the mother when leaving the old cuticle during ecdysis (Suzuki, 2003; see video: https://youtu.be/8WfSs1rB6EM).

As egg carrier

A second peculiar way in which some species of eutardigrades use their old cuticle is as ‘carrier’ to protect and transport the embryonated eggs (Fig. 2C). This use must be considered a form of parental care, which is rare in tardigrades and found mainly in limnic eutardigrade species of the genera *Pseudobiotus*, *Isohypsibius* (Isohypsibioidea), and *Borealibius* (Eohypsibioidea), which carry along their old exuvia containing laid embryonic eggs (Bertolani & Rebecchi, 1999; Pilato et al., 2006). The exuvia containing the embryos is held by the female body, stuck with its anterior opening between the third and fourth pair of legs of the female, in a way that the hind legs of the mother are positioned within the exuvia (Fig. 2C). This allows the female to crawl with her first three pairs of legs without losing the exuvia with its precious load. This carriage allows to keep the eggs in more favourable environmental conditions, at least those affecting the mother. It is not yet clear when the exuvia is released to allow the newborns to exit from the exuvia in which they hatched.

Another example of parental care in tardigrades is represented by the transportation of some eggs on the dorsocaudal part of the body in specimens of the marine genus *Echiniscoides* (Heterotardigrada, Echiniscoidea, Echiniscoididae) (Bertolani & Rebecchi, 1999).

For mating

A third use of the exuvia, in many eutardigrades, is related to the release of the sperms of a mating male within the exuvia of a female, in which she has or has not yet released the eggs (Fig. 2D). This phenomenon is not extensively documented due to the difficulties in observing the mating behaviour in tardigrades (for a review, see Sugiura & Matsumoto, 2021), but it is likely present in most eutardigrade species releasing eggs in the exuvia. Sperms are introduced from the cloaca opening of the exuvia or from the anterior opening produced by the animal during ecdysis; in both cases, the females are still partially or totally inside the old cuticle during male ejaculation (Sugiura & Matsumoto, 2021). In this way, fertilization occurs in a protected environment and very probably, sperms increase their chance to get in contact with eggs. Then, the exuvia is released by the female and the fertilized eggs remain in it until complete development. A variant of this behaviour was observed in an eutardigrade species of the freshwater genus *Pseudobiotus*, in which males introduce the sperms in the empty exuvia still attached to the female; then, the sperms rapidly reach the female cloaca (Bertolani & Rebecchi, 1999), representing a case of external insemination, protected by means of the exuvia, but with an internal fertilization.

In diapause

A fourth use of the exuvia relies in its protective function and consists in protecting animals during periods of potential physiological stress (Figs. 1E, H, I and 2E). Tardigrades are also peculiar animals due to the variety of forms of dormancy they exhibit. Other than cryptobiosis, diapause in the form of encystment and cyclomorphosis can be found in several phylogenetic lines of eu- and heterotardigrades (for a review, see Guidetti & Møbjerg, 2018).

In tardigrades, diapause is controlled by exogenous (e.g. temperature and oxygen tension) and endogenous stimuli; this process involves the suspension of growth and development accompanied by a reduction in metabolic activity, in response (and probably in advance) to adverse environmental conditions. Diapause leads to successive phases of deep morphological and physiological transformations of the animals. Specifically, encystment is characterised by tardigrades in diapause, with lower levels of metabolism, within one or more (up to three) retained cuticular coats (one or two exuviae sclerotize, becoming dark; Fig. 2H). Morphological modifications occurring to animals happen during consecutive moults, during which the animals do not leave old cuticles. These morphological changes, and consequently those of the exuvia, can be very deep. According to the species and the type of cysts, they range from claw reduction to feeding apparatus simplification, loss of claws, arriving to loss of both legs, and typical body shape (the animal is not anymore recognisable as a tardigrade; Fig. 1I) (Guidetti & Møbjerg, 2018; Guidetti et al., 2006). Encystment is likely widespread in limno-terrestrial species and in the marine *Echiniscoides sigismundi* (Schultze, 1865) (Clausen et al., 2014), but is reported for a limited number of species belonging to both classes (Guidetti & Møbjerg, 2018). Moreover, the same species can produce more than one type of cysts, and cryptobiosis is still possible when the animal is encysted (Guidetti et al., 2008). The animals of *Bertolanius volubilis* (Durante Pasa & Maucci 1975) take ca. 2 weeks to produce a cyst, which can last from 35 days to 9 months (in lab conditions) (Guidetti et al., 2008).

Cyclomorphosis is present in the marine eutardigrade *Halobiotus crispae* Kristensen, 1982, and it is characterised by seasonal morphological changes involving four stages, two of them characterised by an extra protecting exuvia (becoming dark by sclerotization) and a modified feeding apparatus (Kristensen, 1983). Unlike encysted animals, those within the exuvia (i.e. the stage called pseudosimplex 1) can crawl (muscles are still connected to the attachment sites of the exuvia). Animals in pseudosimplex 1 are gregarious forming clusters often containing several hundreds of tardigrades (Guidetti & Møbjerg, 2018). Cyclomorphosis and the different lengths of its phases are linked to environmental factors and should be viewed as adaptations to withstand low temperatures (e.g. during winter in Greenland), or heat stress and oxygen depletion (e.g. during the Danish summer) (Møbjerg et al., 2007). Although the active stage of *H. crispae* tolerates quite low temperatures, the pseudosimplex 1 stage is freeze tolerant (Halberg et al., 2009).

Tardigrades in diapause minimize exchange with the environment by producing extra cuticles (exuviae). Encystment may occur as part of cyclomorphic phases, with individual animals undergoing cyclic and reversible morphological modifications. Therefore, both diapause phenomena (encystment and cyclomorphosis) are associated with unconventional moults, not immediately followed by ecdysis, and not directly associated with the growth of animals.

In the case of tardigrades, the diapause processes must be considered an expensive process at both individual (diapausing animals need extra cuticular structures and feeding apparatuses) and population levels (diapausing animals do not reproduce), but represent nonetheless widespread adaptation strategies to survive environmental stress.

Exaptations

The function of tardigrade cuticle as exoskeleton has to be considered an ‘adaptation’, because natural selection shaped this character for a current use (Gould & Vrba, 1982). The old cuticle (exuvia) released by tardigrades during moulting has to be considered an ‘exaptation’ when performing one of the functions that are listed above (e.g. keeping egg, carrying eggs, mating ‘assistant’, and cyst walls), because this character (i.e. the cuticle), previously shaped by natural selection for a particular function (i.e. as exoskeleton), is coopted for new uses (Gould & Vrba, 1982). The use of the old cuticle for purposes other than those of an exoskeleton has an ‘effect’ (see Gould & Vrba, 1982) which produces consequently an increase in fitness that is not directly derived from the developing of that character by natural selection. What is interesting and deserves more attention is that such exaptations raised independently one or more times in the evolution of tardigrades. According to the phylogenetic tree in Fig. 3, the use of the exuvia as egg keeper evolved independently in terrestrial heterotardigrades and in eutardigrades; the use of the exuvia as egg carrier for parental care evolved independently in three genera of two superfamilies in Eutardigrada; the use of the exuvia as protection layers during encystment and cyclomorphosis evolved independently at least in both terrestrial heterotardigrades and in the order Parachela (although how spread this phenomenon is in the parachelan clades is not clear).

Evolution of the egg deposition strategies

Tardigrades colonised marine, freshwater, and terrestrial environments. Marine environments are inhabited by Heterotardigrada species of the polyphyletic order Arthrotardigrada (Grollmann et al., 2023), the family Echiniscoididae (Echiniscoidea, Heterotardigrada), and the Eutardigrada genus *Halobiotus* (Isohypsibioidea, Parachela). This last colonisation is considered secondary, starting from a limno-terrestrial ancestor (Kristensen, 1982). Limno-terrestrial environments are colonised by the other species of Echiniscoidea and by the class Eutardigrada (Fig. 2).

Marine heterotardigrades release smooth eggs surrounded by a sticky shell and laid freely, terrestrial heterotardigrades produce smooth shelled eggs laid in the exuvia, while freshwater and terrestrial eutardigrades produce either smooth shelled eggs laid in the exuvia, or ornamented eggs laid freely (Bertolani & Rebecchi, 1999; Fig. 2).

When present, the ornamentations surround the egg and can have different shapes (e.g. spines, cones, pillars, laminae, and reticulations), size, and densities. Although these ornamentations gained high taxonomic value because they are species specific and useful for species identification, little attention has been paid to the relationships between form and function of these structures and to the reason why there are so many forms even considering very close evolutionary lineages (e.g. sister taxa) (e.g. see Kaczmarek & Michalczyk, 2017, for the *hufelandi*-type of eggs). It has been suggested that these ornamentations would keep the eggs from being swept away from the substrate by external agents (Ramazzotti & Maucci, 1983) and protect the egg in more unstable conditions when continental environments were colonised (Bertolani et al., 1996).

Little investigated is also the reason why three strategies for egg deposition have been developed in tardigrades. Bertolani et al. (1996), based on the phylogenetic knowledge of the time, proposed an evolutionary scenario for the different kinds of egg deposition within the phylum, which was characterised by the following steps: (i) smooth eggs laid freely (ancestral state), (ii) acquisition of ornamentation (when the sea was abandoned), (iii) ornamented eggs laid freely, (iv) exploitation of the exuvia for oviposition (synchronizing egg maturation with moulting), (v) loss of the ornamentation of the eggs within the exuvia, and (vi) smooth eggs laid within the exuvia.

According to the most updated molecular phylogenies within Tardigrada (Bertolani et al., 2014; Fleming & Arakawa, 2021; Fujimoto et al., 2017; Grollmann et al., 2023; Guil & Giribet, 2012; Guil et al., 2013, 2019; Jørgensen et al., 2010, 2011, 2018), it is possible to draw a phylogenetic tree representing the relationships between the main taxa of the phylum (Fig. 2). While the relationships within Eutardigrada are well supported, the relationships within Heterotardigrada remain debated. The position of the Echiniscoidea with respect to the different lines of the polyphyletic Arthotardigrada changes according to the studies. Anyway, Echiniscoididae representatives are found nearly always as basal of Echiniscoidea (Guil & Giribet, 2012; Guil et al., 2013, 2019; Jørgensen et al., 2010, 2011, 2018). This new phylogenetic knowledge suggests a different scenario. Tardigrades originated in the sea (Guidetti & Bertolani, 2018; Kristensen, 1981; Renaud-Mornant, 1982), and the most supported hypothesis sees marine heterotardigrades as the most ancestral groups within the phylum (Kristensen, 1976; Kristensen & Higgins, 1984; Renaud-Mornant, 1982). For these reasons, the first step of the scenario proposed by Bertolani et al. (1996) can be supported, considering the smooth eggs laid freely as the ancestral state. Nevertheless, based on the more updated phylogeny of tardigrade taxa and applying the parsimony criterion (Fig. 2), most of the following steps of Bertolani et al. (1996) remain plausible, but with a different order.

Evolutionary scenarios are useful to explain the different steps for which evidences are present and to develop different hypotheses for the steps not yet clear that can be verified. The newly inferred scenario involves the following five schematic steps (Fig. 3):

1. Smooth eggs laid freely (ancestral state).

   Present in the ancestor of tardigrades.
2. Synchronization of egg maturation with moulting.

   Developed in the ancestor of tardigrades in the sea (although there is no evidence of this synchronization in marine species, the parsimony criterion leads to support this hypothesis); this would also explain the convergence between Echiniscoidea and Eutardigrada in the strategy of egg deposition within the exuvia.
3. Utilization of the exuvia for oviposition, smooth eggs laid within the exuvia.

   Developed convergently in Echiniscoidea (Heterotardigrada) and in the ancestor of Eutardigrada, the selective advantage that led to this step can be related to the enhanced mechanical and physiological protection of eggs by the exuvia (e.g. exuvia could reduce predation and/or water evaporation rate on egg surface) and to the reduction of the washout of the smooth eggs from the more unstable and extreme limno-terrestrial habitats, but none of these speculations have been tested yet.

4. Acquisition of egg ornamentation.
5. Ornamented eggs laid freely.

   Developed in the ancestor of the eutardigrade superfamilies Macrobiotoidea and Eohypsibioidea and convergently in the family Ramazzottidae and in a species of the genus *Acutuncus* (both taxa belong to the eutardigrade superfamily Hypsibioidea) and in the freshwater heterotardigrade genus *Oreella* (Echiniscoidea).

The convergent evolution of these two last evolutionary steps (4 and 5) happened within a wide range of systematic levels, ranging from very distant (Heterotardigrada and Eutardigrada) to very close (within a genus) phylogenetic lines. This is indicative of the action of strong selective forces. The evolutionary passage from smooth eggs within the exuvia to freely ornamented eggs can find an explanation in a bet-hedging strategy, a risk-spreading strategy: it is less risky to spread the eggs singularly in the substrate or in small clutches than to lay all of them in a single place (i.e. within the exuvia). Moreover, the development of egg ornamentation shifted the burden of egg protection, substrate adhesion, and low dehydration rate from the exuvia. In fact, the egg ornamentations could reduce the evaporation rate of the water from the egg surface due to the adhesion forces between water and ornamentations. For example, in *Acutuncus* (Hypsibioidea, a superfamily releasing smooth eggs in the exuvia), some species lay eggs with small ornamentations in the exuvia, while a species (*Acutuncus antarcticus* (Richters, 1904)) lays freely deposited eggs with larger ornamentations (Vecchi et al., 2023). *Acutuncus antarcticus* lives in the unstable and extreme habitats of Antarctica, where a bet-hedging strategy and larger ornamentations can be more adaptative. A testimony of the transition from eggs in the exuvia to free eggs in *Acutuncus* could be related to the finding (although rare) of ornamented eggs of *A. antarcticus* laid within the exuvia (Altiero et al., 2015; McInnes, 1995; Utsugi & Ohyama, 1989).

Thanatochresis in tardigrades

Thanatochresis is the exploitation (not as food source) of dead bodies or part of them by living organisms of other species. Amazing cases of thanatochresis related to egg deposition have been recorded in tardigrades. Clusters of dozens of eggs of the freshwater species *Murrayon pullari* (Murray, 1907) (Eutardigrada) were found in exuviae of ostracods (Guidetti et al. 2022; Fig. 1D, G), and a cluster of about 200 eggs, all containing embryos at the same stage, of the marine genus *Echiniscoides* (Heterotardigrada, Echiniscoididae) was found in an exuvia of a barnacle on which the species lives as epibiont (Kristensen & Hallas, 1980).

Murrayon species release free eggs with small ornamentations on their surface, while *Echiniscoides* species release free smooth eggs. The deposition of so many eggs in the same place (exuviae of crustaceans) in two distant phylogenetic lines which live in different environments certainly developed convergently. It is possible to speculate on the selective advantages of this strategy, with two possible benefits that are not mutually exclusive. One could be related to a more efficient egg protection, although this is not in agreement with a bet-hedging strategy. A second could be related to an increase of the dispersal capabilities, as crustacean exuviae disperse easily (representing vectors), as proposed by Kristensen and Hallas (1980).

For each deposition, *Murrayon* species release no more than two eggs (personal observation) while *Echiniscoides* species release from four to eight eggs (Kristensen & Hallas, 1980). Therefore, the presence of so many eggs in the exuvia of a crustacean would have required either a single animal to release egg several times in the same exuvia or multiple animals to release eggs in the same exuvia. In both cases, the nature of this phenomenon is intriguing and very interesting, because in the first case (less probable, due to the same stage of the *Echiniscoides* embryos in the barnacle exuvia), a tardigrade should have been able to find the same site several times, and in the second case, some kind of ‘signal’ should have led many animals to release eggs in the same site (and in a very short time span).

Conclusions

The exoskeleton and the related moulting process of tardigrades represent characteristics that allowed these animals to develop several strategies to cope with different environments and habitats. The exuvia has been used differently and convergently in different evolutionary lines for different purposes. All the uses of the exuvia reported above and the diapausing forms (cyclomorphosis and encystment) find their starting point in the moulting process. Moulting process, synchronization of egg maturation with moulting, eggs released in the exuvia, and cyclomorphosis and encystment processes are related to endocrine system with the production of hormones. Species recognition and specimens’ aggregation (see cluster of specimens in *H. crispae*), mating, and release of free eggs in a same site (see thanatochresis examples) are related to exocrine system with the production of pheromones. All these behaviours show how little we know about tardigrade ethology and glandular systems. Unfortunately, endocrine and exocrine systems receive very little attention. In the only study devoted to this topic, Koziol (2018) reveals the remarkable similarity in the complement of neuropeptides and their sequences between arthropods and tardigrades, further underlying the role of the latter as key group for understanding the evolution of the Ecdysozoa.

Environmental selection and phylogenetic constrains are involved in the evolution of the egg deposition strategies in tardigrades; an emblematic example can be found in Echiniscoidea. This order represents a phylogenetic line in which the species developed new egg deposition strategies in relation to the colonisation of new environments. Echiniscoidea originated in the sea, but only Echiniscoididae (to which *Echiniscoides* belongs) remains the extant family in this environment and still releases free smooth eggs, while the ancestor of all the other families of the order colonised limno-terrestrial habitats changing egg deposition strategies.

Supplementary information

The video linked in the text are available at the YouTube Channel of the Evolutionary Zoology Lab: https://www.youtube.com/channel/UCUE7-_VizjkblZa0Pmh1HbQ/

Acknowledgements I want to thank Sara Brandoli (University of Modena and Reggio Emilia) for the critical reading of the paper and for making her videos of tardigrades available for publication.

Funding Open access funding provided by Università degli Studi di Modena e Reggio Emilia within the CRUI-CARE Agreement. The project was funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4–Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union–NextGenerationEU, Award Number: Project Code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP E93C22001090001, Project Title 'National Biodiversity Future Center–NBCF'. The project was supported by the University of Modena and Reggio Emilia.

Data availability Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Declarations

Ethics approval Work on tardigrades does not require specific ethics.

Competing interests The author declares no competing interests.

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References

Aguinaldo, A. M. A., Turbeville, J. M., Linford, L. S., Rivera, M. C., Garey, J. R., Raff, R. A., & Lake, J. A. (1997). Evidence for a clade of nematodes, arthropods and other moulting animals. *Nature, 387*(6632), 489–493.

Altiero, T., Giovannini, I., Guidetti, R., & Rebecchi, L. (2015). Life history traits and reproductive mode of the tardigrade *Acutuncus antarcticus* under laboratory conditions: Strategies to colonize the Antarctic environment. *Hydrobiologia, 761*, 277–291.

Baumann, H. (1961). Der Lebensablauf von *Hypsibius* (*H.*) *convergens* Urbanowicz (Tardigrada). *Zoological Anzeiger, 167*, 363–381.

Bellés, X. (2020). *Insect metamorphosis: From natural history to regulation of development and evolution.* Academic Press.

Bertolani, R., & Rebecchi, L. (1999). Tardigrada. In E. Knobil, & J. D. Neill (Eds.). *Encyclopedia of reproduction, 4*, 703–718. Academic Press.

Bertolani, R., Guidetti, R., Marchioro, T., Altiero, T., Rebecchi, L., & Cesari, M. (2014). Phylogeny of Eutardigrada: New molecular data and their morphological support lead to the identification of new evolutionary lineages. *Molecular Phylogenetics and Evolution, 76*, 110–126.

Bertolani, R., Rebecchi, L., & Claxton, S. K. (1996). Phylogenetic significance of egg shell variation in tardigrades. *Zoological Journal of the Linnean Society, 116*(1–2), 139–148.

Buřič, M., Fořt, M., Bláha, M., Veselý, L., Kozák, P., & Kouba, A. (2016). Crayfish bury their own exuviae: A newly discovered behavioral pattern in decapods. *Springerplus, 5*(1), 1–4.

Clausen, L. K., Andersen, K. N., Hygum, T. L., Jørgensen, A., & Møbjerg, N. (2014). First record of cysts in the tidal tardigrade *Echiniscoides sigismundi*. *Helgoland Marine Research, 68*, 531–537.

Crowe, J. H. (1972). Evaporative water loss by tardigrades under controlled relative humidities. *The Biological Bulletin, 142*(3), 407–416.

Crowe, J. H., Newell, I. M., & Thomson, W. W. (1971). Fine structure and chemical composition of the cuticle of the tardigrade, *Macrobiotus areolatus* Murray. *Journal of Microscopy, 11*, 107–120.

Czerneková, M., & Vinopal, S. (2021). *The Tardigrade Cuticle. Limnological Review, 21*(3), 127–146.

Durante Pasa, M. V., & Maucci, W. (1975). Descrizione di tre nuove specie di Tardigradi della Scandinavia. *Atti Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano, 116*, 244–250.

Fahrbach, S. E. (2019). Experimental approaches to hormones and behavior. In J. Chun Choe (Ed.) Invertebrates. Encyclopedia of animal behavior (Second Edition) (pp. 462–469). Elsevier Ltd.

Fleming, J. F., & Arakawa, K. (2021). Systematics of Tardigrada: A reanalysis of tardigrade taxonomy with specific reference to Guil et al. (2019). *Zoologica scripta, 50*(3), 376–382.

Fujimoto, S., Jørgensen, A., & Hansen, J. G. (2017). A molecular approach to arthrotardigrade phylogeny (Heterotardigrada, Tardigrada). *Zoologica Scripta, 46*(4), 496–505.

Giovannini, I., Manfrin, C., Greco, S., Vincenzi, J., Altiero, T., Guidetti, R., Giulianini, P., & Rebecchi, L. (2023). Increasing temperature-driven changes in life history traits and gene expression of an Antarctic tardigrade species. *Frontiers in Physiology, 14*, 1258932.

Gould, S. J., & Vrba, E. S. (1982). Exaptation—A missing term in the science of form. *Paleobiology, 8*(1), 4–15.

Greven, H. (1984). Tardigrada. In J. Bereiter-Hahn, A.G. Matoltsy, K. S., & Richards (Eds.), *Biology of the integument 1. Invertebrates* (pp. 714–727). Springer Berlin Heidelberg.

Grollmann, M. M., Jørgensen, A., & Møbjerg, N. (2023). Actinarctus doryphorus (Tanarctidae) DNA barcodes and phylogenetic reinvestigation of Arthrotardigrada with new. A doryphorus and Echiniscoididae sequences. *Zootaxa, 5284*(2), 351–363.

Guidetti, R., Altiero, T., Marchioro, T., Sarzi Amadè, L., Avdonina, A. M., Bertolani, R., & Rebecchi, L. (2012). Form and function of the feeding apparatus in Eutardigrada (Tardigrada). *Zoomorphology, 131*, 127–148.

Guidetti, R., Altiero, T., & Rebecchi, L. (2011). On dormancy strategies in tardigrades. *Journal of Insect Physiology, 57*(5), 567–576.

Guidetti, R., & Bertolani, R. (2018). Paleontology and molecular dating. In R. O. Schill (Ed.), *Water bears: The biology of tardigrades* (pp. 131–143). Springer.

Guidetti, R., Bertolani, R., & Rebecchi, L. (2013). Comparative analysis of the tardigrade feeding apparatus: Adaptive convergence and evolutionary pattern of the piercing stylet system. *Journal of Limnology, 72*, 24–35.

Guidetti, R., Boschini, D., Altiero, T., Bertolani, R., & Rebecchi, L. (2008). Diapause in tardigrades: A study of factors involved in encystment. *Journal of Experimental Biology, 211*(14), 2296–2302.

Guidetti, R., Boschini, D., Rebecchi, L., & Bertolani, R. (2006). Encystment processes and the "Matrioshka-like stage" in a moss-dwelling and in a limnic species of eutardigrades (Tardigrada). *Hydrobiologia, 558*, 9–21.

Guidetti, R., Giovannini, I., Del Papa, V., Ekrem, T., Nelson, D. R., Rebecchi, L., & Cesari, M. (2022). Phylogeny of the asexual lineage Murrayidae (Macrobiotoidea, Eutardigrada) with the description of Paramurrayon gen. nov. and Paramurrayon meieri sp. nov. *Invertebrate Systematics, 36*(12), 1099–1117. https://doi.org/10.1071/IS22031

Guidetti, R., & Møbjerg, N. (2018). Environmental adaptations: Encystment and cyclomorphosis. In R. O. Schill (Ed.), *Water bears: The biology of tardigrades* (pp. 249–271). Springer.

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

Guil, N., Jørgensen, A., Giribet, G., & Kristensen, R. M. (2013). Congruence between molecular phylogeny and cuticular design in Echiniscoidea (Tardigrada, Heterotardigrada). *Zoological Journal of the Linnean Society, 169*(4), 713–736.

Guil, N., Jørgensen, A., & Kristensen, R. (2019). An upgraded comprehensive multilocus phylogeny of the Tardigrada tree of life. *Zoologica Scripta, 48*(1), 120–137.

Halberg, K. A., Persson, D., Ramløv, H., Westh, P., Kristensen, R. M., & Møbjerg, N. (2009). Cyclomorphosis in Tardigrada: Adaptation to environmental constraints. *Journal of Experimental Biology, 212*(17), 2803–2811.

Hengherr, S., & Schill, R. O. (2018). Environmental adaptations: Cryobiosis. In R. O. Schill (Ed.), *Water bears: The biology of tardigrades* (pp. 295–310). Springer.

Hvidepil, L. K., & Møbjerg, N. (2023). New insights into osmobiosis and chemobiosis in tardigrades. *Frontiers in Physiology, 14*, 1274522.

Jönsson, K. I. (2020). Tardigrades-Evolutionary explorers in extreme environments. In J. Seckbach & H. Stan-Lotter (Eds.), *Extremophiles as astrobiological models* (pp. 255–274). Scrivener Publishing LLC.

Jørgensen, A., Faurby, S., Hansen, J. G., Møbjerg, N., & Kristensen, R. M. (2010). Molecular phylogeny of Arthrotardigrada (Tardigrada). *Molecular Phylogenetics and Evolution, 54*(3), 1006–1015.

Jørgensen, A., Kristensen, R. M., & Møbjerg, N. (2018). Phylogeny and integrative taxonomy of Tardigrada. In R. O. Schill (Ed.), *Water bears: The biology of tardigrades* (pp. 95–114). Springer.

Jørgensen, A., Møbjerg, N., & Kristensen, R. M. (2011). Phylogeny and evolution of the Echiniscidae (Echiniscoidea, Tardigrada)–An investigation of the congruence between molecules and morphology. *Journal of Zoological Systematics and Evolutionary Research, 49*, 6–16.

Kaczmarek, Ł., & Michalczyk, Ł. (2017). The *Macrobiotus hufelandi* group (Tardigrada) revisited. *Zootaxa, 4363*(1), 101–123.

Kaczmarek, Ł, Roszkowska, M., Fontaneto, D., Jezierska, M., Pietrzak, B., Wieczorek, R., Poprawa, I., Kosicki, J. Z., Karachitos, A., & Kmita, H. (2019). Staying young and fit? Ontogenetic and phylogenetic consequences of animal anhydrobiosis. *Journal of Zoology, 309*(1), 1–11.

Koziol, U. (2018). Precursors of neuropeptides and peptide hormones in the genomes of tardigrades. *General and Comparative Endocrinology, 267*, 116–127.

Kristensen, R. M., & Hallas, T. E. (1980). The tidal genus Echiniscoides and its variability, with erection of Echiniscoididae fam. n. (Tardigrada). *Zoologica Scripta, 9*(1–4), 113–127.

Kristensen, R. M. (1982). New aberrant eutardigrades from homothermic springs on Disko Island, West Greenland. In Nelson D. R. (Ed.), *Proceedings of the third international symposium on the Tardigrada, August 3–6, 1980* (pp. 203–220). East Tennessee State University Press.

Kristensen, M. (1976). On the fine structure of *Batillipes noerrevangi* Kristensen, 1976. 1. *Tegument and Moulting Cycle. Zoologischen Anzeiger, 197*, 129–150.

Kristensen, R. M. (1981). Sense organs of two marine arthrotardigrades (Heterotardigrada, Tardigrada). *Acta Zoologica, 62*, 27–41.

Kristensen, R. M. (1983). The first record of cyclomorphosis in Tardigrada based on a new genus and species from Arctic meiobenthos. *Journal of Zoological Systematics and Evolutionary Research, 20*(4), 249–270.

Kristensen, R. M., & Higgins, R. P. (1984). A new family of Arthrotardigrada (Tardigrada: Heterotardigrada) from the Atlantic coast of Florida, USA. *Transactions of the American Microscopical Society, 103*, 295–311.

Marcus, E. 1929. Tardigrada. Bronns Klassen und Ordnungen Tier-Reichs 5 (4. Abt., Buch 3), viii+608 pp.

Massa, E., Rebecchi, L., & Guidetti, R. (2023). Composition and structural organization of tardigrades feeding apparatus focusing on chitin and other autofluorescent molecules. *Zoological Journal of the Linnean Society*, zlad028.

McInnes, S. J. (1995). Tardigrades from Signy Island, South Orkney Islands, with particular reference to freshwater species. *Journal of Natural History, 29*(6), 1419–1445.

Mira, A. (2000). Exuviae eating: A nitrogen meal? *Journal of Insect Physiology, 46*(4), 605–610.

Møbjerg, N., Jørgensen, A., Eibye-Jacobsen, J., Halberg, K. A., Persson, D., & Kristensen, R. M. (2007). New records on cyclomorphosis in the marine eutardigrade *Halobiotus crispae* (Eutardigrada: Hypsibiidae). *Journal of Limnology, 66*, 132–140.

Møbjerg, N., Jørgensen, A., Kristensen, R. M., & Neves, R. C. (2018). Morphology and functional anatomy. In R. O. Schill (Ed.), *Water bears: The biology of tardigrades* (pp. 57–94). Springer.

Murray, J. (1907). XXIV.—Scottish Tardigrada, collected by the Lake Survey. *Transactions of the Royal Society of Edinburgh, 45*, 641–668.

Murray, J. (1910). Part V Tardigrada. British Antarctic Expedition 1907–09, under the command of Sir E.H. Shackleton, c.v.o. Reports on the scientific investigations. W. Heinemann Publisher, London. Volume 1. *Biology, 5*, 1–185.

Nelson, D. R., Guidetti, R., & Rebecchi, L. (2015). Phylum Tardigrada. In J. H. Thorp, & D. Christopher Rogers (Eds.), *Thorp and Covich's freshwater invertebrates* (pp. 347–380). Academic Press.

Pilato, G., Guidetti, R., Rebecchi, L., Lisi, O., Hansen, J. G., & Bertolani, R. (2006). Geonemy, ecology, reproductive biology and morphology of the tardigrade *Hypsibius zetlandicus* (Eutardigrada: Hypsibiidae) with erection of *Borealibius* gen. n. *Polar Biology, 29*, 595–603.

Rackauskas, C., Koranda, J., Allen, S., Burries, R., Demski, K., Gore, L., Jung, T., Kane, K., Subaitis, C., Urban, B., & Whitman, D.

W. (2006). Molting inhibits feeding in a grasshopper. *Journal of Orthoptera Research, 15*(2), 187–190.

Ramazzotti, G., & Maucci, W. (1983). Il Phylum Tardigrada. III Edizione riveduta e aggiornata. *Memorie Dell'istituto Italiano Di Idrobiologia, 41*, 1–1012.

Rebecchi, L., Boschetti, C., & Nelson, D. R. (2020). Extreme-tolerance mechanisms in meiofaunal organisms: A case study with tardigrades, rotifers and nematodes. *Hydrobiologia, 847*(12), 2779–2799.

Renaud-Mornant, J. (1982). Species diversity in marine Tardigrada. In Nelson D. R. (Ed.), *Proceedings of the third international symposium on the Tardigrada, August 3–6, 1980* (pp. 149–178). East Tennessee State University Press.

Richters, F. (1904). Vorläufiger Bericht über die anktartische Moosfauna. *Verhandlungen Der Deutschen Zoologischen Gesellschaft, 14*, 236–239.

Schill, R. O., & Hengherr, S. (2018). Environmental adaptations: Desiccation tolerance. In R. O. Schill (Ed.), *Water bears: The biology of tardigrades* (pp. 273–293). Springer.

Schultze, M. (1865). *Echiniscus sigismundi*, ein Arctiscoide der Nordsee. *Archiv Für Mikroskopische Anatomie, 1*, 428–437.

Sugiura, K., & Matsumoto, M. (2021). Sexual reproductive behaviours of tardigrades: A review. *Invertebrate Reproduction & Development, 65*(4), 279–287.

Suzuki, A. C. (2003). Life history of *Milnesium tardigradum* Doyère (Tardigrada) under a rearing environment. *Zoological Science, 20*(1), 49–57.

Utsugi, K., & Ohyama, Y. (1989). Antarctic Tardigrada. *Proceedings of the NIPR Symposium on Polar Biology, 2*, 190–197.

Vecchi, M., Tsvetkova, A., Stec, D., Ferrari, C., Calhim, S., & Tumanov, D. (2023). Expanding *Acutuncus*: Phylogenetics and morphological analyses reveal a considerably wider distribution for this tardigrade genus. *Molecular Phylogenetics and Evolution, 180*, 107707.

Walz, B. (1982). Molting in Tardigrada. A review including new results on cuticle formation in *Macrobiotus hufelandi*. In Nelson D. R. (Ed.), *Proceedings of the third international symposium on the Tardigrada, August 3–6, 1980* (pp. 129–147). East Tennessee State University Press.

Wright, J. C. (1989). The tardigrade cuticle II. Evidence for a dehydration-dependent permeability barrier in the intracuticle. *Tissue and Cell, 21*(2), 263–279.

Wright, J. C. (1988). Structural correlates of permeability and tun formation in tardigrade cuticle: An image analysis study. *Journal of Ultrastructure and Molecular Structure Research, 101*(1), 23–39.

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