Tardigrades (Tardigrada) in Baltic States

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The tardigrades (Tardigrada), one of the micrometazoans groups, are reviewed. Tardigrades are distributed almost everywhere: in moss, lichens, soil, in niches of freshwater and seawater. The ability of tardigrades to undergo cryptobiosis has aroused interest among world scientists who would like to apply this mechanism to other biological systems that might benefit from dry storage in extreme conditions. This paper reviews the latest information on tardigrades taxonomy and phylogenetic position, distribution, some aspects of their biology, reproduction and life history. The present work also provides the data of research of tardigrades in the Baltic states.

Key words: tardigrada, eutardigrada, heterotardigrada, freshwater, life history, anhydrobiosis, cryptobiosis

INTRODUCTION

Scientific knowledge of invertebrates varies from group to group. The best-studied groups are insects, crustaceans and mollusks. Even in these well-studied groups, there are huge information gaps, especially for smaller animals and those living in developing countries or underground habitats (Strayer, 2006). Little is known about "minor" phyla: bryozoans (Ryland, 2005; Dunn et al., 2008; Massard, Geimer, 2008), gastrotrichs (Balsamo et al., 2008; Kieneke et al., 2012; Paps, Riutort, 2012), nematomorphs (Hanelt et al., 2005; Poinar, 2008) and freshwater tardigrades (McFatter et al., 2007; Garey et al., 2008; Meyer, 2011). Meanwhile some

of these groups are widespread and abundant. For example, gastrotrichs probably occur in most freshwater habitats throughout the world (Strayer, 2006). The tardigrades are one of most abundant invertebrates in moss and lichens, meanwhile little is known about the tardigrades in the Baltic countries.

Some of these micrometazoans could be used as bioindicators, as model organisms for studies of development mechanisms or for experiments in the open space.

Tardigrades are referred to as one of the "lesser-known" groups of micrometazoans (Nelson, 2002; Romano, 2003). Presently, the exact number of various Tardigrada species is unknown. In order to increase knowledge about these invertebrates and also to explore the possible biotechnological

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and biomedical applications of their biology, more economic and human resources need to be focused on this group (Guil, Cabrero-Sañudo, 2007). The aim of this study was to summarize the latest information on tardigrades biology, significance and provide the most recent research data on tardigrades in the Baltic States.

Some aspects of tardigrade biology

Tardigrada is known as "water bear" that bears this descriptive name based on the animal's lumbering gait (*tardi* – slow, *grade* – walker) and also because these invertebrates usually live in water. Tardigrades are one of the smallest metazoa and their size is close to that of rotifera, gastrotricha or microscopic nematodes. Body length of adult tardigrade is approximately 250–500 μm (Nelson, 2002). Like in the arthropods, the body of tardigrades is covered by cuticle. External layer of cuticle is soft, lacks calcium and is built from a protein albuminoid which differs from chitin (Gagyi-Palffy, Stoian, 2011). The inner layer of the cuticle contains chitin. Tardigrades molt and change their cuticle like the arthropods. Molting occurs every five to ten days throughout the tardigrade's life (Nelson, 2002).

Water bear may be detritovorious, bacteriovorous, carnivorous and herbivorous (Romano, 2003). Irrespective of the wide range of food consumption, tardigrades do not display a great variation in the organization of the digestive system. The tardigrade buccal–pharyngeal apparatus that is used to suck out the content of cells is a complex structure with a considerable taxonomic significance among taxa belonging to several taxonomic levels, particularly in the eutardigrades (Pilato, Binda, 2010). The buccal–pharyngeal apparatus consists of cuticular structures such as a buccal ring, a buccal tube with apophyses for muscle attachments, stylets and a muscular sucking pharynx (containing placoids) (Nelson et al., 2010; Guidetti et al., 2012) (Fig. 1).

During the last decade various aspects of anatomy and physiology of Tardigrada have been studied. Greven (2007) provided information on the visual organs of tardigrada. Many Eutardigrada and some of Echiniscidae possess inverse pigment-cup ocelli, which are located in the outer lobe of the brain, and probably are of cerebral origin (Fig. 2). Depending on the species, response

to light (photokinesis) is negative, positive or indifferent and may change during the ontogeny. Greven (2007) states that the homology of the tardigrade eyes with the visual organs of other bilaterians is difficult to establish and further comparative studies are needed.
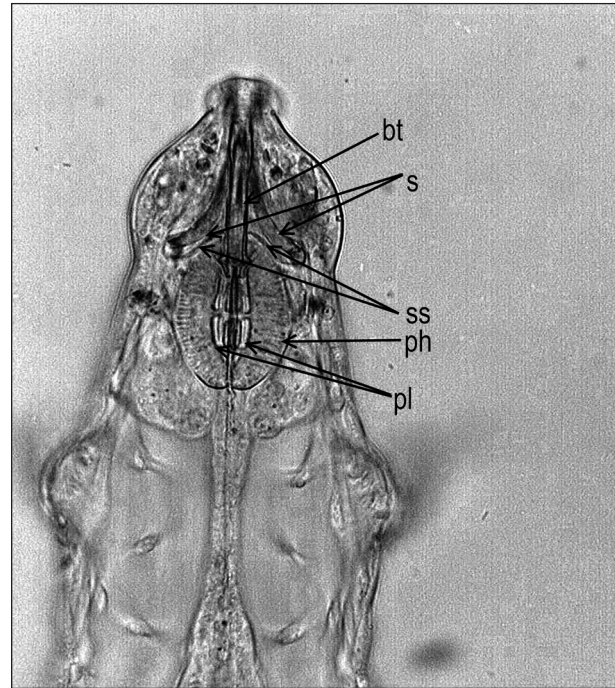

Fig. 1. General structure of buccal apparatus of eutardigrade. *m*, mouth; *bt*, buccal tube; *s*, stylets (which serve as a tool to pierce plant cells); *ss*, stylet supports; *ph*, pharynx; *pl*, placoids
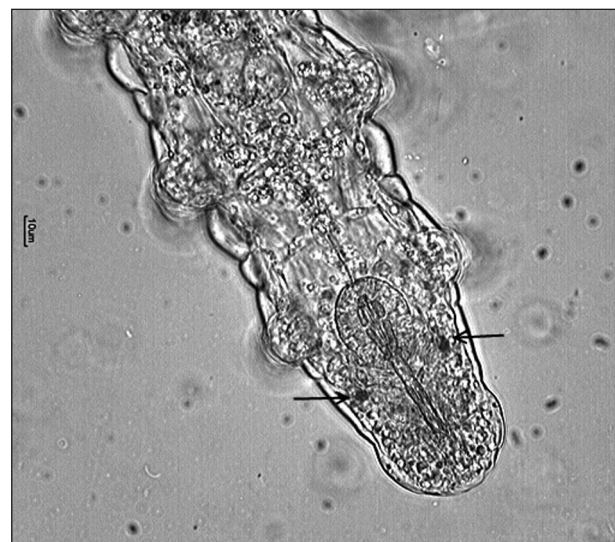

Fig. 2. The anterior part of the body of tardigrade with two eyes (arrows)

The analysis of the nervous system of Tardigrada plays an important role considering the phylogenetic afinities of the groups (Dewel, Dewel, 1996, 1997; Nielsen, 2001). Although the central nervous system of tardigrades is generally described as belonging to the rope-ladder-like type comprised of ganglia with connectives and commissures, unambiguous documentation of commissures is almost absent in the literature (Zantke et al., 2008). Meanwhile the use of more neuroanatomical data of Tardigrada should contribute to our understanding of tardigrade phylogenetic afinities in a more global approach considering a variety of morphological and molecular data (Zantke et al., 2008).

Considering the reproduction system of tardigrades it is noteworthy that it is variable and species-dependent, it may be dicoeciuos, hermaphroditic and may reproduce in sexual or parthenogenetical way. Using cryptobiosis and passive dispersal, parthenogenesis is favorable in colonizing new, isolated and unstable habitats, as beginning with a single individual is all that is required (Altiero et al., 2006). For marine species (mainly heterotardigrades) parthenogenesis is unknown, meanwhile in limno-terrestrial tardigrades always appear continuous (Bertolani, 2001). Hermaphroditism in limno-terrestrial species occurs in several eutardigrade families (Pilato et al., 2006) and in Heterotardigrada only one species is hermaphroditic (Bertolani, 2001). Hermaphroditism is not common in tardigrades, indicating that this model of reproduction is a less frequent sexual condition. The findings of a hermaphroditic species confirm that in tardigrades this condition develops independently in different evolutionary lines (Pilato et al., 2006).

Sexual dimorphism was established for one species of Heterotardigrada – *Echiniscus mauccii* (Mitchell, Romano, 2007) and three other *Echiniscus* species (Miller et al., 1999). Females, distinguished by their gonopore, are larger than males (Mitchel, Romano, 2007). Similar sexual dimorphism (females are larger than males) is observed in two eutardigrada species: *Macrobiotus richtersi* and *Hypsibius convergens* (Guidetti et al., 2007). Data covering the gametogenesis of Tardigrada is especially scarce (Poprawa, 2005).

Reproduction of tardigrades is coupled with molt. The female subsequently moults, laying her

eggs in the shed cuticle (Fig. 3). Gonad size is constrained by the cuticle capacity and by the gut size (food source) that competes for the space within the body cavity (Guidetti et al., 2007).

All tardigrada produce eggs, which consist of polysaccharides, peptides and lipids (Poprawa, 2005) and are often essential for a correct identification of tardigrada species (Fig. 4a, 4b, 4c) (Cromer et al., 2007).

The behavior and chemical communication at the time of reproduction of tardigrades are unknown. The information on the origin and rele-
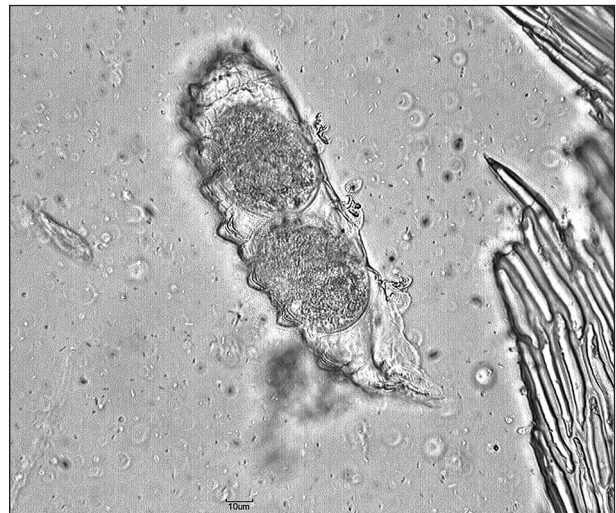

Fig. 3. Many tardigrades store their eggs underneath the cuticle, afterwards shed them along with the cuticle when moulting
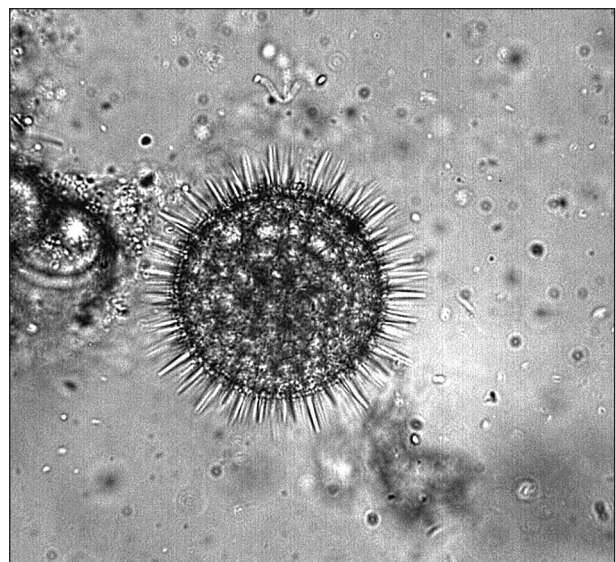

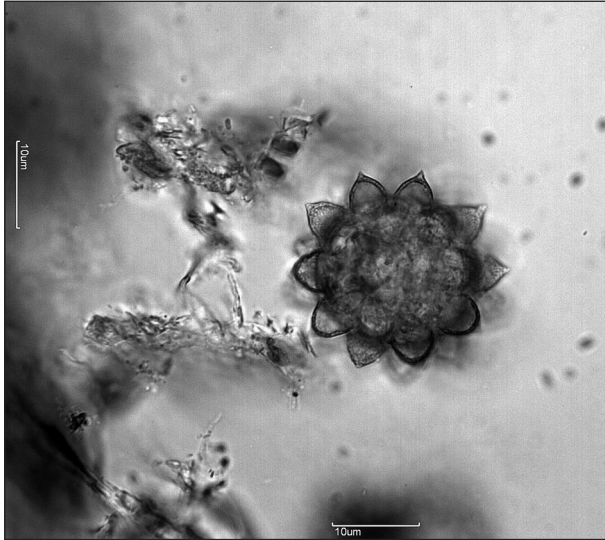**Fig. 4a.** The egg of *Richtersius coronifer* found in moss *Bryum* sp.

Fig. 4b. The egg of *Macrobiotus richtersii* (?) found in moss *Pleurozium schreberi*
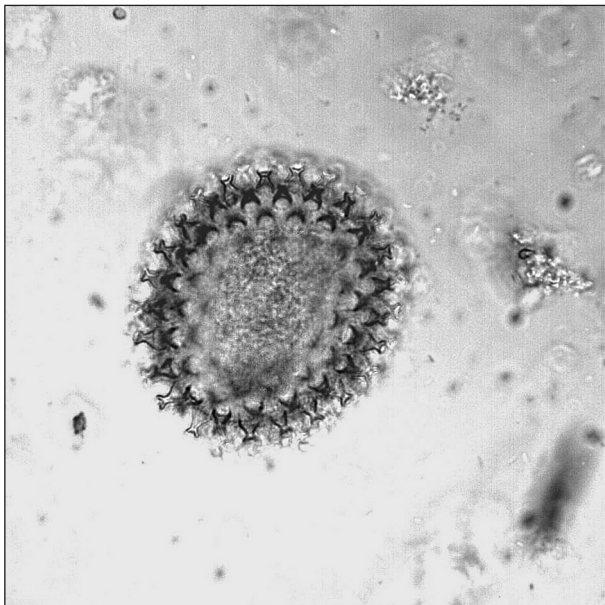

Fig. 4c. The egg of *Macrobiotus hufelandi* found in moss *Bryum* sp.

vance of sex steroid receptors in invertebrates are still limited (Köhler et al., 2007).

Parental care is not common in tardigrades, however Pilato et al. (2006) mentioned that newborns of *Hypsibius zetlandicus* (Eutardigrada) are still within the old cuticle of the parent and this could be an indication of the possibility of "parental care".

Time of reproduction is not clear for many species of tardigrades. Mitchel and Romano (2007) observed that sexual reproduction of

Echiniscus mauccii continues for the entire year and suggested that this species has classic K-selected traits.

Life history traits are always very interesting subjects in the study of animal adaptation and evolution. Despite this importance, literature data on the life history traits and population dynamics of freshwater and semiterrestrial species of tardigrades are scanty and fragmentary (Mitchell, Romano, 2007), while actually no data are available for marine species (Altiero et al., 2006).

Distribution and habitats of tardigrades

The phylum Tardigrada has a worldwide distribution (Nelson, 2002; Bertolani et al., 2004) that is related with the ability of Tardigrada to survive in extreme environments and to use cryptobiosis together with passive dispersal and parthenogenesis. Theoretically, parthenogenesis allows the colonization of new and remote territories beginning with a single individual (Miller, Heatwole, 1996; Bertolani, 2001). Despite this possibility to spread, a very high number of tardigrada species have limited geographical distribution and are adapted to restricted microenvironmental conditions (Pilato et al., 2006).

Tardigrades use various habitats: marine and estuarine, freshwater and terrestrial. Information on the distribution and ecology of limnoterrestrial Tardigrada is scarce (McFatter et al., 2007). They are benthic or crawl on water vegetation. Most species live in the littoral zone (Nelson, 2002). The majority of these organisms are not real hidrophylous and are found in terrestrial and aquatic environments. Numerically, limnoterrestrial tardigrades comprise a minor component of benthic invertebrate communities. Knowledge is lacking about their tropic relationships or dispersal in benthic habitats (McFatter et al., 2007).

Terrestrial tardigrades are inhabitants of mosses, lichens, soil and leaf litter. Tardigrades in leaf litter from beech forests (Guidetti et al., 1999) and soil (Bertolani et al., 1996; Nelson, 2002) exhibit high species diversity and high densities, but the evidence of tardigrades for substrate specificity is weak. Many species of tardigrades may be present in soil and leaf litter, but few were found only in these substrates (Meyer et al., 2007). Jönsson (2003) indicated that different mosses' growth

form might have an impact on tardigrada abundance. Meyer (2006; 2007) noted that some tardigrada species were significantly associated with mosses or foliose lichens in general, but no significant association was detected between a tardigrada species and a substrate species.

Tardigrada densities in soil are high and may vary from 300 to 33,600 individuals in m² (Hohberg, 2006). Soil-inhabiting tardigrades have high metabolic rates, hence they are sensitive to subtle environmental variances and respond to structural changes in the soil (Harada, Ito, 2006).

There are few studies on the relation of tardigrada diversity and richness and season. Some scientists have proposed that tardigrada communities are stable through time and there are not significant differences comparing the tardigrada density and community structure between different seasons (Peluffo et al., 2006). Meanwhile McFatter (2007) mentioned that the density of Nearctic freshwater tardigrada species peaks up in the spring and / or fall. This disagreement indicates that more studies and more data are needed in this field.

Taxonomy and phylogeny

More than one thousand of Tardigrada species were included in the recently published checklist (Degma et al., 2009–2012). Based on morphological characteristics, the phylum of tardigrada is divided into two major classes: Heterotardigrada (armored tardigrades) and Eutardigrada (naked tardigrades) (Nichols et al., 2006).

The exact number of species of tardigrades is not known because of insufficient efforts invested in the tardigrades compared to other invertebrates (such as Insecta: Coleoptera), and that there is a need of engagement of more taxonomists and more extensive sampling areas (Guil, Cabrero-Sañudo, 2007).

Dormancy of tardigrades: anhydrobiosis

Dormancy is an important adaptation strategy that allows many organisms to survive in unfavorable environmental conditions. Tardigrades have two forms of dormancy, namely cryptobiosis and encystment.

Tardigrades and some others organisms (Nematoda, Rotifera) are capable of entering a latent state (cryptobiosis) when environmental conditions are unfavorable, e. g. freezing, desiccation, low oxygen tension, and salinity variations. When tardigrada are in a latent state, their metabolism is reduced. In contrast to death, cryptobiosis is a reversible state and as soon as environmental conditions change, tardigrada return to life (Neuman, 2006). Five types of cryptobiosis are distinguished: encystment, anoxybiosis, cryobiosis, osmobiosis and anhydrobiosis (Nelson 2002; Bertolani et al., 2004; Watanabe, 2006).

To remain active, all tardigrades require water. One type of cryptobiosis, namely anhydrobiosis, is induced by water loss and occurs in eggs, juveniles and adults of terrestrial eutardigrades and echiniscids (Nelson, 2002; Rebecchi et al., 2007). Anhydrobiotic tardigrades always shrink in structure resembling "tun" when dehydrated (Wright, 2001; Bertolani et al., 2004; Watanabe, 2006). When tardigrades are desiccated at a low relative humidity or under anoxia they cannot form tun and be revived (Watanabe, 2006). Various species of tardigrada are characterized by interspecific differences in cryptobiotic (anhydrobiosis and cryobiosis) survival possibility (Jönsson et al., 2001; Bertolani et al., 2004).

Jönsson and Rebecchi (2002) indicate that the phenotypic state of the individuals has an impact on the probability to survive a period of anhydrobiosis. Effects of body size on anhydrobiotic survival suggest age – specific selection, and effects of energy status indicate that energy allocations to anhydrobiotic functions are also object to selection of evolution (Bertolani et al., 2004).

Biochemical mechanism of cryptobiosis (anhydrobiosis) is also an object of interest. Trehalose is known as a common compatible solute in anhydrobiotic organisms from unicellular organisms to invertebrates and higher plants. Trehalose may provide effective protection against desiccation because of its superior biochemical and physicochemical properties for stabilizing membranes and biomolecules including proteins and lipids (more about anhydrobiosis see review by Watanabe, 2006).

Trehalose is produced prior to the stages of anhydrobiosis and cryobiosis. Recently, it has become clear that the phenomenon of cryptobiosis is much more complex than initially thought to be. New results show that it is not only trehalose that is responsible for survival during cryobiosis

and anhydrobiosis. Some species of tardigrades produce trehalose in small amounts (2–3% of dry weight) compared to other invertebrates capable to devolve anhydrobiotic state (Jönsson, 2007). Hengherr et al. (2008) revealed significant differences between eight tardigrada species (Heterotardigrada and Eutardigrada) on the trehalose levels in different states of hydratation and dehydratation. Trehalose accumulation was found in some species of Eutardigrada but not detected in the species *Milnesium tardigradum* and no change in trehalose level had been observed in any species of Heterotardigrada (Hengherr et al., 2008). The study of Jönsson and Schill (2007) suggests that the desiccation cycles of some species of tardigrades are related with stress proteins of the family Hsp70. These proteins participate in unfolding and recolonization of proteins damaged by stresses and protect newly synthesized proteins from denaturation and aggregation (Watanabe, 2006). This group of proteins may play a role in the post-anhydrobiotic repair process (Jönsson, 2007). The phenomenon of cryptobiosis, when a tardigrada can go into a reversible death (ametabolic stage) for many years, and after a few minutes of rehydration can climb around again, might propose some hints to explain how life developed on earth.

Significance of tardigrades

Tardigrades share many characteristics with *Caenorhabditis elegans* and *Drosophila* that could make them useful as laboratory models, but there have been few studies of long-term culturing of tardigrades (Suzuki, 2003). Gabriel et al. (2007) demonstrated that the tardigrade *Hypsibius dujardini* can be cultured continuously for decades and can be cryopreserved. It has been reported that *H. dujardini* has a compact genome, a little smaller than that of *C. elegans* or *Drosophila* and can serve as a model for studying the evolution of developmental mechanisms (Gabriel et al., 2007).

Other significant feature of tardigrades is their tolerance to very high doses of ionizing radiation. Hydrated tardigrades have shown similar or higher tolerance to irradiation compared to dehydrated, anhydrobiotic tardigrades (Jönsson, 2007). This suggests that radiation tolerance in these animals is not restricted to biochemical protection mechanisms of the dry cells (Jönsson, 2007).

Tardigrades are relatively more vulnerable to UV irradiation than to γ-irradiation in the hydrated state (Jönsson, 2007). The observed tolerance to ionizing radiation, particularly in active tardigrades, may rely on an efficient DNA repair system. However, this mechanism of DNA repair will remain speculative and still must be verified (Jönsson, 2007).

Tardigrades with abilities to stand complete desiccation, cold and high levels of ionizing and UV radiation provide opportunity for studies on the response of living multicellular organisms exposed to open space (Jönsson, 2007).

Moreover, tardigrades may even be of economic interest due to their ability to undergo cryptobiosis, an environmentally resistant state, when conditions are unfavorable; the substances involved in cryptobiosis have potential applications in biomedicine and biotechnology (Guil, Cabrero-Sañudo, 2007). The pharmaceutical industry has been very interested in the role of sugar trehalose that tardigrades produce prior to the stages of anhydrobiosis and cryobiosis. Trehalose appears to protect the cellular membranes of tardigrades against the damage of freezing and dehydration and therefore provide stability for biological products (Crowe et al., 1996). Vaccines and restriction enzymes can be stored in a trehalose formulated dry state at +70 °C for one month without lost activity (Colaco et al., 1992). A number of biological products (monoclonal antibodies; pharmaceutical, foodstuff) have been stabilized using trehalose (Guo et al., 2000). Trehalose may be used in organ transplantation to avoid freezing damage and to safely preserve human eggs and those of endangered species, giving better options to young women facing cancer therapies that may leave them infertile and others who simply want to delay reproduction. Introduction of sugars into cells and into oocytes can protect them against freezing-associated stresses. Researchers injected the eggs of mouse with trehalose, cooled them to liquid nitrogen temperature, thawed them and exposed them to sperm. They got healthy babies at a similar rate to unfrozen controls (Eroglu et al., 2003).

These microscopic metazoans are significant as bioindicators of environmental pollution. Most terrestrial tardigrades live in mosses and lichens. Numerous studies proved that mosses are valuable bioaccumulators and the concentrations of the

heavy metals in mosses closely correlate to atmospheric deposition (Vargha et al., 2002). Elevated heavy metal contents decrease the number of water bear species and of specimens, and abundance of tardigrada strongly depends on air pollution (Vargha et al., 2002). The moss-dwelling fauna could be a sufficiently sensitive tool for measuring the ecological consequences of pollution on the soil biota (Peluffo et al., 2006).

Investigations of terrestrial tardigrades in the Baltic States

The investigation of tardigrades in the Baltic States is insufficient. Two eutardigrade species groups: *Paramacrobiotus richtersi* and *Macrobiotus hufelandi* were recorded in Latvia (Ziemelis et al., 2012). Two species of genus *Isohypsibius*: *Eremobiotus alicatai* (Binda, 1969) and *Isohypsibius marcellinoi* (Binda, Pilato, 1971) were found in Estonia (Zawierucha, Kaźmierski, 2012). Little is known about tardigrades in Lithuania. Two genera: *Macrobiotus* sp. and *Ramazzottius* sp. have been reported in Lithuania two years ago (Šatkauskienė, Vosyliūtė, 2010) and eight genera of tardigrades belonging to four families of eutardigrades have been found in Lithuania last year (Šatkauskienė, unpublished data, 12th International Symposium on Tardigrada, 2012).

CONCLUSIONS

Tardigrada are micrometazoans organisms with a cryptobiotic ability to survive in unfavourable environmental conditions. However, information of many aspects of their biology remain unclear. Irrespective of the most recent molecular investigations, phylogenetic position of these organisms still remains at the level of controversy. Data of the tardigrada relation with habitat type, substrate specificity and community ecology are not comprehensive. Little is known about their sexual dimorphism, sexual behaviour and gametogenesis. The tardigrades belonging to freshwater and marine species are especially scarcely described. Despite of the fact that they are common in moss, lichens and soil, the available information on tardigrada in Lithuania so far is very sparse.

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Ingrida Šatkauskienė

BALTIJOS ŠALIŲ LĖTŪNAI

Santrauka

Straipsnyje apžvelgiama viena mikroskopinių daugialąsčių organizmų grupė – lėtūnai (Tardigrada), išplitę įvairioje aplinkoje: samanose, kerpėse, dirvožemyje, gėlame ir jūriniame vandenyje. Dėl kriptobiotinių biologinių savybių ir jų pritaikymo lėtūnai pastaruoju metu sulaukia nemažai pasaulio mokslininkų dėmesio. Straipsnyje pateikiami kelerių pastarųjų metų duomenys apie lėtūnų paplitimą, taksonomiją ir filogeniją, aptariami kai kurie biologijos, dauginimosi ir ekologijos ypatumai. Pateikiama informacija apie lėtūnų ištyrimą Baltijos šalyse.

Raktažodžiai: lėtūnai, anhidrobiozė, kriptobiozė, gėlavandeniai, eutardigrada, heterotardigrada