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MEIOFAUNA IN DECIDUOUS SLOPE FOREST BRYOPHYTES: A CASE STUDY

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Abstract
We did a pilot study to evaluate the abundance of bdelloids in *Anomodon* sp. cushions in relation to vertical distribution on a tree stem and to evaluate the importance of tree location and tree diameter in nematode and bdelloid distribution in *Anomodon* sp. cushions in deciduous slope forest. We found that bdelloid abundance was significantly higher on upper part of tree stems. Nematode and bdelloid distribution is related to tree diameter and tree location on a slope. Our results show that bdelloids and nematodes are highly related to tree local environmental characteristics that could show indirectly the importance of humidity and interactions with other meiofauna species.

Keywords: meiofauna, nematode, rotifers, deciduous forests.

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INTRODUCTION

In latest studies, meiofauna is defined as ecological communities of tiny free-living animals and protists in aquatic sediments and on plants, algae, and sometimes on other animals. Another definition notes that meiofauna are animals that can go through 1 mm (not 45 μm) sieve (Laumer 2024).
Meiofauna may contribute significant part of the biomass in several ecosystems. Importantly, meiofauna have short lifespans and can turn over biomass very fast. Meiofauna play also important role in trophic chains ensuring connection between microbes and higher-level representatives in a trophic chain. Therefore, meiofauna can be used as ecological indicators (Laumer 2024).
Studies show that the most abundant meiofauna representatives in bryophytes are Nematoda and Rotifera, less Tardigrada, Acari, Oligochaeta and Insecta groups (Sohlenius & Boström 2006, Glime 2017a). Both communities, bryophytes and meiofauna are humidity dependent. The life cycle of meiofauna is highly dependent on the humidity of bryophytes (Glime 2017a). Rotifers occur with bryophytes in both aquatic and terrestrial habitats. Bdelloid rotifers (Bdelloidea) are usually among the dominant meiofaunal groups in bryophytes with tardigrades and nematodes (Kutikova 2005, Bielańska-Grajner et al. 2013, Bielańska-Grajner et al. 2011, Ricci 1987, Ricci & Fontaneto 2009, Glime 2017b). It is noted that no single method has been found so far that would be effective in extracting all invertebrates from different substrates and for living organisms or for preserved samples (Andrew & Rodgerson 1999, Glime 2017b).
The relationship between bryophytes and their meiofauna can be referred to commensalism, because bryophytes do not only provide protection, but also serve as a habitat, where food resources such as bacteria, algae or small invertebrates can co-exist (Glime 2017b).
While past studies were mostly focused on meiofauna in bryophytes in freshwater habitats (Linhart et al. 2002, Schenk & Fontaneto 2020, Kreuzinger-Janik et al. 2022, Jekatierynczuk-Rudczyk & Ejsmont-Karabin 2023), we are lacking the knowledge about meiofauna in bryophytes in drier habitats, as in deciduous forests (Ramsay et al. 2021, Majdi et al. 2024).
The aim of the present article was to summarize the information about meiofauna in bryophytes, with a particular emphasis on bdelloid rotifers.

MATERIAL AND METHODS

Study sites

Our pilot study was conducted in two study sites in Naujene ravine located in Augšdaugava region, Southeastern Latvia (Fig. 1). In the first site (55°55’29.3”N, 26°42’33.9”) we studied only bdelloids in relation to vertical distribution on trees, but on the second site (55°55’30.9”N, 26°42’37.0”E) we studied nematodes and bdelloids on trees in relation to height on the slope (Fig. 2). The first site was 84 years old broad-leaved forest stand dominated by *Ulmus laevis, Ulmus glabra, Quercus robur* and *Acer platanoides.* The second site was 64 years old broad-leaved forest dominated by *Ulmus glabra* and *Acer platanoides*.

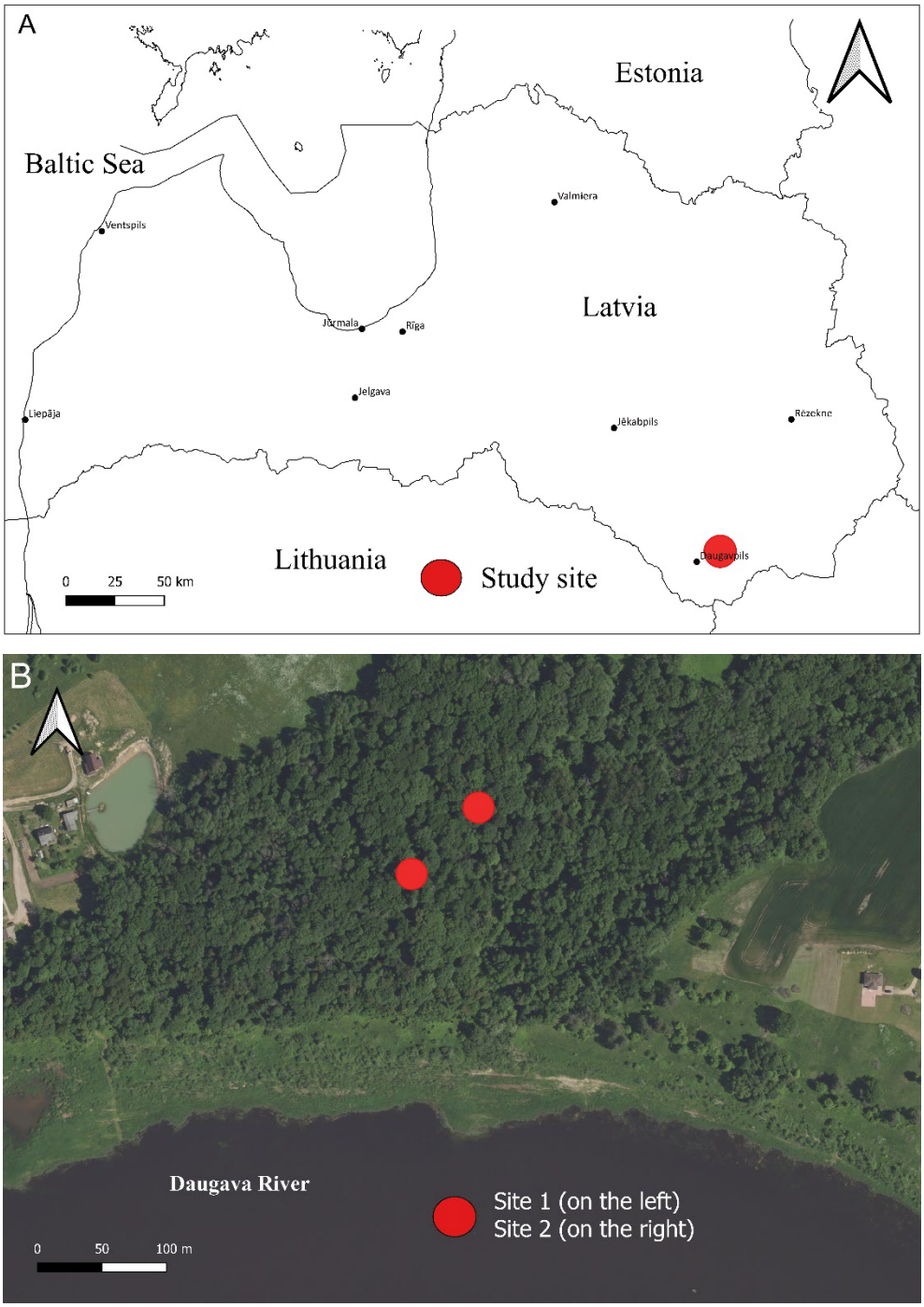

Figure 1. Studied sites (red dots) in Latvia (A, B). Site 1 (55º55'29.3"N, 26º42'33.9" on the left in B), site 2 (55º55'30.9"N, 26º42'37.0"E on the right in B).

Bdelloid vertical distribution on trees

Anomodon sp. samples (approximately 5.0 cm x 5.0 cm each) were taken in May 6th 2022 from each type of microhabitat in a clear day. In total six samples of bryophyte mats (two microhabitats: 0.5 m and 1.5 m in height from the tree base of three *Acer platanoides* L. trees) were collected. Collected samples were immediately delivered to the laboratory for further analysis. Bryophyte samples were not weighed, because for meiofauna more important is size of the substrate patch than a weight due to the movement of meiofauna.

Extraction method by washing of live and soft-bodied organisms was chosen for the pilot study of bryophyte rotifers, which would be easy to implement, and both qualitative and quantitative data could be obtained. It should be economical in both equipment cost and time (Peters et al. 1993, Bielańska-Grajner et al. 2011).

The pilot study of rotifers sampling and extracting on terrestrial bryophytes was done according to the method described by Peters et al. (1993), Bielánska-Grajner et al. (2011) and Iakovenko et al. (2013).

In the laboratory, within two days after sampling, rotifers from bryophytes were extracted by substrate washing. Samples should be identified soon after collection, because obtained results of stored samples after long periods of dehydration may be scarce (Fontaneto & Ricci 2004). Before washing bryophyte samples were rehydrated by adding distilled water; approximately after 1 h, samples were carefully rinsed 10 times with distilled water (100 ml of water each time). The washed water was then filtered through a plankton net (mesh size 30 μm), and the residue was preserved with ethanol at least to 75% solution. Ethanol preservation is good for soft-bodied meiofauna. Before sample preservation, a small part of bryophyte sample was taken for living rotifers identification. The sample was transferred to a Petri dish with distilled water for direct observation. The rotifers were identified by using the keys of Kutikova (2005) and Bielańska-Grajner et al. (2013). Since this was a pilot study, particular specimen identification to the lowest taxonomic level was not done due to time and resource constraints.

Before quantitative analysis each sample condensed to 50 ml, rotifers were counted in six subsamples in the 1 ml of gridded Sedgwick-Rafter counting chamber. The mean number of rotifers from six counting chamber was expressed per 1 g of bryophyte dry mass. The analysis of rotifer samples was done using a microscope *ZEISS Axiolab 5* at 100–200x magnification equipped with a camera *Axiocam 208* colour.

We applied Chi-square test in R programme to compare differences in bdelloid vertical abundance on trees (R Core Team 2021).

Nematoda and bdelloid distribution along slope in deciduous forest

We investigated meiofauna relationship with tree diameter along height on a slope in a deciduous forest in Naujene ravine. In total we selected randomly 19 *Acer platanoides* trees in deciduous forest in Naujene along the slope. The forest was abundant with moss *Anomodon* sp. cushions and we selected samples for meiofauna study these cushions. We defined four height classes on a slope (upper, middle, lower, base near creek). We collected *Anomodon* sp. samples (around 5x5 cm) on 4–6 trees in each of height class on a slope. Data were collected in September 13th, 2024 in a cloudy weather. Bryophyte samples were not weighed, because for meiofauna more important is size of the substrate patch than a weight due to the movement of meiofauna.

We measured diameter of each studied tree at breast height (DBH). Average DBH of studied trees was 67 cm.

We collected all samples in one day and assumed that they had similar weight. We counted nematodes and bdelloids in a lab. The modified Baermann funnel method, which is particularly suited for the extraction of active nematode forms across different size ranges, was employed and demonstrated high efficiency in recovering nearly the entire

population from the substrate (Van Bezooijen 2006). The collected moss samples were placed on a large mesh sieve (2x2 mm) in a glass funnel to which a silicone tube was attached, the end of which was closed with a clamp. The funnel was filled with warm (25-30°C) tap water so that the sample was slightly submerged in water.

After an incubation period of 1.5 h, a small volume of the resulting sediment containing moss-associated microfauna was transferred to a Petri dish for microscopic examination and target organism collection. This procedure was repeated until nematodes were detected in the sediment. All representatives of the microfauna were subsequently counted and sorted, with the majority being preserved in 75% ethanol. Microscopic examination and counting were conducted using a *Nikon SMZ800* stereo microscope. Object visualization and detailed study were carried out using a *Nikon Eclipse 90i* microscope with the *NIS-elements Advanced Research 3.2. 64* – bit program.

Nematodes were not identified to a more detailed taxonomic level; however, they were classified according to body size. One group comprised larger nematodes exhibiting pronounced sexual dimorphism, with males averaging 1.04 mm and females averaging 1.51 mm in length. The second group consisted of smaller nematodes (average 0.52 mm), which are likely to represent larval stages.

Data were analyzed with Generalized Linear Model (GLM) with Poisson distribution and Anova ('car' package) in R programme (R Core Team 2021).

RESULTS AND DISCUSSION

Bdelloid vertical distribution on trees

The pilot study by washing bryophyte samples obtained only bdelloids (Fig. 2) from rotifers, also nematodes, tardigrades and Acari were obtained. From bdelloids one of the taxa was *Adineta* sp. This pilot study found that overall, bdelloids were more common than tardigrades. The abundance of bdelloids between microhabitats by heights also differed significantly (Fig. 3). The greatest number of bdelloid was found in the microhabitats of 1.5 m than of 0.5 m. According to studies of tardigrade distribution patterns in trees, tardigrades diversity and density significantly increased with height and greatly increased in epiphytic moss of the limbs or branches of live oak (Chang et al. 2015, Flowers 2022). Other investigations show that rotifers, tardigrades and nematodes had differential distributions across the tree heights due to epiphyte types and each taxa's habitat suitability (Young et al. 2018). The present study has demonstrated that rotifers collection from bryophyte substrates with washing of distilled water maybe a useful technique for further studies. At least 3 samples from each habitat, for example, after location, moisture, bryophyte life forms, different soil types and others are needed for the further studies that would give more reliable results in bdelloid abundance and species diversity.

Figure 2. Bdelloid *Adineta* sp. sample. Image courtesy: J. Paidere.

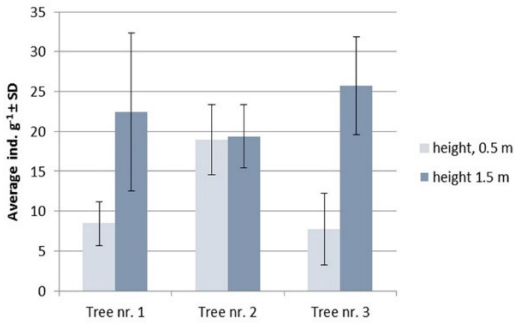

Figure 3. Abundance of bdelloids in bryophytes between trees by height. SD – standard deviation. Significant difference was found in average bdelloid amount between 0.5 m and 1.5 m height (chi-squared = 8.04, df = 2, p-value = 0.02). Empirical data.

Nematodes and bdelloids on trees along height on a slope

We found that interaction between tree location on a slope and tree diameter has a significant influence on number of big nematodes (Tab. 1, Fig. 4, Fig. 5). Number of big nematodes was much higher on smaller trees on upper slope. Number of big nematodes did not show clear pattern in relation to tree diameter in lower parts of slope. Number of small nematodes was decreasing with tree diameter and slope height (Tab. 1., Fig. 6., Fig. 7).

Table 1. Number of big and small nematodes in relation to tree diameter (DBH) and height on a slope after GLM (Poisson distribution) and Anova analysis.

Analysis of Deviance (Type II tests)			
Response: Big nematode			
	LR Chisq	Df	p
DBH	55.22	1	<0.01
Slope height	123.84	3	<0.01
DBH*Slope height	73.14	3	<0.01
Response: Small nematodes			
	LR Chisq	Df	p
DBH	14.75	1	<0.01
Slope height	85.02	3	<0.01

Study about epiphytic moss showed that nematode distribution in mosses could be related to motility that is related to amount of humidity (Merrifield & Ingham 1998). We assume that in lower parts of a slope is higher humidity than in top of a slope and that can also influence the higher small nematode abundance near creek. Especially decrease in number of small nematode on larger trees could be related to competition for space and food with other invertebrates, for instance, tardigrades (Glime 2017c). Probably big nematodes are more resistant than small ones to drier stress conditions.

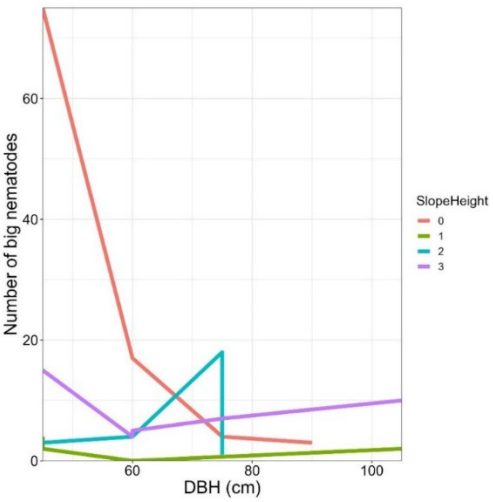

Figure 4. Number of big nematodes in relation to tree diameter (DBH) and location on slope interaction. Slope classes: 0 – upper, 1- middle, 2 – lower, 3 – base near creek. Empirical data.

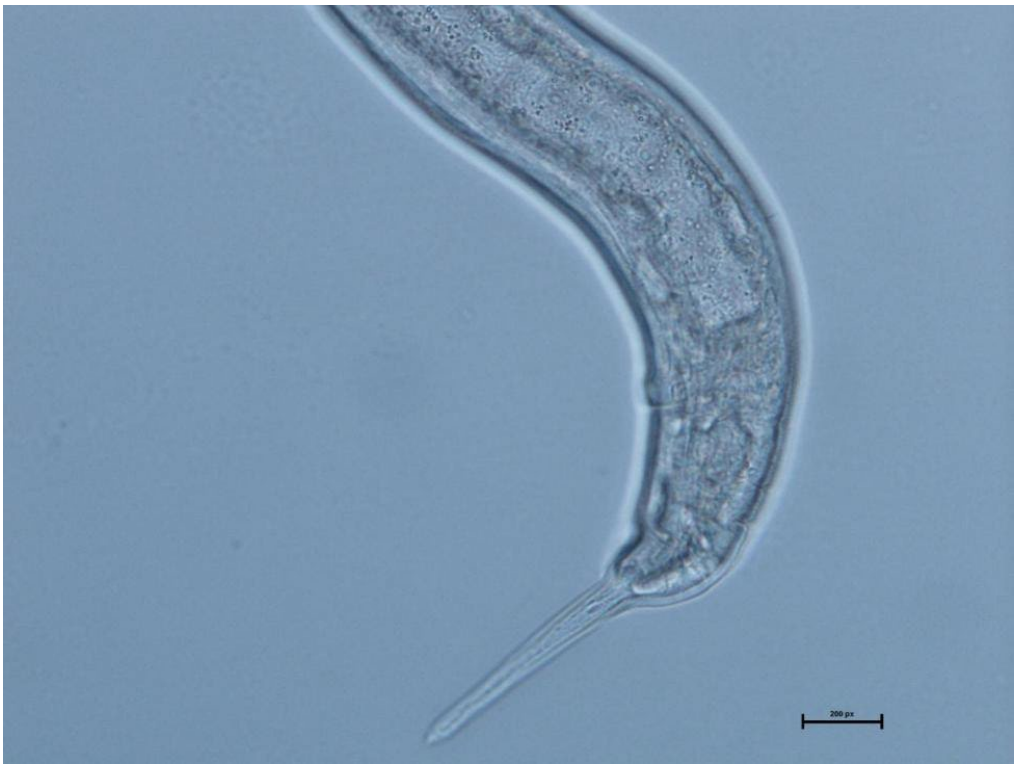

Figure 5. Big nematode. Image courtesy: S. Kecko.

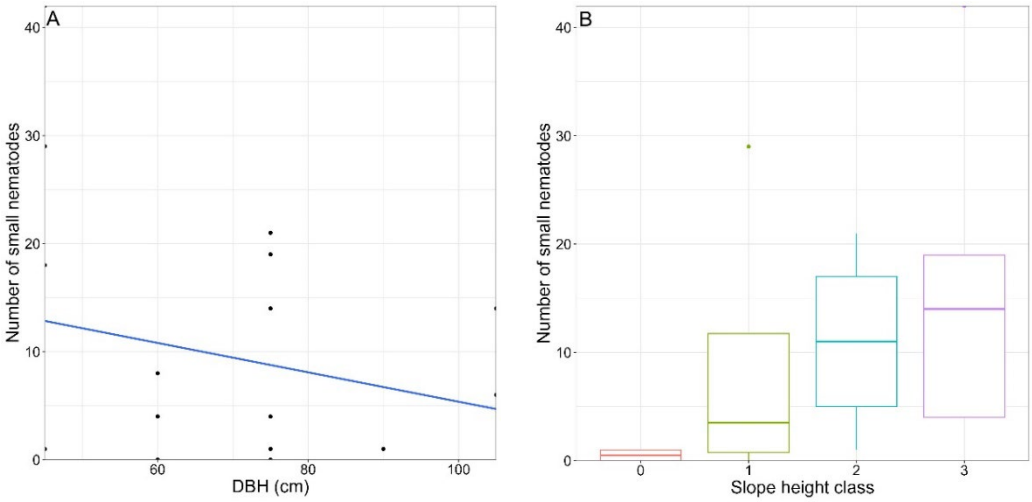

Figure 6. Number of small nematodes in relation to tree diameter (DBH, A) and location on slope (B). Slope classes: 0 – upper, 1- middle, 2 – lower, 3 – base near creek. Empirical data.

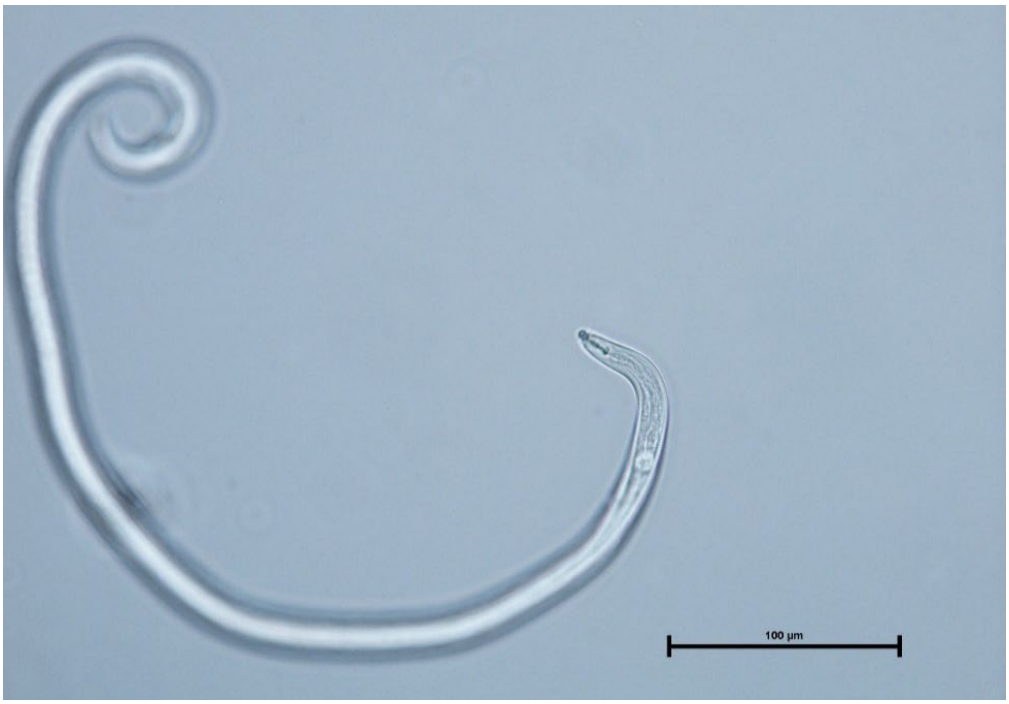

Figure 7. Small nematode. Image courtesy: S. Kecko.

Number of bdelloids was influenced significantly only by slope height, but not by tree diameter (Tab. 2). Bedloids were absent from *Anomodon* sp. samples from upper part of the slope (Fig. 8). Although number of bryophyte samples in each height class on a slope was insufficient for wider conclusions, it is clear that number of bdelloids is increasing in lower parts of the slope (Fig. 8). Higher humidity is in lower part of slope near a creek and bdelloid distribution is highly related to humidity (Ricci 1987). However, as epiphytic bryophytes do not provide permanent humid state, bryophyte dwelling bdelloids have anhydrobiotic ability as they can undergo desiccation by entering in dormant phase (Ricci 1998). It is important to consider that both the moisture level of bryophyte and geographic location significantly contribute to the differences in species composition observed in the middle Arctic tundra subzone of Spitsbergen (Svalbard) (Kaya et al. 2010). Future studies should be focused on meiofauna identification in more detailed taxa level. That would increase the knowledge about particular meiofauna taxa ecological demands in changing environmental conditions. For example, bdelloid studies of a small area of urban plain and adjacent hill in China revealed high bdelloid rotifer diversity with strong habitat preferences between moss and leaf litter habitats, suggesting that bdelloid species can be used as indicators of heterogeneous habitats and giving an ecological basis for exploring bdelloid rotifer species diversity (Wang et al. 2023).

Table 2. Number of small bdelloids in relation to slope height after GLM (Poisson distribution) and Anova analysis.

Analysis of Deviance (Type II tests)			
Response: bdelloids			
	LR Chisq	Df	p
Slope height	18.27	3	<0.01

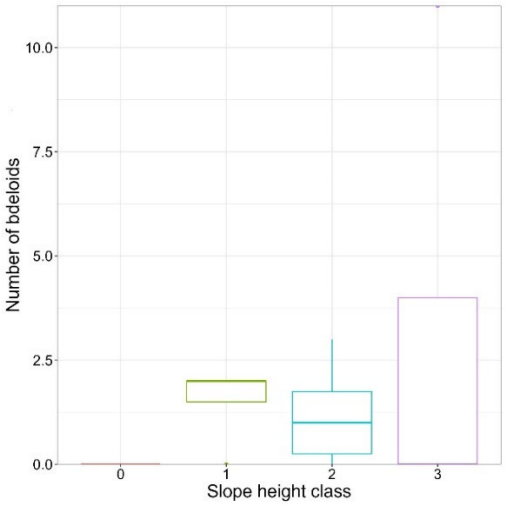

Figure 8. Number of bdelloids in relation to location on slope. Slope classes: 0 – upper, 1- middle, 2 – lower, 3 – base near creek. Empirical data.

CONCLUSIONS

Our study showed that meiofauna representatives as bdelloids and nematodes are highly dependent on microhabitat provided by *Anomodon* sp. depending on tree location on a slope. Therefore, meiofauna can indicate the microclimate of the forest. We studied limited number of samples, more samples are necessary to get more general results about meiofauna relationship with humidity patterns in forests. We also recommend to use DNA methods in nematode and bdelloid detailed taxa identification.

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REFERENCES

Andrew N., Rodgerson L. 1999. Extracting Invertebrates from Bryophytes. *Journal of Insect Conservation* 3: 53–55. https://doi.org/10.1023/A:1009682523054

Bielańska-Grajner I., Cudak A., Mieczan T. 2011. Epiphytic rotifer abundance and diversity associated with moss in bogs and fens in the Polesie National Park (eastern Poland). *International Review of Hydrobiology* 96 (1): 29–38. https://doi.org/10.1002/iroh.201011290

Bielańska-Grajner I., Ejsmont-Karabin J., Iakovenko N. 2013. Fauna słodkowodna Polski, Zeszyt 32C: Wrotki (Rotifera, Bdelloidea). Wydawnictwo Uniwersytetu Łódzkiego, Łódz. 153 pp.

Chang L., Powell D., Miller W.R., Lowman M. 2015. Tardigrades of the canopy: evidence of stratification. *Transactions of the Kansas Academy of Science* 18(3–4): 230–236.

Flowers A. 2022. Population Distribution Analysis of Tardigrades Found on Quercus Virginiana. (Southern Live Oak). Office of Undergraduate Research – our grant recipients 2021-22. Lamar University. 10 pp.

Fontaneto D., Ricci C. 2004. Rotifera: Bdelloidea. In: Yule C.M., Yong H.S. (eds.): Freshwater invertebrates of the Malaysian Region. Academy of Sciences Malaysia, Kuala Lumpur. Pp. 121–126.

Glime J.M. 2017a. The Fauna: A Place to Call Home. Chapt. 1. In: Glime, J. M. Bryophyte Ecology. Volume 2. Bryological 1-1-1 Interaction. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. Last updated 18 July 2020 and available at <http://digitalcommons.mtu.edu/bryophyte-ecology2/>. [Accessed in 01.07.2024].

Glime J.M. 2017b. Invertebrates: Rotifers. Chapt. 4-5. In: Glime, J. M. Bryophyte Ecology. Volume 2. Bryological Interaction. Ebook. Available at http://digitalcommons.mtu.edu/bryophyte-ecology2/Iakovenko N.S., Kašparová E., Plewka M., Janko K. 2013. Otostephanos (Rotifera, Bdelloidea, Habrotrochidae) with the description of two new species. *Systematics and Biodiversity* 24: 1–13. https://doi.org/10.1080/14772000.2013.857737

Glime J.M. 2017c. Invertebrates: Nematodes. Chapt. 4-3. In: Glime, J.M. Bryophyte Ecology. Volume 2. Bryological Interaction. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. Last updated 18 July 2020 and available at <http://digitalcommons.mtu.edu/bryophyte-ecology2/>. [Accessed in 01.07.2025]

Jekatierynczuk-Rudczyk E., Ejsmont-Karabin J. 2023. Rotifers of inter-forest springs. *Diversity* 15(2): 153. https://doi.org/10.3390/d15020153

Kaya M., Smet W.H., Fontaneto D. 2010. Survey of moss-dwelling bdelloid rotifers from middle Arctic Spitsbergen (Svalbard). *Polar Biology* 33: 833-842. https://doi.org/10.1007/s00300-009-0761-8

Kreuzinger-Janik B., Traunspurger W., Majdi N. 2022. Who feeds on whom in semi-aquatic moss ecosystems? *Food Webs* 32: e00237. https://doi.org/https://doi.org/10.1016/j.fooweb.2022.e00237

Kutikova L.A. 2005. The bdelloid rotifers of the fauna of Russia. Russian Academy of Sciences. *Proceedings of the Zoological Institute*. 305 pp.

Laumer C. 2024. Meiofauna. *Current Biology* 34(6): R223–R225. https://doi.org/10.1016/j.cub.2024.02.017

Linhart J., Vlčková Š., Uvíra V. 2002. Bryophytes as a special mesohabitat for meiofauna in a rip-rapped channel. *River Research and Applications* 18(4): 321–330. https://doi.org/10.1002/rra.671

Majdi N., Araujo T.Q., Bekkouche N., Fontaneto D., Garrigue J., Larrieu L., Kamburska L., Kieneke A., Minowa A K., Laumer C., Sabatino R., Sorel D., Stec D., Traunspurger W. 2024. Freshwater and limno-terrestrial meiofauna of the Massane Forest Reserve in the Eastern French Pyrenees. *Biogeographia* 39(1): a032. https://doi.org/10.21426/B639162226

Merrifield K., Ingham R.E. 1998. Nematodes and other aquatic invertebrates in *Eurhynchium oreganum* from Mary's Peak, Oregon Coast Range. *The Bryologist* 101(4): 505–511.

Ramsay B.P.L., Marley N.J., Bilton D.T., Rundle S.D., Ramsay P.M. 2021. The structure of tardigrade communities at fine spatial scales in an Andean *Polylepis* forest. *Neotropical Biodiversity* 7(1): 443–454. https://doi.org/10.1080/23766808.2021.1943216

Ricci C.N. 1987. Ecology of bdelloids: how to be successful. *Hydrobiologia* 147(1): 117–127. https://doi.org/10.1007/BF0002573

Ricci C. 1998. Anhydrobiotic capabilities of bdelloid rotifers. *Hydrobiologia* 387/388: 321–326.

Ricci C., Fontaneto D. 2009. The importance of being a bdelloid: Ecological and evolutionary consequences of dormancy. *Italian Journal of Zoology* 76(3): 240–249. https://doi.org/10.1080/11250000902773484

R Core Team 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. URL https://www.R-project.org/.

Sohlenius B., Boström S. 2006. Patch-dynamics and population structure of nematodes and tardigrades on Antarctic nunataks. *European Journal of Soil Biology* 42(SUPPL. 1): 321–325.

https://doi.org/10.1016/j.ejsobi.2006.07.008

Schenk J., Fontaneto D. 2020. Biodiversity analyses in freshwater meiofauna through DNA sequence data. *Hydrobiologia* 847(12): 2597–2611. https://doi.org/10.1007/s10750-019-04067-2

Wang W., Yang Y., Cui Z. Chen M., Ma X., Wang Q. 2023. High diversity and strong habitat preference of bdelloid rotifers in the moss and leaf litter from a small area of urban plain and adjacent hill in China. *Biodiversity and Conservation* 32: 2769–2789. https://doi.org/10.1007/s10531-023-02630-x

Young A.R., Miller J.E., Villella J., Carey G., Miller W.R. 2018. Epiphyte type and sampling height impact mesofauna communities in Douglas-fir trees. *PeerJ* 6:e569. https://doi.org/10.7717/peerj.5699

Van Bezooijen J. 2006. Methods and techniques for nematology. Wageningen University, Wageningen. 112 pp.

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