An Introduction to phylum *Tardigrada* - Review

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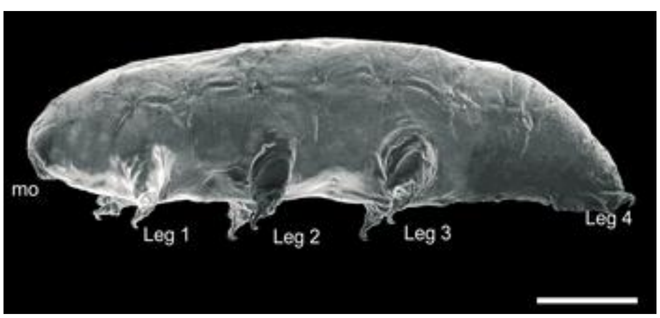
Abstract: **Tardigrades popularly known as water bears are micrometazoans with four pairs of lobopod legs. They are the organisms which can live in extreme conditions and are known to survive in vacuum and space without protection. Tardigardes survive in lichens and mosses, usually associated with water film on mosses, liverworts, and lichens. More species are found in milder environments such as meadows, ponds and lakes. They are the first known species to survive in outer space. Tardigrades are closely related to Arthropoda and nematodes based on their morphological and molecular analysis. The cryptobiosis of Tardigrades have helped scientists to develop dry vaccines. They have been applied as research subjects in transplantology. Future research would help in more applications of tardigrades in the field of science.**

Keywords: **Tardigrades, cryptobiosis, dry vaccines, Transplantology, space research**

I. INTRODUCTION

Tardigrade, a group of tiny arthropod-like animals having four pairs of stubby legs with big claws, an oval stout body with a round back and lumbering gait. They are also known as “water bears”. [1][2][3] These were discovered first by a German zoologist named Johann August Ephraim Goeze in 1773. Tardigrada ("slow stepper") was the name given later by the Italian biologist Lazzaro Spallanzani. [3]. Figure 1 illustrates the lateral view of a tardigrade [40].

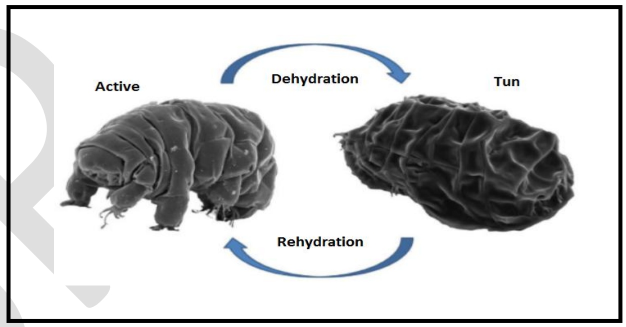
Figure 1: Hydrated specimen- lateral view



The most intriguing part of Tardigrades is their ability to go into *anabiosis*. When this process occurs, the tardigrade retracts its limbs, loses all its water content, and forms a double-walled thick cuticle. Their metabolism processes drop to a very low detectable level. [4][5]

In *cryptobiosis* (extreme form of anabiosis), the metabolism is undetectable and the animal is known as *tun* in this phase. Tuns have been known to survive very harsh environmental conditions such as immersion in helium at -272° C (-458° F) or heating temperatures at 149° C (300° F), exposure to very high ionizing radiation and toxic chemical substances and long durations without oxygen. [4] Figure 2 illustrates the process of transition of the tardigrades[41].

Figure 2: Transition process of Tardigrades



They are theoretically known to survive in space without protection. Some species are identified to survive in complete vacuum and cosmic radiations. The Tardigrades resume their metabolism state within a few hours when life supporting conditions prevail. [2][6]

Johann August Ephraim Goeze, a German pastor originally named the tardigrades as *kleiner Wasserbär (Bärtierchen* today), which means 'little water bear' in German. The name *Tardigrada* means "slow walker" and was coined by Lazzaro Spallanzani in 1776. [3] The name *water bear* is because of their locomotion, identical to a bear's gait. The biggest of adults may reach a body length of 1.5 mm and the smallest below (0.1 mm.). Newly hatched Tardigrades can be smaller than 0.05 mm. They can be found throughout the world, from the Himalayas (above 6,000 m (19,800 ft.)), to the deep sea (below 4,000 m (13,200 ft.)) and from the equator to Polar Regions. [8]

II. HABITAT OF TARDIGRADES

The usual habitats are lichens and mosses. They are often found when a piece of moss is soaked in water. They can be found in other environments such as soil, beaches, sand dunes, and freshwater or marine sediments, (up to 25,000 animals per liter). [9] They are known to live worldwide in moist habitats, along rocky shorelines, and in the bottoms of streams, lakes, and oceans. Tardigrades are most commonly associated with the water film on mosses, liverworts, and lichens. Their densities may reach 2.5 million individuals per sq. m (257,584 per sq. ft.) of moss. Some live in hot springs and a few live in symbiotic relationships with or on the bodies of other organisms. [8][9]

Tardigrades use a needle-like mouth to pierce the walls of plant cells and feed on the liquid inside them. Most of the species are plant eaters, but some are predators that feed on tiny invertebrates or bacteria and a few are detritivores, feeding on dead tissue and debris. [10][11]

III. ANATOMY AND MORPHOLOGY OF TARDIGRADES

Tardigrades have drum-shaped bodies with four pairs of stubby legs. They range from 0.1 to 0.5 mm in length, the largest species are known to reach 1.2 mm. The body consists of head, three body segments with a pair of legs each, and the posterior consists of four pair of legs. [10][14]

The legs have no joints while their feet have four to eight claws each. The cuticle contains chitin and protein and are moulted periodically. They are eutelic in nature, meaning all the adult Tardigrades of the same species have the same number of cells. [10][12][13][14]

The tardigrades typically have two eyespots on its head, each consisting of five or six cells, and one or more pairs of sensory bristles located on head and other regions of the body.[15] Its legs are hollow and each of them end in 1 to 12 toes tipped with claws or adhesive paddings. They move their legs with strands of muscle attached to the inside of the body covering. [10][14][16] The body contains a haemocoel (open circulatory system where blood freely flows inside the body), the only place where a true coelom is found is near the gonad. [16] There is no respiratory system, as gas exchange occurs across the whole body. These have two or three tubular glands usually associated with the rectum; these can be similar to the Malpighian tubules of arthropods, although the details remain unclear.[16] The mouth contains stylets, a needle-like projection which are used to pierce the plant cells, algae, or small invertebrates on which the tardigrades feed, releasing the body fluids or cell contents. The mouth opens into a triradiate (Y-shaped) muscular, sucking pharynx. They lose their stylets when they molt, and a new pair is secreted from a pair of glands present on either side of the mouth. The pharynx extends to a short esophagus, and then to an intestine that occupies major portion of the body, it is the main site of digestion. They also contain separate salivary glands. They also possess a buccopharyngeal apparatus, which, along with the claws, is used to differentiate between the species. [10][14][16] The intestine further extends via a short rectum, to an anus located at the terminal part of the body. Some species only defecate when they molt, leaving the feces behind with the shed cuticle.[16] The nervous system is mainly comprised of a mass of nerve cells in the head that leads to a double ventral nerve cord present in the bottom of the body cavity, with a pair of branches for each pair of legs. [17]

IV. REPRODUCTION IN TARDIGRADES

Tardigrades are of separate sexes, with males and females each having a sac-shaped gonad, or sex organ. In few species, the male deposits sperm under the female's cuticle.

The sperm then fertilizes the eggs as the female molts. She then deposits the eggs into the molted cuticle, laying 1 to 30 eggs at a time. Tardigrades are oviparous, and fertilization occurs externally. Mating occurs during the molt with the eggs being laid inside the shed cuticle of the female and then covered with sperm. Few species have internal fertilization method, where mating occurs before the female fully sheds her cuticle. In most cases, the eggs are left inside the shed cuticle to develop further, but some attach them to nearby substrate. The eggs then hatch after 14 days, with the young ones possessing complete developed adult cells. Growth is influenced by enlargement of individual cells called hypertrophy. They molt up to 12 times in a lifetime. [16][18]

V. PHYSIOLOGY OF TARDIGRADES

Tardigrades have been reported to exist in hot springs, on top of the Himalayan Mountains, under thick layers of ice in polar caps, and in ocean bed rock sediments. Many species are found in much milder environments such as meadows, ponds and lakes. They are mostly observed in moist environments, but stay active wherever and whenever they have little moisture content. [5] Tardigrades have evolved with suite of survival tactics to escape localized and vulnerable environments. Anoxybiosis and encystment, are some of the responses one might observe in a variety of organisms. Cryptobiosis, is a state where metabolism is suspended—an act diagnostic of death. Cryobiosis occurs due to freezing, and anhydrobiosis due to drying. [4][5]

In the latter, the organism surrenders its water content in order to become a desiccated pellet. Both result in the formation of a durable shrunken state called a *Tun*. In this state, tardigrades can survive for many years, resistant to extremities beyond the ones encountered in their natural environments. Depending on the environment they live, they enter cryptobiosis via cryobiosis, anoxybiosis, osmobiosis or anhydrobiosis. Their metabolism lowers to < 0.01% of normal and their water content drops to nearly 1% of normal. [4][5]Their ability to exist desiccated for such a long state is dependent on high levels of the non-reducing sugar trehalose (a non-reducing sugar) that protects the membranes. [4][5]

Tardigrades are able to survive in extreme environment conditions. Tardigrades can survive up to 151 °C (304 °F),[19] or −200 °C (-328 °F).[19] Some even survive cooling at −272 °C (~1 degree above absolute zero or -458 °F). [20] They are able to withstand extremely low pressures of vacuum and pressures, usually more than 1,200 times that of atmospheric pressure. They are able to survive the combined solar radiations and vacuum of space for at least 10 days.[21] Some species are able to withstand high pressures which are nearly six times that of pressures experienced in Mariana Trench. [12]

Anoxybiosis occurs due to low oxygen. Tardigrades are very sensitive to oxygen levels. Prolonged asphyxia results in failure of the osmoregulatory, causing the tardigrade to puff up and float around for a few days until its habitat dries out and it can resume active life. [1] Some tardigrades exhibit effective osmoregulation through Osmobiosis. Osmobiosis is a response to extreme salinity, which causes destructive swelling. Some others escape by forming a tun that is resistant to osmotic transfer. [1] The longest surviving Tardigrades are known to live in dry conditions for nearly 10-12 years,[22][23] although there was one report of a limb movement, not really "survival" technically speaking,[21] in a 120-year-old specimen from dried moss.[24] When exposed to extremely low temperatures nearly -250°C, the body composition reduces from 85% of water to only 3% of water or even less. Due to anomalous expansion of water, dehydration ensures that Tardigrades don't rip apart due to freezing ice.[25] Tardigrades withstand 1,000 times more radiation than any other organism, [26] median lethal doses of 5,000 Gy (ɤ-rays) and 6,300 Gy (heavy ions) in hydrated animals (5 to 10 Gy is fatal to a humans).[27] The only explanation is their ability that lowered water state provides only a fewer reactants for the ionizing radiations.[27] However, later researches showed that Tardigrades, when hydrated, are highly resistant to UV radiation (of lower frequencies) in comparison to other organisms, and they have an ability to efficiently repair DNA damage that results from the radiation exposure.[28] Tardigrades undergo chemobiosis that is tolerance towards high levels of environmental toxins. As of 2001, the laboratory results are yet to be verified.[25] [29]

Tardigrades are the first known species of organisms to survive outer space. On September 2007, dehydrated Tardigrades were taken aboard FOTON-M3. For 10 days, groups of Tardigrades were exposed to harsh vacuum of outer space and solar UV radiation.[2] [6] After rehydrating them back on Earth, more than 68% of the subjects were protected from high-energy UV radiation and quickly revived within 30 minutes, but subsequent mortality was high; producing viable embryos.[21] [30] In contrast, dehydrated samples when exposed to combined effect of vacuum and full solar radiation there was significant reduction in survival, with only 3 subjects of *Milnesium tardigradum* had survived.[21] In 2011, Italian scientists sent Tardigrades to the International Space Station [ISS] along with other extremophiles in STS-134, the final flight of Space shuttle *Endeavour*. [31][32] The conclusion was that microgravity and cosmic radiation "had no significant effect on survival of Tardigrades in flight, confirming that Tardigrades are useful organisms for space research." [33]

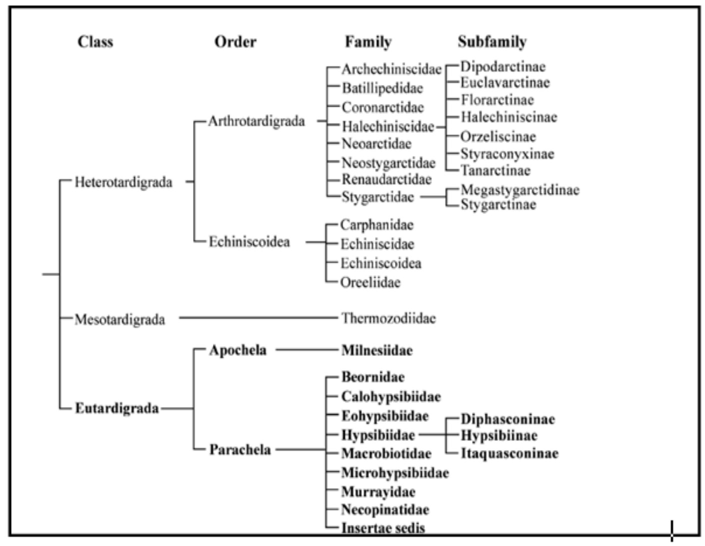
VI. EVOLUTION HISTORY OF TARDIGRADES

Many numbers of morphological and molecular studies have been made to understand the relationship between tardigrades and other ecdysozoan organisms. Two credible situations have been noted: tardigrades are most closely related to Arthropoda ± Onychophora (a result of morphological studies) and tardigrades are mostly related to nematodes (found in some molecular analysis). The latter idea is rejected based on EST analysis.[34] Three types of relationship are possible:

1. (The lobopodia hypothesis) tardigrades are sister to onychophora plus arthropods;
2. (The tactopoda hypothesis) onychophora sister to tardigrades plus arthropods;
3. Onychophora sister to tardigrades.[35]

Recent analysis showed that the panarthropoda group is monophyletic, and tardigrades are sister group of Lobopodia, lineage consisting of both Onychophora and Arthropoda. [36]. Figure 3 indicates the taxonomical features of tardigrades [11].

Figure 3: Taxonomical Features of Tardigrades



Between rare specimens in Cretaceous amber comprise *Milnesium swolenskyi*, from New Jersey, the oldest subject, whose claws and mouthparts are indistinguishable from the living *M. tardigradum*; and two specimens from western Canada, some 15–20 million years younger than *Milnesium swolenskyi.* The latter ones have their own genus and family, *Beorn leggi* (the genus named by Cooper after the character Beorn from *The Hobbit* by J. R. R. Tolkien and the species named after his student William M. Legg); however, it bears a strong resemblance to many living specimens in the family Hypsibiidae. [36] [37]

VII. APPLICATIONS

- Their cryptobiosis have helped scientists to develop 'dry vaccines'. In such vaccines water is replaced with trehalose. Dry vaccines don't require refrigeration and hence are delivered and stored at room temperature. [23]
- Tardigrades are used as research subjects in field of transplantology. Because of their cryptobiotic behaviour, they can be revived at any given time. [4]
- Panspermia Hypothesis – It is a theory that life on planets originates from organisms or chemical agents brought by meteorites from outer space which further

initiate life on planets. Tardigrades can be used to prove the above hypothesis. They can be used for long distance space travelling as they undergo cryptobiosis. [4][6][21][38]

- Their impeccable ability to repair heavily damaged DNA can help in treatment of cancers. [39]

VIII. CONCLUSION

Tardigrades, the “water bears” are water residents having four pairs of legs. They are known to survive in extreme conditions like vacuum and space without protection. The morphological and anatomical features are well studied and have applications in medical field in development of dry vaccines. They have been applied in Transplantology because of theor cryptobiotic behavior. Though there are studies emphasizing on their identification and importance, there is a need for more research to understand their relevance in different fields of science.

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