# Epiphyte type and sampling height impact mesofauna communities in Douglas-fir trees

Alexander R. Young[1], Jesse E.D. Miller[2], John Villella[3], Greg Carey[3] and William R. Miller[4]

[1] Department of Forest and Natural Resource Management College of Environmental Science and Forestry, State University of New York (SUNY), Syracuse, NY, United States of America
[2] Department of Environmental Science and Policy, University of California, Davis, CA, United States of America
[3] Siskiyou Biosurvey, Ashland, OR, United States of America
[4] Department of Biology and Chemistry, Baker University, Baldwin City, KS, United States of America

## ABSTRACT

Branches and boles of trees in wet forests are often carpeted with lichens and bryophytes capable of providing periodically saturated habitat suitable for microfauna, animals that include tardigrades, rotifers, nematodes, mites, and springtails. Although resident microfauna likely exhibit habitat preferences structured by fine-scale environmental factors, previous studies rarely report associations between microfaunal communities and habitat type (e.g., communities that develop in lichens vs. bryophytes). Microfaunal communities were examined across three types of epiphyte and three sampling heights to capture gradients of microenvironment. Tardigrades, rotifers, and nematodes were significantly more abundant in bryophytes than fruticose lichen or foliose lichen. Eight tardigrade species and four tardigrade taxa were found, representing two classes, three orders, six families, and eight genera. Tardigrade community composition was significantly different between bryophytes, foliose lichen, fruticose lichen, and sampling heights. We show that microenvironmental factors including epiphyte type and sampling height shape microfaunal communities and may mirror the environmental preferences of their epiphyte hosts.

## INTRODUCTION

Tree canopies house a tremendous diversity of life and create horizontal and vertical heterogeneity in forest ecosystems (*Nadkarni, 1994*; *Lowman & Rinkner, 2004*). Major forms of life in temperate canopies include mammals, birds, and epiphyte mats. In addition to large organisms, small organisms occupy canopy soil and epiphyte material including the phyla Tardigrada, Rotifera, and Nematoda (collectively microfauna), which are often overlooked in forest ecology and biodiversity studies (*Glime, 2013*; *Voegtlin, 1982*; *Wilson, 2002*). Microfauna living in bryophytes and lichens are affected by humidity and condensation and will desiccate without regular precipitation and re-animate when sufficient moisture returns (*Kinchin, 1994*; *Nelson, 1982*; *Boothby et al., 2017*). The global

Corresponding author
Alexander R. Young,
alexyoung.116@gmail.com

distribution of tardigrades, rotifers, and nematodes in ecosystems, and the roles they play as primary and secondary consumers of canopy food webs, make them useful for comparing micro-scale community dynamics (*Sánchez-Moreno, Ferris & Guil, 2008*; *Collins & Bateman, 2001*; *Harada & Ito, 2006*; *Sohlenius, Boström & Jönsson, 2004*). However, the extent to which geographic variation in microfaunal communities is due to random dispersal processes, or is a reflection of habitat suitability, remains an open question. Relatively few ecological studies explore the impact of macro and micro environmental factors impact on microfaunal communities (*Guil et al., 2009*; *Mitchell, Miller & Davis, 2009*; *Chang et al., 2015*; *Porazińska et al., 2012*; *Zawierucha et al., 2015*).

Forest canopies create vertical gradients of microclimate which can be characterized by heterogeneity in humidity, sunlight, airflow, and nutrient availability (*Geiger, 1967*). These forces result in stratified habitats with distinct microclimates and create an elegant system for examining microclimate factors associated with canopy height, and differences between epiphyte habitat (*McCune, 1993*; *Donoso, Johnston & Kaspari, 2010*). Gradients of light availability, $CO_2$ concentration, humidity, and desiccation rates occur along vertical axes in tree canopies with tree tops experiencing higher light availability and lower humidity (*McCune, 1993*; *Geiger, 1967*). The "similar gradients hypothesis" suggests that drivers of epiphyte distribution such as forest age, regional precipitation, and the vertical gradient of height could also impact other canopy organisms by creating similar habitat attributes or dispersal methods via different causal mechanisms (*McCune et al., 1997*). Furthermore, while previous studies suggest macro-environmental factors (e.g., elevation) shape macrofaunal communities, this response may be elicited indirectly through changes in vegetation communities or changes in habitat growth form which elicit microfaunal community response, rather than altitude directly (*Wright, 1991*; *Jönsson, 2003*; *Richardson, Richardson & Soto-Adames, 2005*; *Guil et al., 2009*; *Zawierucha et al., 2016*).

Studies report contrasting microfaunal responses to macro-environmental factors (*Dastych, 1987*; *Nelson, 1975*; *Kathman & Cross, 1991*; *Young & Clifton, 2015*). The factors governing small-scale distributions of microfauna remain elusive despite our awareness of their global distribution (*McInnes, 1994*). Local micro-environmental factors such as humidity may be more informative than regional factors such as elevation to explain species distribution due to taxa-level micro-climate suitability (*Collins & Bateman, 2001*; *Guil et al., 2009*; *Degma, Katina & Sabatovicova, 2011*; *Zawierucha et al., 2015*). For example, studies conducted on mountain slopes show both positive and negative impacts of altitude on tardigrade communities (*Dastych, 1987*; *Beasley, 1988*; *Collins & Bateman, 2001*; *Zawierucha et al., 2015*) while laboratory based studies support groupings of tardigrade species based on varying affinities for moisture (*Ramazzotti & Maucci, 1983*; *Wright, 1989*). Additionally, in one of the few studies of tardigrade phenology, *Schuster & Greven (2013)* tracked the body length and reproduction statistics including # of gravid females over 5 years and found that humidity was negatively correlated with # of oocytes and juveniles, while # of hours of sunlight was negatively correlated with body length and the percentage of juveniles present (*Schuster & Greven, 2013*). Field studies have an important place in advancing research on tardigrada, specifically in directing efforts to better culture tardigrade taxa in laboratory settings.

Tardigrades, nematodes and rotifers exhibit differences in stress tolerant dormant stages associated with dispersal strategies which support varying habitat suitability for taxa that can persist after arrival (*Bongers & Ferris, 1999*; *Fontaneto, Melone & Ricci, 2003*; *Guil, Sánchez-Moreno & Machordom, 2008*; *Ramazzotti & Maucci, 1983*; *Wright, 1989*). Nematodes vary in their sensitivity to disturbance but have been shown to have higher abundance with increasing soil porosity (*Harada & Ito, 2006*; *Bongers & Ferris, 1999*) and increased mortality rates with higher soil salinity (*Poage et al., 2008*). Rotifers display geographic distributions that suggest habitat preference, although species distributions were highly variable (*Fontaneto & Ricci, 2006*). Tardigrades were linked to tree species substrate and demonstrated higher abundance in the tops of trees in a mixed deciduous forest in Kansas, U.S.A. (*Mitchell, Miller & Davis, 2009*; *Miller, Gallardo & Clark, 2013*; *Chappell et al., 2015*; *Chang et al., 2015*). Also, tardigrade communities in leaf litter of Beech forests in Modenese Apennine (Italy) and Roan Mountain (TN, U.S.A.) had similar species composition (*Guidetti, Bertolani & Nelson, 1999*). Challenges to understanding limno-terrestrial microfaunal ecology include their patchy distributions (*Meyer, 2006*) and the difficulty of species identification which may be influenced by ontogeny, cryptic species diversity, and a poor understanding of population clustering (*Miller, Miller & Heatwole, 1994*; *Degma, Katina & Sabatovicova, 2011*; *Morek et al., 2015*).

In this study, we document the density and diversity of tardigrades, rotifers, and nematodes in a Douglas-fir forest canopy in Northern California, USA (Fig. 1). To test the factors of epiphyte type and abiotic factors associated with height, we collected a factorial combination of multiple epiphyte types at multiple sampling heights in nine Douglas-fir trees. We expect microfaunal populations to respond to epiphyte type and sampling height because of the differences in water availability which represent barriers to micro-population establishment.

## METHODS

### Site description

Field sampling took place in September, 2015 in Six Rivers National Forest of California, USA (41.871, −123.846). The site has a Mediterranean climate with an annual average annual precipitation of 200 cm. The soil is primarily composed of deep, well-drained soils formed in material weathered from metasedimentary rock (*Natural Resources Conservation Service, 2017*). Nine mid-sized diameter at breast height (60–75 cm DBH) Douglas-fir trees (average height 26 m, min: 20 m, max 35 m) were selected at random and spaced at least 500 m apart and within 30 m of unpaved access roads. Structurally unsound trees were avoided. Trees were climbed using minimally invasive and safe climbing techniques (*Miller & Lowman, 2012*; *Anderson et al., 2015*).

Where present, fist-sized (approx. 5 gram) or smaller patches of three epiphyte types (foliose lichens, fruticose lichens, and bryophyte) were collected and stored in paper bags (*McCune et al., 1997*). Sample collection was stratified into three sampling heights: below any branches on the bole (low, ∼10 m), the middle canopy (mid, ∼15 m), and the top 8 m of the tree (top, ∼25 m). Where possible one patch of foliose lichen and fruticose lichen were collected, and two bryophyte patches were collected from each tree at the three
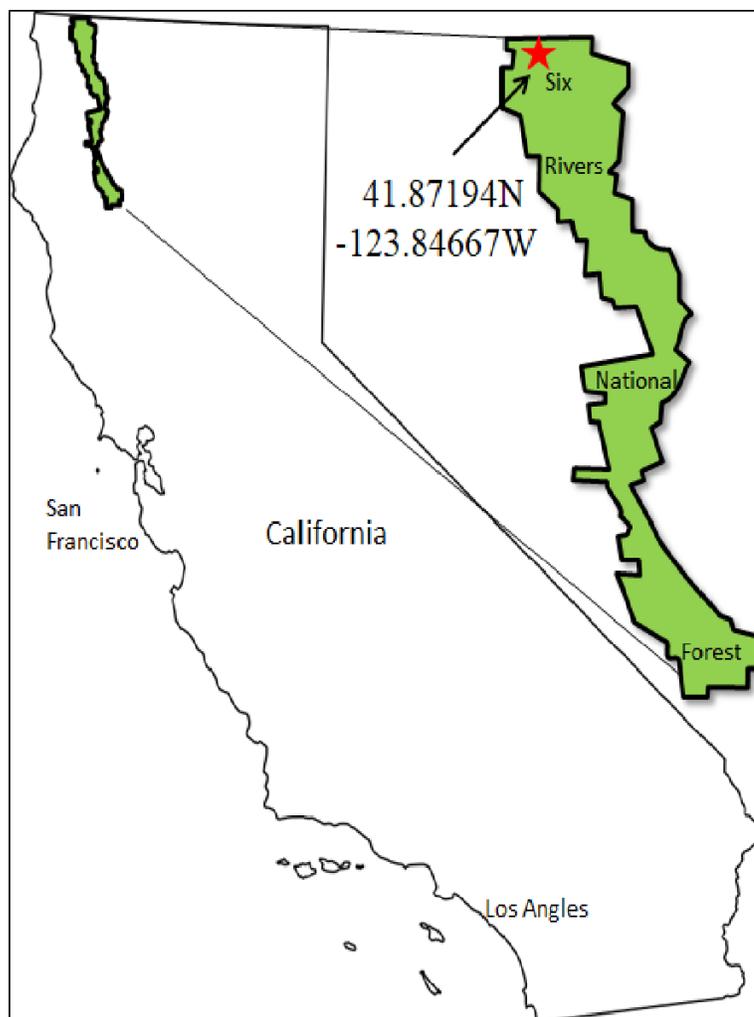
**Figure 1  A map of the study area.**

vertical locations. Not all epiphyte types were present at each sampling location and not all epiphytes were identified to species level.

## Processing samples for microfauna

A portion (0.3–1.9 grams, mean 0.95 grams) of each epiphyte sample was hydrated with 20 mL of commercially bottled spring water for 12 h to enable microfauna to become active. For each sample, three 1 mL aliquots were visually searched with a dissecting microscope at 20× magnification for nematodes, rotifers, and adult tardigrades. The abundance of nematodes, rotifers, and tardigrades was counted for each epiphyte sample and divided by the mass of the sample following *Mitchell, Miller & Davis (2009)* methodology.

Tardigrade specimens were deposited into a drop of polyvinyl alcohol media on a microslide (*Salmon, 1951*) with an Irwin loop (*Schram & Davidson, 2012*). A glass coverslip was placed over the medium, dried for three days, and nail polish was applied to seal the PVA mounting media.

## Species identification

Epiphytes were identified using standard techniques (*Brodo, Sharnoff & Sharnoff, 2001*) and identification guides (*Norris & Shevock, 2004*; *McCune & Geiser, 2009*) on a Zeiss 45–50–52 dissecting scope and a Leica ACT 2000 light microscope. Dominating genera for Bryophyte were: *Dicranum, Isothecium myosurides*; for foliose lichens: *Hypogymnia* and *Platismatia;* and for fruticose lichens: *Usnea, Sphaerophorus* (Table S1).

Tardigrades were identified using an Olympus BX60 DIC (differential interference contrast) microscope at 1,000× magnification. Morphological features including claws, buccopharyngeal apparatus, cuticle design, and other characteristics were used for species identification (*Ramazzotti & Maucci, 1983*; *Pilato & Binda, 2010*; *Kaczmarek & Michalczyk, 2017*; *Stec et al., 2018*). Nomenclature was based on *Guidetti & Bertolani (2005)*, *Degma & Guidetti (2007)*, *Bertaloni et al. (2014)* and *Degma, Bertolani & Guidetti (2009–2018)*.

## Statistical analysis

Univariate three by three factorial analyses of variance for tardigrade, rotifer, and nematode density were blocked by tree with sampling height and epiphyte type as two interacting factors. The density of tardigrade, rotifer, or nematode populations and resulting microfaunal diversity per gram of epiphyte material was tested for differences across sampling heights, epiphyte types, and for interaction between sampling height and epiphyte type. Type III sum of squares was used for uneven replication and Tukey's honestly significant difference was used to determine the magnitude and direction of statistical differences ($\alpha = 0.05$). Pearson correlation coefficients of microfaunal density and mass of the portion of each sample did not reveal significant relationship of sample mass to microfaunal density ($p > 0.98$).

Tardigrade community composition was analyzed using a permutation multivariate analysis of variance (PERMANOVA) with the R package *vegan* (*Oksanen et al., 2015*). Dissimilarities in tardigrade community composition was visualized with non-metric multi-dimensionally scaled (NMDS) ordination using the Bray-Curtis dissimilarity method (*Bray & Curtis, 1957*; *Dufrêne & Legendre, 1997*). Ellipses representing 95% confidence intervals were displayed around the centroid of each epiphyte type to visualize significant differences in tardigrade composition. Tardigrade species richness was calculated as the count of each tardigrade taxa found in a sample. Tardigrade diversity and microfaunal diversity are reported using simpsons diversity index.

Additionally, each tardigrade species was tested for association with sampling heights or epiphyte types with the R package *indicspecies* (*De Caceres & Legendre, 2009*). All statistical analyses were performed with the program R ver. 3.3 (*R Core Team, 2018*), and visualized with the R package *ggplot2* (*Wickham, 2009*).

## RESULTS

A total of 68 nematodes, 411 rotifers, and 231 tardigrades were found in 51 samples, with 89% of samples containing at least one nematode, rotifer, or tardigrade (Table 1). Analyzed epiphyte material included 18 lichen and bryophyte taxa (Table S1). Epiphyte type was significant in explaining differences in nematode ($p = 0.03$), rotifer ($p = 0.01$),

**Table 1 Summary of microfauna density and epiphyte types.** A summary of raw data that provides the % of positive samples, mean microfauna density, and tardigrade community data including species richness and average Simpson's diversity index for each epiphyte type and sampling location.

| Epiphyte type | Height | N | % positive samples | | | Density per sample | | | Tardigrade Community | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Tardigrade | Rotifer | Nematode | Tardigrade mean, sd | Rotifer mean, sd | Nematode mean, sd | Species Richness | Diversity mean |
| Foliose lichen | Top | 8 | 88% | 88% | 50% | 15.4, 10.3 | 2.2, 2.6 | 0.6, 0.9 | 11 | 0.7 |
| | Mid | 9 | 89% | 89% | 67% | 8.1, 6.5 | 1.3, 1.0 | 0.7, 0.7 | 7 | 0.5 |
| | Low | 7 | 29% | 71% | 14% | 1.2, 1.1 | 2.7, 3.0 | 0.2, 0.4 | 7 | 0.3 |
| Fruticose lichen | Top | 8 | 50% | 38% | 38% | 2.6, 6.1 | 0.4, 0.8 | 0.2, 0.3 | 3 | 0.3 |
| | Mid | 9 | 33% | 56% | 0% | 0.9, 1.4 | 0.6, 1.0 | 0.0, 0.0 | 6 | 0.2 |
| | Low | 8 | 38% | 25% | 38% | 1.0, 1.9 | 0.0, 0.2 | 0.1, 0.2 | 1 | 0.2 |
| Bryophytes | Top | 1 | 100% | 100% | 100% | 14.3, NA | 19.0, NA | 3.3, NA | 4 | 1.3 |
| | Mid | 4 | 75% | 100% | 50% | 10.7, 10.9 | 5.3, 1.9 | 1.5, 1.7 | 5 | 0.4 |
| | Low | 9 | 100% | 89% | 67% | 17.9, 12.2 | 11.2, 15.9 | 1.0, 1.2 | 4 | 0.3 |

and tardigrade density ($p = 0.04$). Nematode and rotifer density was higher in bryophytes than foliose lichen or fruticose lichen ($p < 0.01$), while tardigrade density was higher in bryophyte and foliose lichen than fruticose lichen ($p < 0.01$, $p = 0.02$). Microfaunal richness was also significantly higher in bryophytes and foliose lichen than fruticose lichen ($p < 0.01$). The mass of the portion of epiphyte used in analysis did not impact the density of nematodes, rotifers, or tardigrades ($p = 0.98$, $p = 0.98$, $p = 0.99$). Overall, nematodes were less common than rotifers or tardigrades (Fig. 2).

Sampling height had a significant interaction with epiphyte type on nematode density, with mid and top bryophytes having significantly higher nematode density than mid and top fruticose lichen ($p = 0.03$, $p < 0.01$). However, rotifer and tardigrade density were not significantly impacted by sampling height ($p = 0.6$, $p = 0.63$). Of the nine trees accessed, only one bryophyte was found in the top sampling location which also supported the highest nematode density in the collection (Fig. 2). Three tardigrade species were significantly associated with the top sampling height (*Pilatobius nodulosus, p >* 0.01; *Echiniscus quadrispinosus, p = 0.03; Milnesium sp. 2, p = 0.04*), and *Ramazzottius oberhauseri* significantly associated with the top and mid sampling heights ($p = 0.05$). Epiphyte species richness was lowest in the top position (6 species), and similar at the middle (11 species) and lowest sampling height (10 species).

Eight species of tardigrade and four tardigrade taxa were found, representing two classes, three orders, six families and eight genera (Table 2). Tardigrade taxa in the *Macrobiotus hufelandi* group comprised 31% of the collection, while the second most common species, *Echiniscus quadrispinosus* compromised 22% of the collection. Tardigrade diversity was significantly higher in fruticose lichen than bryophyte and foliose samples ($p < 0.01$). Furthermore, tardigrade community composition was significantly impacted by epiphyte type ($p < 0.01$, Fig. 3) and sampling height ($p = 0.02$). The *Macrobiotus hufelandi* group was significantly associated with bryophyte samples ($p < 0.01$). In contrast, tardigrade species *Echiniscus quadrispinosus* and *Ramazzottius oberhauseri* were significantly associated with foliose lichen, and fruticose lichen ($p < 0.01$, $p = 0.02$).
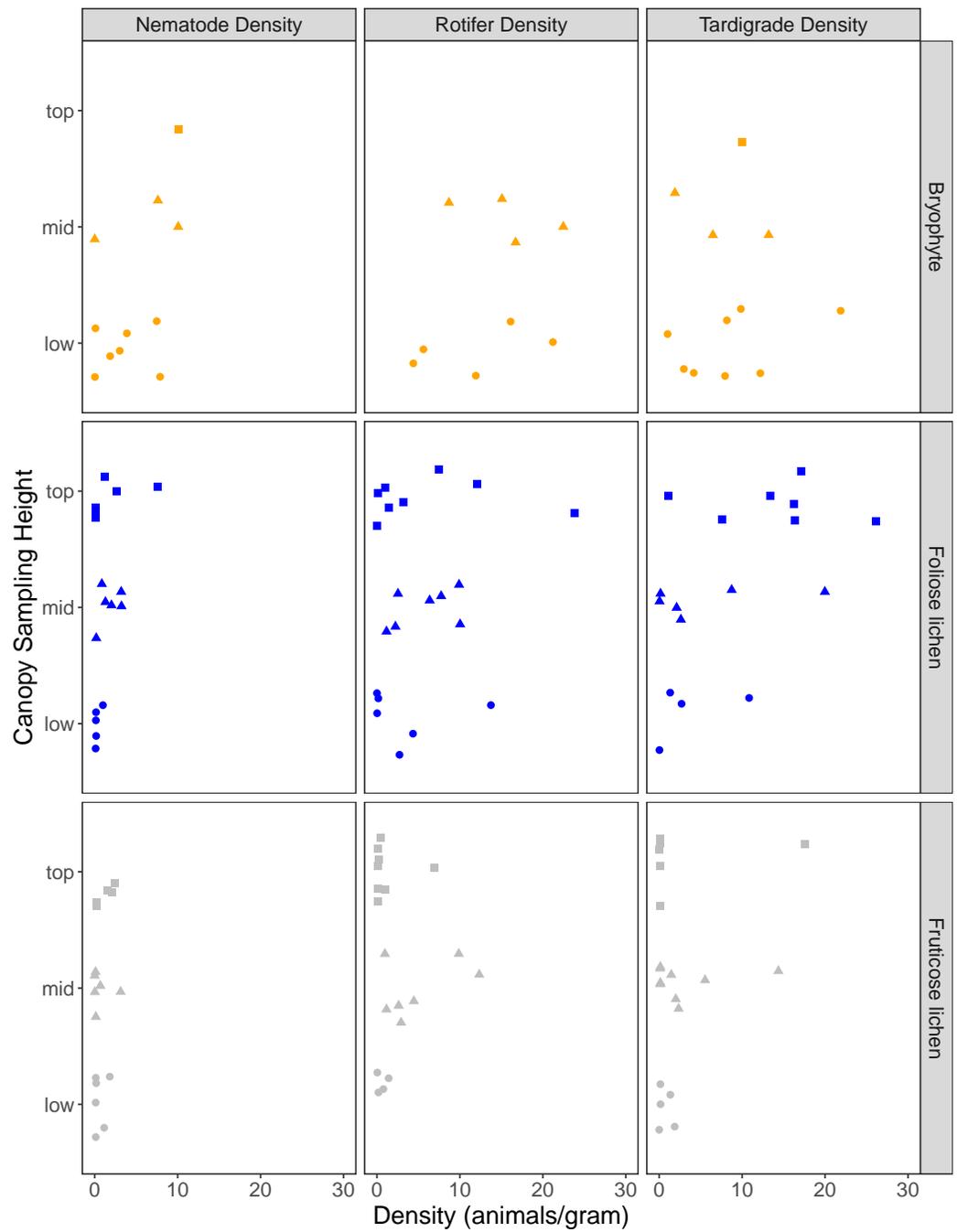
**Figure 2** **Nematode, rotifer, and tardigrade density (animals per gram) from three epiphyte types, and three canopy sampling locations within Douglas-fir trees.** Square, triangle, circle data points represent microfauna density for samples that came from top, mid, or low sampling heights. Orange, blue or gray shapes represent bryophyte, foliose lichen, or fruticose lichen epiphyte types.

**Table 2  Tardigrade species found.**

| Class, Order, SuperFamily, Family Genus species | Foliose Lichen $n = 22$ mean, sd | Bryophytes $n = 14$ mean, sd | Fruticose Lichen $n = 27$ mean, sd | Total $N = 63$ |
|---|---|---|---|---|
| Eutardigrada, Apochela, Milnesiidae | | | | |
| *Milnesium eurystomum* (Maucci, 1991) | 0.1, 0.2 | 0 | 0.8, 1.5 | 4 |
| *Milnesium* sp.1 | 0.1, 0.3 | 0.1, 0.3 | 0.3, 0.5 | 9 |
| *Milnesium* sp. 2 | 0.3, 0.8 | 0.3, 0.9 | 0 | 4 |
| Eutardigrada, Parachela, Hypsibiidae | | | | |
| *Pilatobius nodulosus* (Ramazzotti, 1957) | 0.6, 1.2 | 0.3, 0.6 | 0 | 14 |
| Eutardigrada, Parachela, Itaquasconinae | | | | |
| *Itaquascon* sp. | 0.1, 0.2 | 0 | 0 | 1 |
| Eutardigrada, Parachela, Isohypsibidea, Isohypsibioiidae | | | | |
| *Ramazzottius sp.* (Doyere, 1840) | 1.0, 1.4 | 0.1, 0.3 | 1.0, 1.4 | 24 |
| Eutardigrada, Parachela, Macrobiotoidea, Macrobiotidae | | | | |
| *Macrobiotus hufelandii* group | 0.7, 1.4 | 4.9, 6.1 | 0 | 70 |
| *Mesobiotus harmsworthi* (Murray, 1907) | 0.9, 1.7 | 0.5, 0.9 | 0 | 22 |
| Heterotardigrada, Echiniscoidea, Echiniscoididae | | | | |
| *Echiniscus arctomys* group (Ehrenberg, 1853) | 0.6, 1.0 | 0.2, 0.4 | 0 | 12 |
| *Echiniscus horningi* (*Schuster & Grigarick, 1971*) | 0.8, 2.0 | 0 | 0 | 15 |
| *Echiniscus quadrispinosus* (Richters, 1902) | 2.4, 3.1 | 0.1, 0.3 | 1.3, 1.3 | 50 |
| *Multipseudechinisus raneyi* (Grigarick, Mihelčič, & Schuster 1964) | 0.2, 0.6 | 0.1, 0.3 | 0 | 15 |

**Notes.**

Mean, average density of each species in each epiphyte type; sd, standard deviation; $n$, the number of epiphyte type samples; $N$, total samples.

All identifications are based on morphological approaches.

## DISCUSSION

Tardigrades, nematodes, and rotifers had differential distributions across the three sampling heights and epiphyte types studied, suggesting varying habitat suitability for different phyla. These patterns may be due to epiphyte water-retention characteristics, secreted secondary metabolites, light availability (*Wright, 1991*), or food availability and feeding habit limitations (e.g., filter feeding rotifers require higher humidity to feed) (*Guidetti et al., 2012*; *Hallas & Yeates, 1972*). While microfaunal populations varied substantially within and between trees a general trend was that fewer nematodes, rotifers, and tardigrades were found in the fruticose lichen. In contrast, overall microfaunal density was highest in bryophytes growing in the low sampling height, suggesting that the consistently humid habitat combined with optimal resource availability (e.g., photosynthetic cells) is sufficient for microfaunal communities to thrive and epiphyte growth form is relevant to microfaunal communities (*Jönsson, 2003*).

Nematode, rotifer, and tardigrade density had contrasting responses to sampling height, with nematode density increasing with sample height, while rotifer and tardigrade density was not significantly different across sampling heights. The trend we report has not been found for Nematoda, but previous studies document similar responses of tardigrade density to tree height (*Miller, Gallardo & Clark, 2013*; *Chang et al., 2015*) and
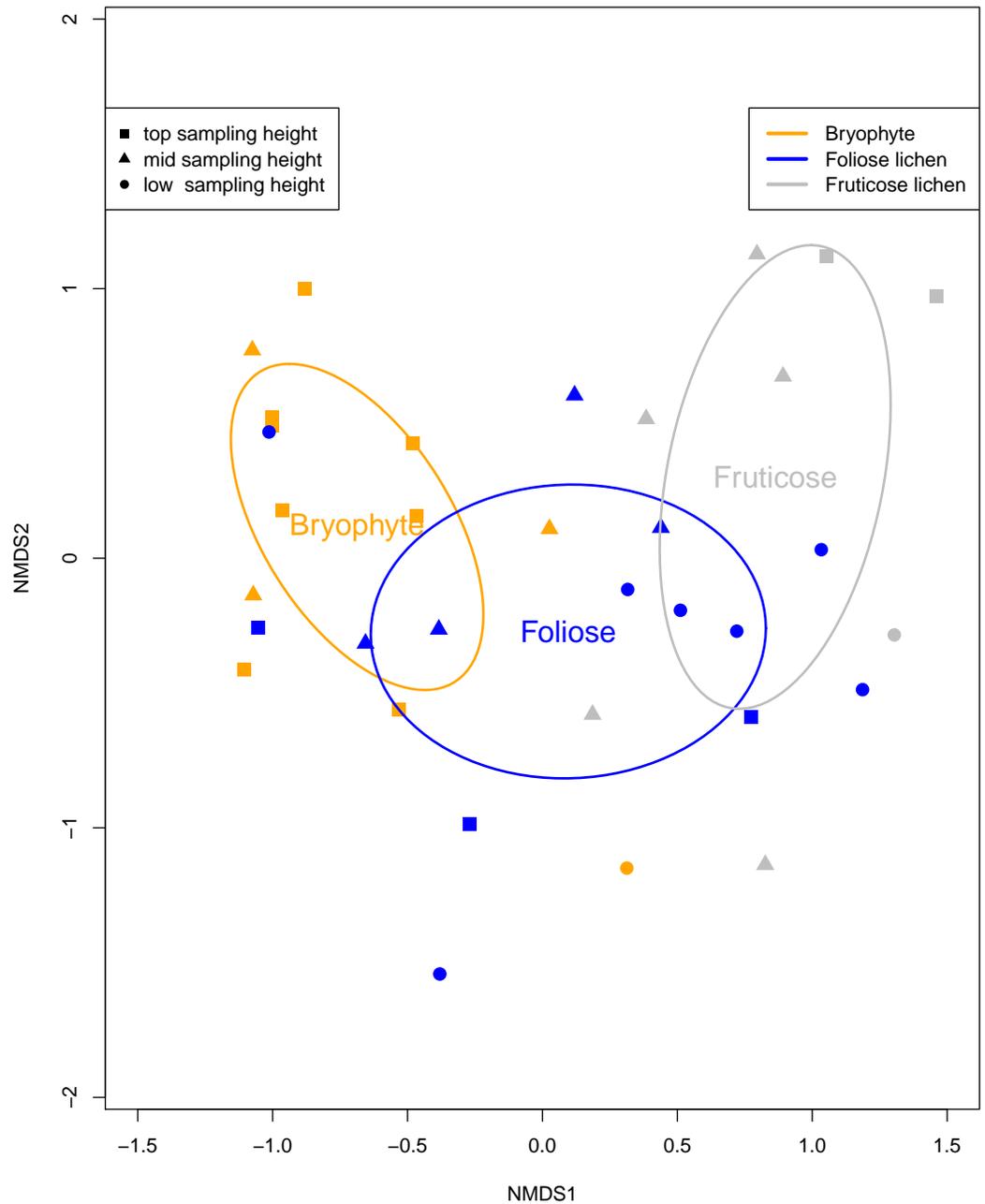
**Figure 3** **A non-metric multi-dimensional scaling (NMDS) ordination of tardigrade community composition with 95% confidence ellipses surrounding Bryophyte, Foliose lichen, and Fruticose lichen samples.** Each data point represents a bryophyte (orange), foliose lichen (blue), or fruticose lichen (gray) sample. Squares represent samples from the top sampling location, triangles represent the mid sampling location, and circles represent the low sampling location. Similarity between the tardigrade communities found in each sample can be interpreted by the proximity of each symbol. Tardigrade communities were significantly different between epiphyte types ($p < 0.01$) and sampling heights ($p = 0.02$).

four tardigrade species significantly associated with top canopy positions suggests that microfaunal communities are likely impacted by sampling height, in addition to epiphyte type.

Although fruticose lichens were sparsely populated, *Echiniscus quadrispinosus* and *Ramazzottius oberhaeuseri* were mainly found in fruticose lichens over bryophyte and foliose lichen (Table 1). Two of the four tardigrade species found in fruticose samples feed on microbes (*Echiniscus quadrispinosus* and *Ramazzottius oberhaeuseri*) while the other two are predatory (*Milnesium eurystomum* and *Milnesium.* sp 1). The "hair-lichen" morphology of fruticose lichen may represent a relatively xeric, high-stress environment, with implications for those taxa which can successfully colonize (*Grime, 1977*; *Guil et al., 2009*; *Bartels, Nelson & Exline, 2011*). All of the tardigrade taxa found in this study were documented in *Schuster & Grigarick (1965)* and *Schuster & Grigarick (1971)* suggesting population stability during the 50 years between each study. This highlights the utility of co-locating field sites with previous studies to inform rates of long-term immigration and emigration and provides further evidence that phylum Tardigrada is relatively species poor (*Bartels et al., 2016*).

Epiphytes in tree canopies seem to support a similar tardigrade species richness as epiphytes found at ground level, and our observation of higher microfaunal diversity in bryophytes than lichen is supported by *Bartels & Nelson (2007)*, *Guil et al. (2009)*, *Zawierucha et al. (2016)* and *Zawierucha et al. (2017)*. An understanding of regional tardigrade community structure in North America is beginning to emerge (*Meyer, 2013*; *Kaczmarek, Michalczyk & McInnes, 2016*).

The distribution of microfaunal communities is complex, but may be explained by immigration events (*Mogle et al., 2018*; *Zawierucha et al., 2018*), reproduction rates (*Tsujimoto, Imura & Kanda, 2016*; *Bingemer, Hohberg & Schill, 2016*), lifespan and life history traits (*Schuster & Greven, 2013*), suitable abiotic environment (*Wright, 1989*), and site specific biotic factors (*Kinchin, 1994*; *Guil et al., 2009*; *Glime, 2013*) including feeding behavior (*Guil et al., 2009*; *Sánchez-Moreno, Ferris & Guil, 2008*; *Miller, Horning & Heatwole, 2001*; *Guidetti et al., 2012*; *Guil & Sanchez-Moreno, 2013*). Although there are likely numerous interacting forces that contribute to microfaunal species distributions, disentangling the impact of epiphyte growth form and abiotic micro-climate on microfaunal abundance is challenging because epiphyte species and microfaunal distributions may respond similarly to gradients. Experimental manipulations of habitat characteristics using factorial treatment designs may be useful to decipher microfaunal habitat preferences. Habitat manipulations could help identify mechanisms driving microfaunal distribution.

## CONCLUSION

Microfaunal populations respond to epiphyte type, and to a lesser extent, sampling height. This evidence suggests that microfauna are more strongly influenced by biotic micro-environmental forces such as epiphyte growth form and water retention characteristics than abiotic micro-environmental forces alone. Additionally, it may be more informative to view microfaunal population dynamics through the lens of their habitat morphology, as

conserved functional traits of epiphyte morphology may mirror local micro-environmental forces on which microfaunal micro-population establishment is dependent. Future ecological studies on microfauna could benefit from carefully considering epiphyte morphology.

## ACKNOWLEDGEMENTS

The authors would like to thank Six Rivers National Forest for supporting this research, the California Academy of Sciences for archiving the resulting specimens, Nils Nelson for help with field work, Dr. Ruth Yanai's Forestry 694 writing class at the State University of New York College of Environmental Science and Forestry, and Dr. Noemi Guil for critical review.

### Funding

This work was supported by the Baker University NSF:REU:14161005. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

### Grant Disclosures

The following grant information was disclosed by the authors:
Baker University: NSF:REU:14161005.

### Competing Interests

John Villella and Greg Carey are employed by Siskiyou Biosurve, Ashland, OR, USA.

### Author Contributions

- Alexander R. Young conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Jesse E.D. Miller analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- John Villella and Greg Carey helped conceive and design the experiments authored or reviewed drafts of the paper, approved the final draft, epiphyte species identification.
- William R. Miller analyzed the data, authored or reviewed drafts of the paper, approved the final draft, tardigrade species identification.

### Data Availability

 The raw data are provided in the Supplemental Files.

### Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.5699#supplemental-information.

# REFERENCES

**Anderson DK, Koomjian W, French B, Altenhoff SR, Luce J. 2015.** Review of rope-based access methods for the forest canopy: safe and unsafe practices in published information sources and a summary of current methods. *Methods in Ecology and Evolution* **6(8)**:856–872 DOI 10.1111/2041-210X.12393.

**Bartels PJ, Apodaca JJ, Mora C, Nelson DR. 2016.** A global biodiversity estimate of a poorly known taxon: phylum Tardigrada. *Zoological Journal of the Linnean Society* **178(4)**:730–736 DOI 10.1111/zoj.12441.

**Bartels PJ, Nelson DR. 2007.** An evaluation of species richness estimators for tardigrades of the Great Smoky Mountains National Park, Tennessee and North Carolina, USA. *Journal of Limnology* **66**:104–110 DOI 10.4081/jlimnol.2007.s1.104.

**Bartels PJ, Nelson DR, Exline RP. 2011.** Allometry and the removal of body size effects in the morphometric analysis of Tardigrades. *Journal of Zoological Systematics and Evolutionary Research* **49(1)**:17–25 DOI 10.1111/j.1439-0469.2010.00593.x.

**Beasley CW. 1988.** Altitudinal distribution of Tardigrada of New Mexico with the description of a new species. *The American Midland Naturalist* **120**:436–440 DOI 10.2307/2426016.

**Bertaloni R, Guidetti R, Marchioro T, Altiero T, Rebecchi L, Cesari M. 2014.** Phylogeny of Eutardigrada: new molecular data and their morphological support lead to the identification of new evolutionary lineages. *Molecular Phylogenetic Evolution* **76**:110–126 DOI 10.1016/j.ympev.2014.03.006.

**Bingemer J, Hohberg K, Schill RO. 2016.** First detailed observations on tardigrade mating behavior and some aspects of the life history of Isohypsibius dastychi Pilato, Bertaloni & Binda 1982 (Tardigrada, Isohypsibiidae). *Zoological Journal of the Linnaean Society* **178(4)**:856–862 DOI 10.1111/zoj.12435.

**Bongers T, Ferris H. 1999.** Nematode community structure as a bioindicator in environmental monitoring. *Trends in Ecology and Evolution* **14(6)**:224–228 DOI 10.1016/S0169-5347(98)01583-3.

**Boothby TC, Tapia H, Brozena AH, Piszkiewicz S, Smith AE, Giovannin I, Re-becchi L, Pielak GJ, Koshland D, Goldstein B. 2017.** Tardigrades use intrinsically disordered proteins to survive desiccation. *Molecular Cell* **65(6)**:975–984 DOI 10.1016/j.molcel.2017.02.018.

**Bray JR, Curtis JT. 1957.** An ordination of the upland forest communities of Southern Wisconsin. *Ecological Monographs* **27(4)**:325–349 DOI 10.2307/1942268.

**Brodo IM, Sharnoff SD, Sharnoff S. 2001.** *Lichens of North America.* New Haven: Yale University Press.

**Chang L, Powell D, Miller WR, Lowman MD. 2015.** Tardigrades of the canopy: evidence of stratification. *Transactions of the Kansas Academy of Science* **188(3 & 4)**:220–236 DOI 10.1660/062.118.0306.

**Chappell BM, Parry D, Miller WR, Lowman MD. 2015.** Tardigrades of the Canopy: *Doryphoribius gibber* Beasley and Pliato, 1987 (Eutardigrada: Parachela: Hypsibiidae)

new records from eastern Kansas, U.S.A. *Transactions of the Kansas Academy of Science* **118(1–2)**:48–52 DOI 10.1660/062.118.0106.

**Collins M, Bateman L. 2001.** The ecological distribution of tardigrades in Newfoundland. *Zoologischer Anzeiger* **240(3–4)**:291–297 DOI 10.1078/0044-5231-00036.

**Dastych H. 1987.** Altitudinal distribution of Tardigrada in Poland. In: Bertolani R, ed. *Biology of tardigrades. Selected symposia and monographs U. Z. I. Vol. 1.* Modena: Mucchi Editore, 169–176.

**De Caceres M, Legendre P. 2009.** Associations between species and groups of sites: indices and statistical inference. *Ecology* **90(12)**:3566–3574 DOI 10.1890/08-1823.1.

**Degma P, Bertolani R, Guidetti R. 2009–2018.** Actual checklist of Tardigrada species. Ver. 3.3. *Available at* http://www.tardigrada.net/register/taxonomy.htm (accessed on 14 August 2018).

**Degma P, Guidetti R. 2007.** Notes to the current checklist of Tardigrada. *Zootaxa* **1579**:41–53.

**Degma P, Katina S, Sabatovicova L. 2011.** Horizontal distribution of moisture and Tardigrada in a single moss cushion. *Journal of Zoological Systematics and Evolutionary Research* **49(S1)**:71–77 DOI 10.1111/j.1439-0469.2010.00602.

**Donoso DA, Johnston MK, Kaspari M. 2010.** Trees as templates for tropical litter arthropod diversity. *Oecologia* **164(1)**:201–211 DOI 10.1007/s00442-010-1607-3.

**Dufrêne M, Legendre P. 1997.** Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs* **67**:345–366 DOI 10.1890/0012-9615(1997)067[0345:SAAIST]2.0.CO;2.

**Fontaneto D, Melone G, Ricci C. 2003.** Structure of bdelloid rotifer metacommunities in two alpine streams in Northern Italy. *Acta Biologica, Studi Trentini di Scienze Naturali* **80**:23–26.

**Fontaneto D, Ricci C. 2006.** Spatial gradients in species diversity of microscopic animals: the case of bdelloid rotifers at high altitude. *Journal Biogeography* **33**:1305–1313 DOI 10.1111/j.1365-2699.2006.01502.x.

**Geiger R. 1967.** *The climate near the ground.* Translation of 4th German edition. Cambridge: Harvard University Press.

**Glime JM. 2013.** Tardigrade densities and richness, chapters 5–5. In: Glime JM, ed. *Bryophyte ecology. Vol. 2. Bryological 5-5-1 interaction.* MI: Michigan Technological University and the International Association of Bryologists.

**Grime JP. 1977.** Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist* **111**:1169–1194 DOI 10.1086/283244.

**Guidetti R, Altiero T, Marchioro T, Sarzi Amade L, Avdonina AM, Bertolani R, Rebecchi L. 2012.** Form and function of the feeding apparatus in Eutardigrada (Tardigrada). *Zoomorphology* **131**:127–1 DOI 10.1007/s00435-012-0149-0.

**Guidetti R, Bertolani R. 2005.** Tardigrade taxonomy: an updated check list of the taxa and a list of characters for their identification. *Zootaxa* **845**:1–46 DOI 10.11646/zootaxa.845.1.1.

**Guidetti R, Bertolani R, Nelson DR. 1999.** Ecological and faunistic studies on tardigrades in leaf litter of beech forests. *Zoological Anzeiger* **238**:215–223.

**Guil N, Hortal J, Sánchez-Moreno S, Machordom A. 2009.** Effects of macro and micro-environmental factors on the species richness of terrestrial tardigrade assemblages in an Iberian mountain environment. *Landscape Ecology* **24**:375–390 DOI 10.1007/s10980-008-9312-x.

**Guil N, Sanchez-Moreno S. 2013.** Fine-scale patterns in micrometazoans: tardigrade diversity, community composition and trophic dynamics in leaf litter. *Systematics and Biodiversity* **11**:181–193 DOI 10.1080/14772000.2013.798370.

**Guil N, Sánchez-Moreno S, Machordom A. 2008.** Local biodiversity patterns in micrometazoans: are tardigrades everywhere? *Systematics and Biodiversity* **7(3)**:259–268.

**Hallas, Yeates. 1972.** Tardigrada of the soil and litter of a Danish beech forest. *Pedobiologia* **12**:287–304.

**Harada H, Ito MT. 2006.** Soil-inhabiting tardigrade communities in forests of Central Japan. *Hydrobiologia* **588(1)**:119–127.

**Jönsson KI. 2003.** Population density and species composition of moss-living tardigrades in a boreo-nemoral forest. *Ecography* **26**:356–364 DOI 10.1034/j.1600-0587.2003.03458.x.

**Kaczmarek Ł, Michalczyk L. 2017.** The Macrobiotus hufelandi group (Tardigrada) revisited. *Zootaxa* **4363(1)**:101–123 DOI 10.11646/zootaxa.4363.1.4.

**Kaczmarek Ł, Michalczyk Ł, McInnes S. 2016.** Annotated zoogeography of non-marine Tardigrada. Part III: North America and Greenland. *Zootaxa* **4203(1)**:1–249 DOI 10.11646/zootaxa.4203.1.1.

**Kathman RD, Cross SF. 1991.** Ecological distribution of moss dwelling tardigrades on Vancouver Island, British Columbia Canada. *Canadian Journal of Zoology* **69**:122–129 DOI 10.1139/z91-018.

**Kinchin IM. 1994.** *The biology of tardigrades.* London: Portland Press, 96–98.

**Lowman MD, Rinkner HB. 2004.** *Forest canopies.* 2nd edition. Oakland: University of California Press.

**McCune B. 1993.** Gradients in epiphyte biomass in three *Pseudotsuga-Tsuga* forests of different ages in western Oregon and Washington. *The Bryologist* **96(3)**:405–411 DOI 10.2307/3243870.

**McCune B, Amsberry KA, Camacho FJ, Clery S, Cole C, Emerson C, Felder G, French P, Greene D, Harris R, Hutten M. 1997.** Vertical profile of epiphytes in a Pacific Northwest old-growth forest. *Northwest Science* **71(2)**:145–152.

**McCune B, Geiser L. 2009.** *Macrolichens of the Pacific Northwest.* 2nd edition. Corvallis: Oregon State University Press.

**McInnes SJ. 1994.** Zoogeographic distribution of terrestrial/freshwater tardigrades from current literature. *Journal of Natural History* **28**:257–352 DOI 10.1080/00222939400770131.

**Meyer HA. 2006.** Small scale spatial distribution variability in terrestrial tardigrade populations. *Hydrobiologia* **558**:133–139 DOI 10.1007/s10750-005-1412-x.

**Meyer HA. 2013.** Terrestrial and fresh water tardigrada of the Americas. *Zootaxa*
**3747(1)**:1–71 DOI 10.11646/zootaxa.3747.1.1.

**Miller WR, Gallardo L, Clark T. 2013.** Chapter 30. Do water bears climb trees too?
In: Lowman M, ed. *Tree tops at risk: the challenges of global canopy ecology and
conservation.* New York: Springer, 307–311.

**Miller WR, Horning DS, Heatwole HF. 2001.** Tardigrades of the Australian Antarctic:
Macquerie Island, sub-Antarctica. *Zoologischer Anzeiger* **240**:473–489.

**Miller WR, Lowman M. 2012.** Finding tardigrades in forest canopies. In: Lowman M,
Schowalter T, Franklin J, eds. *Methods in forest canopy research.* Oakland: University
of California Press, 240.

**Miller WR, Miller JD, Heatwole HF. 1994.** Tardigrades of the Australian Antarctic
territories: assessing diversity within a sample. *Memoirs of the Queensland Museum*
**36(1)**:13–145.

**Mitchell C, Miller WR, Davis B. 2009.** Tardigrades of North America: influence of
substrate on habitat selection. *Journal of the Pennsylvania Academy of Science*
**83(1)**:10–16.

**Mogle MJ, Kimball SA, Miller WR, McKown RD. 2018.** Evidence of avian-mediated long
distance dispersal in American tardigrades. *PeerJ* **6**:e5035 DOI 10.7717/peerj.5035.

**Morek W, Gasiorek P, Stec D, Blagden B, Michalczyk L. 2015.** Experimental taxonomy
exposes ontogenetic variability and elucidates the taxonomic value of claw configura-
tion in *Milnesium* Doyère, 1840 (Tardigrada: Eutardigrada: Apochela). *Contributions
to Zoology* **85(2)**:173–200.

**Nadkarni NM. 1994.** Diversity of species and interactions in the upper tree canopy of
forest ecosystems. *American Journal of Zoology* **34**:70–78 DOI 10.1093/icb/34.1.70.

**Natural Resources Conservation Service (NRCS). 2017.** Web Soil Survey. *Available at
https://websoilsurvey.sc.egov.usda.gov/* (accessed on 05 March 2017).

**Nelson DR. 1975.** Ecological distribution of tardigrades on Roan Mountain, Tennessee-
North Carolina. *Memorie dell'Istituto italiao id Idrobiologia* **32**:225–276.

**Nelson DR. 1982.** Developemental biology of the Tardigrada. In: Harrison F, Cowden R,
eds. *Developmental biology of freshwater invertebrates.* New York: Alan R. Liss.

**Norris DH, Shevock JR. 2004.** Contributions toward a bryoflora of California: a key to
the mosses. *Madroño* **51**:133–269.

**Oksanen J, Guillaume Blanchet F, Kindt R, Legendre P, Minchin PR, O'Hara RB,
Simpson GL, Solymos P, Stevens MHH, Wagner H. 2015.** vegan: community
ecology package. R package version 2.3-0. *Available at https://CRAN.R-project.org/
package=vegan.*

**Pilato G, Binda MG. 2010.** Definition of families, subfamilies, genera and subgenera of
the Eutardigrada and keys to their identification. *Zootaxa* **2404**:1–54.

**Poage MA, Barrett JE, Virginia RA, Wall DH. 2008.** The influence of soil geochemistry
on nematode distribution, McMurdo Dry Valleys, Antarctica. *Arctic, Antarctic, and
Alpine Research* **40**:119–128 DOI 10.1657/1523-0430(06-051)[POAGE]2.0.CO;2.

**Porazińska DL, Giblin-Davis RM, Powers TO, Thomas WK. 2012.** Nematode spatial and ecological patterns from tropical and temperate rainforests. *PLOS ONE* **7(9)**:e44641 DOI 10.1371/journal.pone.0044641.

**R Core Team. 2018.** R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. *Available at https://www.R-project.org/* .

**Ramazzotti G, Maucci W. 1983.** *Il Philum Tardigrada. Memorie dell'Istituto italiao id Idrobiologia*, 3rd edition. Vol. 41. Italy, 1–1012.

**Richardson BA, Richardson MJ, Soto-Adames FN. 2005.** Separating the effects of forest type and elevation on the diversity of litter invertebrate communities in a humid tropical forest in Puerto Rico. *Journal of Animal Ecology* **74**:926–936 DOI 10.1111/j.1365-2656.2005.00990.x.

**Salmon JT. 1951.** Polyvinyl alcohol as a mounting medium in microscopy. *The Microscope* **8(6)**:139–142.

**Sánchez-Moreno S, Ferris H, Guil N. 2008.** Role of tardigrades in the suppressive service of a soil food web. *Agriculture, Ecosystems and Environment* **124**:187–192 DOI 10.1016/j.agee.2007.09.011.

**Schram MD, Davidson PG. 2012.** A history and method of constructing homemade loops. *Transactions of the Kansas Academy of Science* **115(1–2)**:35–40 DOI 10.1660/062.115.0106.

**Schuster R, Greven H. 2013.** Reproductive traits of Macrobiotus hufelandi during a longterm field study with notes on Paramacrobiotus richtersi and Diphascon pingue (Eutardigrada). *Journal of Limnology* **72**:166–174.

**Schuster RO, Grigarick AA. 1965.** *Tardigrada from western North America: with emphasis on the fauna of California.* Oakland: University of California Press.

**Schuster RO, Grigarick AA. 1971.** Two new species of *Echiniscus* from the pacific northwest (Tardigrada: Echiniscidae). *Entomological Society of Washington* **73(2)**:104–111.

**Sohlenius B, Boström S, Jönsson KI. 2004.** Occurrence of nematodes, tardigrades, and rotifers on ice-free areas in East Antarctica. *Pedobiologia (Jena)* **48**:395–408 DOI 10.1016/j.pedobi.2004.06.001.

**Stec D, Morek W, Gąsiorek P, Michalczyk Ł. 2018.** Unmasking hidden species diversity within the Ramazzottius oberhaeuseri complex, with an integrative redescription of the nominal species for the family Ramazzottiidae (Tardigrada: Eutardigrada: Parachela). *Systematics and Biodiversity* **16(4)**:357–376.

**Tsujimoto M, Imura S, Kanda H. 2016.** Recovery and reproduction of an Antarctic tardigrade retrieved from a moss sample frozen for over 30 years. *Cryobiologia* **72**:76–81.

**Voegtlin DJ. 1982.** Invertebrates of the H.J. Andrews Experimental Forest, western Cascade Mountains, Oregon: a survey of arthropods associated with the canopy of old-growth Pseudotsuga menziesii. *Forest Research Laboratory, Oregon State University, Special Publication* **4**:1–31.

**Wickham H. 2009.** *ggplot2: elegant graphics for data analysis.* New York: Springer-Verlag.

**Wilson EO. 2002.** *The future of life.* New York: Alfred A. Knopf.

**Wright JC. 1989.** Desiccation tolerance and water retentive mechanisms in Tardigrades. *Journal of Experimental Biology* **142**:267–292.

**Wright JC. 1991.** The significance of four xeric parameters in the ecology of terrestrial tardigrada. *Journal of Zoology* **224**:59–77 DOI 10.1111/j.1469-7998.1991.tb04788.x.

**Young A, Clifton K. 2015.** Tardigrades inhabit lichen and moss in Smith Rock State Park, Oregon. *Bulletin of the California Lichen Society* **22(2)**:48–53.

**Zawierucha K, Smykla J, Michalczyk Ł, Gołdyn B, Kaczmarek Ł. 2015.** Distribution and diversity of Tardigrada along altitudinal gradients in the Hornsund, Spitsbergen (Arctic). *Polar Research* **34**:24168 DOI 10.3402/polar.v34.24168.

**Zawierucha K, Węgrzyn M, Ostrowska M, Wietrzyk P. 2017.** Tardigrada in Svalbard lichens: diversity, densities and habitat heterogeneity. *Polar Biology* **40(7)**:1385–1392 DOI 10.1007/s00300-016-2063-2.

**Zawierucha K, Zmudczyńska-Skarbek K, Kaczmarek Ł, Wojczulanis-Jakubas K. 2016.** The influence of a seabird colony on abundance and species composition of water bears (Tardigrada) in Hornsund (Spitsbergen, Arctic). *Polar Biology* **39(4)**:713–723 DOI 10.1007/s00300-015-1827-4.

**Zawierucha Z, Stec D, Lachowska-Cierlik D, Takeuchi N, Li Z, Michalczyk Ł. 2018.** High mitochondrial diversity in a new water bear species (Tardigrada: Eutardigrada) from mountain glaciers in central Asia, with the erection of a new genus Cryoconicus. *Annales Zoologici* **68(1)**:179–201 DOI 10.3161/00034541ANZ2018.68.1.007.