Urban Tardigrades from Plovdiv City and Some Ecological Remarks

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Abstract. There is insufficient data on urban tardigrade distribution, diversity and density. The present paper is based on preliminary data of an ongoing research of tardigrade diversity and ecology in Plovdiv region. Specimens from family Macrobiotidae Thulin, 1928, from genus Milnesium Doyère, 1840, from Ramazzotius Binda and Pilato, 1986 and from genus Echiniscus Schultze, 1840 were discovered.

Key words: Tardigrada, urban tardigrades, Plovdiv, Bulgaria.

Introduction
The phylum Tardigrada consists of meiofaunal organisms, up to 500 μm long, found on every continent, in marine, freshwater and terrestrial habitats such as sand, algae, aquatic vegetation, moss, lichen, soil, and leaf litter. They are known to occur from high altitude to abyssal depths. Tardigrades are herbivorous on bacteria, algae, mosses, and lichens or carnivorous on protozoans, rotifers, nematodes, mites, and other tardigrades (JOHANSSON, 2009).

The non-urban communities of tardigrades have been well and broadly studied, while little is known about urban tardigrades. Several studies have been conducted by SEMÉRIA (1981; 1982) in France, MEININGER et al. (1985) and JOHANSSON et al. (2011) in USA, UTSUGI (1986) in Japan, STEINER (1994a; b; c) in Switzerland, PELUFFO et al. (2007) and MOLY DE PELUFO et al. (2006) in Argentina. Recent tardigrade studies are mainly focused on taxonomy and zoogeography, with few researches on their ecology.

According to MOLY DE PELUFO et al. (2006) urban tardigrades provoke a number of questions on rapid urbanization and the consequences of this process, such as: “a) what is the diversity of tardigrades in a city? b) is it different from that in the surrounding rural areas? c) can an internal gradient or inter-site differences be detected within a city? d) do different cities host different faunas? e) if so, how different are these faunas? f) do populations of the same species living in different cities show morphometric variations? and g) are such differences due to phenotypic plasticity or genetic differences?”.

JOHANSSON et al. (2011) compared urban and rural tardigrade community richness and whether it is determined by pH. The findings of the study confirmed the negative relationship between urban sites and tardigrade richness. However, it was not explained by the lower pH usually associated with urban environment.

Up to date one of the unanswered questions in tardigrade studies is the uneven pattern of distribution. Despite the fact that they are
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found everywhere in the world, they are not found in every moss or lichen sampled and when found, abundances vary widely. Moreover, even within close areas a great variation in abundance can be observed (e.g. NELSON & ADKINS, 2001; MEYER, 2006). The biotic and abiotic factors that play a role in defining the tardigrade habitat are poorly known and rarely studied (GUIL et al., 2009).

Data on tardigrade fauna in Bulgaria, including Plovdiv Region, is scarce. Two species were reported by ICHAROS (1982) – *Macrobiotus richtersi* J. Murray, 1911 and *Hypsibius microps* Thulin, 1928 from Plovdiv City, but the exact locality is unknown.

The information presented in this paper is based on preliminary data of an ongoing research of tardigrade diversity and ecology in Plovdiv region.

**Material and Methods**

Literature survey was conducted considering tardigrades found in Plovdiv Region. Moss and lichens samples were collected from 41 different localities. 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 in glycerol. All species were identified 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). Systematic follows DEGMA et al. (2017).

The material was collected from the following localities:

1. Central Post Office, 42 08 44.59N, 24 45 38.16E, 1 cm³ moss from pavement, leg. M. Yankova;
2. Concrete area in front of apartment building, 42 07 33.53N, 24 44 43.85E, moss from pavement, leg. D. Georgiev;
3. Djumaya Mosque, 42 08 52.26N, 24 44 53.63E, 0,5 cm³ moss from wall, leg. M. Yankova;
4. Eastern District, Kamenitza, 42 08 44.35N, 24 45 35.51E, 1 cm³ moss from edge stone, leg. M. Yankova;
5. Eastern District, Kamenitza, Plovdiv City, 42 08 42.32N, 24 45 41.38E, 1 cm³ moss from shaft, leg. M. Yankova;
6. “Geo Miley” Str. and “Han Tervel” Str. Corner, 42 08 44.59N, 24 45 38.16E, 2 cm³ moss from brick wall, leg. M. Yankova;
7. “Geo Miley” Str., 42 08 44.81N, 24 45 39.29E, 1 cm³ moss from pavement in front of auto service, leg. M. Yankova;
8. Commodity Station, 0,5 cm³ moss from *A. altissima*, leg. D. Georgiev;
9. Institute of Fishery and Aquaculture, 211 18.88N, 24 45 03.03E, 0,6 cm³ moss from pavement, leg. M. Yankova;
10. Institute of Fishery and Aquaculture, 211 18.88N, 24 45 03.03E, lichens from *Prunus domestica*, leg. M. Yankova;
11. Institute of Fisheries and Aquaculture, 42 11 18.88N, 24 45 03.03E, 0,5cm³ moss from *Morus nigra*, leg. M. Yankova;
12. Institute of Fisheries and Aquaculture, 42 11 18.88N, 24 45 03.03E, 0,5 cm³ moss from pavement, leg. M. Yankova;
13. Institute of Fisheries and Aquaculture, 42 11 18.88N, 24 45 03.03E, 0,5 cm³ moss from window wooden frame, leg. M. Yankova;
14. Institute of Fisheries and Aquaculture, 42 11 18.88N, 24 45 03.03E, 1 cm³ moss from concrete wall, leg. M. Yankova;
15. Institute of Fisheries and Aquaculture, 42 11 18.88N, 24 45 03.03E, 5 cm³ moss from base of a trunk of *Acacia* sp., leg. M. Yankova;
16. Institute of Fisheries and Aquaculture, 42 11 18.88N, 24 45 03.03E, 5 cm³ moss and lichens from *Populus* sp., leg. M. Yankova;
17. Institute of Fisheries and Aquaculture, 42 11 18.88N, 24 45 03.03E, 5 cm³ moss and lichens from *Platanus* sp., leg. M. Yankova;
18. “Maria Luiza” Blvd., 42 08 44 85N, 24 45 34 43E, 3 cm³ moss from *Platanus* sp., leg. M. Yankova;
19. “Maria Luiza” Blvd., 42 08 49.26N, 24 45 54.98E, 3 cm³ moss from pavement, leg. M. Yankova;
20. Nature monument (NM) “Mladezhki halm” Hill, 0.5 cm³ moss, leg. D. Georgiev;
21. NM “Hulm Bunardzhik” Hill, 42°08'42.05"N, 24°44'26.42"E, 1 cm³ moss from rock, leg. M. Yankova;
22. NM “Hulm Bunardzhik” Hill, 42°08'42.09"N, 24°44'26.39"E, 1 cm³ moss from rock, leg. M. Yankova;
23. Old Town Plovdiv, 42°08'50.85"N, 24°45'07.36"E, 1 cm³ moss from shaft, leg. M. Yankova;
24. Old Town Plovdiv, 42°08'51.41"N, 24°45'01.96"E, 0.5 cm³ moss from wall, leg. M. Yankova;
25. Old Town Plovdiv, 42°08'51.09"N, 24°45'01.91"E, 0.5 cm³ moss from wall, leg. D. Georgiev;
26. Old Town Plovdiv, 42°08'52.47"N, 24°44'59.68"E, 0.5 cm³ moss from wall, leg. M. Yankova;
27. Old Town Plovdiv, Biological Faculty of University of Plovdiv, 42°08'51.42"N, 24°45'02.01"E, 0.9 cm³ moss from pavement, leg. M. Yankova;
28. Old Town Plovdiv, Church of Saint Nicolas, 42°08'51.46"N, 24°45'00.23"E, 1.5 cm³ moss from pavement, leg. M. Yankova;
29. Old Town Plovdiv, Church of Saint Paraskeva, 42°08'47.32"N, 24°45'11.82"E, 0.5 cm³ moss from granite wall, leg. M. Yankova;
30. Old Town Plovdiv, Church of Saint Paraskeva, 42°08'47.32"N, 24°45'11.82"E, 0.2 cm³ moss from pavement, leg. M. Yankova;
31. Old Town Plovdiv, Church of Saint Paraskeva, 42°08'47.32"N, 24°45'11.82"E, 1 cm³ moss from brick wall, leg. M. Yankova;
32. Old Town Plovdiv, Church of the Holy Mother of God, 42°08'51.71"N, 24°45'01.73"E, 2 cm³ moss between pavement, leg. M. Yankova;
33. Old Town Plovdiv, Church of the Holy Mother of God, 42°08'52.70"N, 24°45'00.05"E, 1.25 cm³ moss from pavement, leg. M. Yankova;
34. Old Town Plovdiv, City Art Gallery, 42°08'53.24"N, 24°45'09.65"E, 0.6 cm³ moss from pavement, leg. M. Yankova;
35. “Ponedelnik Pazar” Marketplace, 42°08'43.00"N, 24°45'11.47"E, 1 cm³ moss from pavement, leg. M. Yankova;
36. “Ponedelnik Pazar” Marketplace, DKC 1, 2 cm³ moss from wall, leg. M. Yankova;
37. “Trihalmie” Hills, 42°08'47.8"N, 24°45'12.8"182E, moss (Grimmia sp.) from brick wall, leg. D. Georgiev;
38. “Trihalmie” Hills, 42°08'47.8"N, 24°45'12.8"182E, moss from rock (granite) crevice, leg. D. Georgiev;
39. “Tsar Simeonova gradina” Park, 42°08'26.94"N, 24°44'51.98"E, 1 cm³ moss from shaft, leg. M. Yankova;
40. Unspecified locality, 1 cm³ moss, leg. D. Georgiev;
41. Unspecified locality, moss (Grimmia sp.) 0.5 cm³ from wall, leg. D. Georgiev.

Results and Discussion
Tardigrades were found in 20 (48.8%) of the 41 localities sampled. Moss and lichen samples were taken from urban paved areas, walls and trees, exposed to heavy, medium or no vehicle traffic. More than 49 specimens from family Macrobiotidae Thulin, 1928, 49 from genus Milnesium Doyère, 1840, 31 from Ramazzotius Binda and Pilato, 1986 and 2 from genus Echiniscus Schultze, 1840 were discovered. Due to bad preservation or fixation of the specimens 47 eutardigrades were unidentified. Free laid eggs and exuvia with or without eggs were found in 19.5% of the samples.

All mentioned taxa has been reported from previous studies of urban tardigrades. MOLY DE PELUFFO et al. (2006) state that similarities among tardigrade taxocenoses in cities suggest that these animals are also undergoing a process of biotic homogenization linked to urbanization, in a different measure according to the level of organization and spatial scale considered, and it appears that homogenization reaches a taxonomic level at a regional scale.

Registered Tardigrades in the city of Plovdiv:
Phylum: Tardigrada Doyère, 1840
Class: Heterotardigrada Marcus, 1927
Order: Echiniscoidea Richters, 1926
Family: Echiniscidae Thulin, 1928
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Genus: **Echiniscus** Schultze, 1840
*Echiniscus testudo* (Doyère, 1840) - Locality 29 and 30.

Class: Eutardigrada Marcus, 1927
Family: Macrobiotidae Thulin, 1928 – Locality 7, 33, 39
Genus: **Macrobiotus** Schultze, 1834
*Macrobiotus hufelandi* group – Locality 27.
Genus: **Paramacrobiotus** Guidetti, Schill, Bertolani, Dandekar & Wolf, 2009
*Paramacrobiotus richtersi* (Murray, 1911) – *Macrobiotus richtersi* J. Murray, 1911: Plovdiv City (Iharos, 1982).
Family: Hypsibiinae Pilato, 1969
Family: Hypsibiinae Pilato, 1969
Genus: **Hypsibius** Ehrenberg, 1848
*Hypsibius microps* Thulin, 1928 – Plovdiv City (Iharos, 1982).
Family: Microhypsibiidae Pilato, 1998
Genus: **Ramazzottius** Binda and Pilato, 1986
*Ramazzottius cf. oberbaeuseri* (Doyère, 1840) – Locality 11, 17, 18, 21, 31 and 38.
Family: Milnesiidae Ramazzotti, 1962
Genus: **Milnesium** Doyère, 1840 – Locality 14, 16, 17, 21, 28, 29, 30.


In the present study individuals from Macrobiotidae were predominantly found in moss collected from pavement, whereas *Milnesium* sp. were mostly discovered in moss from walls and *Ramazzottius cf. oberhauseri* dominated in moss and lichen samples from trees (Fig. 1). This might be due to different factors or their combination (e.g., pH, moisture, substrate, exposure to sun). Moly de Peluffo et al. (2006) found *Macrobiotus areolatus* Murray, 1907 in paved areas with medium to heavy traffic. They also established that *Ramazzottius oberhauseri* was absent from samples collected from area with heavy traffic, unlike *Milnesium cf. tardigradum* which was the only species found in heavy traffic areas. In another research by Peluffo et al. (2007), *R. oberbaeuseri* and *M. cf. tardigradum* were the most frequent species. They recorded that *R. oberbaeuseri* dominates in peri-urban areas with high suspension dust and exposure to sun and *M. cf. tardigradum* dominates on paved streets with intense vehicle traffic. These findings correlate with the results from the current study.

*Milnesium* sp. and *Ramazzottius cf. oberbauseri* were registered in NM “Hulm Bunardzhik” Hill, and several unidentified eutardigrades in NM “Mladezhki halm” Hill.

![Fig. 1. Number of individuals per 1 cm³ moss and lichens from different habitats in the city of Plovdiv.](image-url)
In 10% of the samples Milnesium sp. were found together with Ramazzottius cf. oberbaueri. Such association might be a result of selective predation or similar preferences towards specific environmental conditions of the microhabitat (e.g. WRIGHT, 1991).

Conclusions
Both micro and macro habitat features should be considered as factors potentially influencing the distribution and abundance of tardigrades (e.g. MOLY DE PELUFFO et al., 2006; GUIL et al., 2009; JOHANSSON et al., 2011).

PELUFFO et al. (2007) support the hypothesis of the relationship between air quality and tardigrade diversity in urban environment.

There is insufficient data on urban tardigrade distribution, diversity and density. Further research and systematic sampling is needed in order to establish these parameters and the mechanisms that produce them. Another interesting and neglected aspect of tardigrade research is their possible role as bioindicators (e.g. VARGHA et al., 2002).

Acknowledgements. The authors would like to express their gratitude to the tardigradologists at Adam Mickiewicz University, Department of Animal Taxonomy and Ecology, Poznań and Jagiellonian University, Institute of Zoology and Biomedical Research, Kraków, Poland for their constant help and support.

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