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# On the occurrence and density of some tardigrade taxa in the city area of Plovdiv, Bulgaria

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**Abstract**. There is insufficient data on tardigrades' distribution, diversity and density. Moss and lichen samples were collected from four different substrates – ground, walls, rocks and trees. Specimens from genus *Echiniscus* Schultze, 1840, genus *Hypsibius* Ehrenberg, 1848, genus *Milnesium* Doyère, 1840, genus *Paramacrobitous* Guidetti, Schill, Bertolani, Dandekar & Wolf, 2009, genus *Ramazzottius* Binda & Pilato, 1986 and family Macrobiotidae Thulin, 1928 were found.

Key words: Tardigrada, Plovdiv city, habitats.

### Introduction

Tardigrades, also called water bears or moss piglets, are small metazoans within the size range of 0.1 – 1 mm. They are found on every continent, from marine, freshwater and terrestrial habitats. Tardigrades are best known for their ability to undergo cryptobiosis and survive unfavourable condition, such as desiccation up to 10 years (Jönsson & Bertolani 2001), -272.95°C for 20 hours or -200°C for 20 months (Miller 1997), 151°C for a few minutes (Lindahl & Balser 1999), pressures of 6000 atmospheres (Seki & Toyoshima 1998) and the vacuum and UV radiation of space (Jönsson *et al.* 2008).

Despite their ability to survive extreme environmental conditions, little is known about their ecology, behaviour, densities and the structure of their populations, especially at human settlements. Different factors, such as temperature, moisture, pH of the substrate, altitude, food availability, air pollution, inter and intraspecific competition, combined or separately, may play role in tardigrades densities and distribution. Random dispersal can also be considered as factor affecting water bears' populations.

Quantitative and qualitative research on tardigrades' populations are generally focused on the relation between abundance and altitude, different microhabitats, urban and rural areas. Some studies on substrate specificity and spatial distribution of terrestrial tardigrades established that there is weak substrate specificity and that the variation in tardigrades' diversity and abundance is high (Meyer 2006a, 2006b).

The aim of this study is to provide information on moss and lichen dwelling limnoterrestrial tardigrades in a city environment, and the importance of the base substrate on their occurrence and specimen-densities.



## **Material and Methods**

A total number of 87 moss and lichens samples were collected from Plovdiv city and Plovdiv region. The sampling was mainly focused on urban habitats (55.2%) with fewer samples collected from suburban (22.9%) and from habitats in Plovdiv vicinity (21.8%). The samples were collected from four main substrate types – ground (29.9% of all samples), walls (24.1%), rocks (11.5%) and trees (32.2%).

At the laboratory the samples were soaked in tap water for 6 to 24 hours. After this period, water containing tardigrades, their eggs and sample particles was decanted and further examined under stereomicroscope and light microscope. Specimens were mounted on microscope slides with glycerol or Hoyer's medium. A total of 252 tardigrade specimens from 6 genera and 1 family were registered.

All species were identified to genus level using original descriptions and modern keys mainly by Ramazzotti & Maucci (1983), Guidetti & Bertolani (2005); Michalczyk *et al.* (2012a, b); Kaczmarek *et al.* (2011); Morek *et al.* (2016), Kaczmarek & Michalczyk (2017), Kaczmarek *et al.* (2017). Systematic follows Degma *et al.* (2018).

## **Results and Discussion**

Tardigrades were found in 46% of all collected samples, with individuals from genus *Milnesium* found in 17.8% of the samples. Water bears were discovered in 50% of the urban samples, in 36.8% of the suburban and in 50% of the vicinities' samples. Individuals belonging to genus *Echiniscus*, *Hypsibius*, *Paramacrobitous*, *Ramazzottius*, family Macrobiotidae and unidentified eutardigrades were also found in the samples.

Genus *Echiniscus* had average density of  $3.5 \text{ ind./cm}^3$  for the urban samples and was registered in one suburban locality with density of 6 ind./cm<sup>3</sup>. The average density of *Milnesium* spp. from the urban habitats was  $6.9 \text{ ind./cm}^3$ , for the suburban –  $2.7 \text{ ind./cm}^3$  and for the samples collected from Plovdiv vicinity –  $1.8 \text{ ind./cm}^3$ . The average density of family Macrobiotidae was  $12.8 \text{ ind./cm}^3$  for urban habitats and 6 ind./cm<sup>3</sup> for Plovdiv vicinity, with no individuals identified from the suburban habitats. Specimens from genus *Hypsibius* and *Paramacrobiotus* were registered with densities of 1 individuals/cm<sup>3</sup> and were found in few localities. The specimens from genus *Ramazzottius* had average of  $5.1 \text{ ind./cm}^3$  from urban samples,  $5 \text{ ind./cm}^3$  from suburban and  $2 \text{ ind./cm}^3$  from samples from Plovdiv vicinity.

*Echiniscus* specimens had average density of 4 ind./cm<sup>3</sup> for rocks and were established in one sample from ground substrate with density of 5 ind./cm<sup>3</sup>, with no individuals found from walls and trees. In the present study highest average density of *Milnesium* spp. was established in samples collected from rocks (8.5 ind./cm<sup>3</sup>) and lowest – from trees (3 ind./cm<sup>3</sup>). The individuals of family Macrobiotidae had highest average density for samples from ground substrate (16 ind./cm<sup>3</sup>) and lowest - from rock substrate (3 ind./cm<sup>3</sup>). The higher density for ground substrates is due to sample containing more than 40 ind./cm<sup>3</sup>. Individuals from genus *Hypsibius* were established in two tree samples with average density of 1 ind./cm<sup>3</sup> and specimens from genus *Paramacrobiotus* were present in one tree sample with density of 1 ind./cm<sup>3</sup> and lower for rock formations (3 ind./cm<sup>3</sup>), trees (2.8 ind./cm<sup>3</sup>), with no individuals found from ground substrates (Fig. 1).

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Fig. 1. Individuals per 1cm3 moss or lichens samples from different substrates.

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