Tardigrades inhabit lichen and moss in Smith Rock State Park, Oregon

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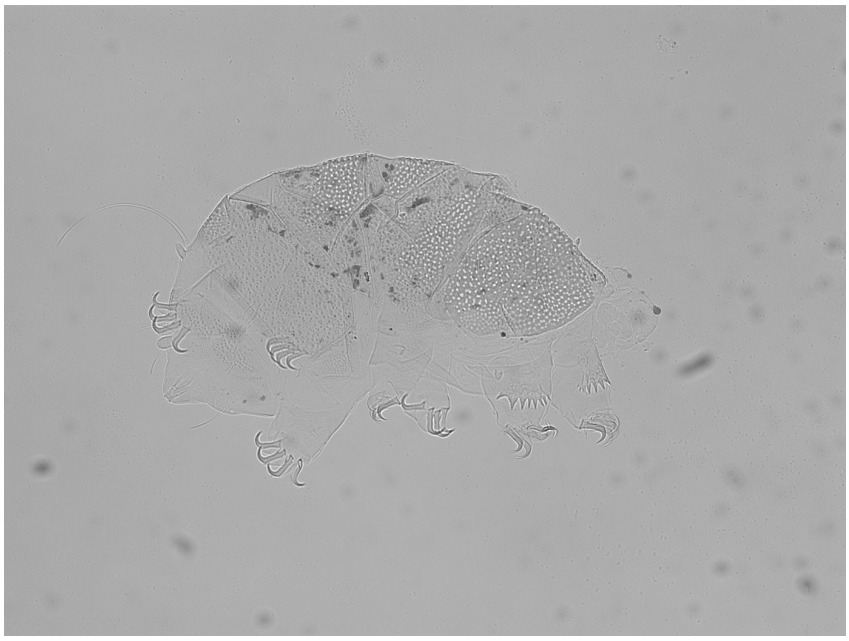


ALEXANDER YOUNG

Heterotardigrade *Echiniscus*

Abstract

One moss and twelve lichen samples collected from exposed rock cliffs at Smith Rock State Park in central Oregon contained 117 tardigrade specimens representing three orders, five families, and five genera, providing the first documentation of the phylum Tardigrada at this location. While tardigrades are widely known for their capacity to survive in extreme environments, little is known about species level habitat preferences. Previous research suggests that microclimatic differences in humidity may influence habitat suitability and determine species distribution. We examined tardigrade community composition in saxicolous moss and lichen at Smith Rock State Park in two small areas separated vertically by 122 meters. We examined if sample diversity was related to the species of moss or lichen (habitat), elevation, or weight of a sample. Tardigrades were preserved on microscope slides, and identified to Genus or species level. Wide variation was observed between seemingly identical habitats, with no apparent effect of elevation, habitat, or sample weight. The tardigrade species *Echiniscus trisetosus* had the highest abundance, and the lichen species *Umbilicaria phaea* and *Rhizoplaca melanophthalma* supported a majority of tardigrade individuals.

Introduction

Rock outcrops support the establishment and growth of saxicolous lichen and moss communities and provide physical refuge for a whole world of meiofaunal organisms including nematodes, rotifers, colembola, and tardigrades. The phylum Tardigrada, or water bears, is composed of small invertebrate animals ranging from 0.2 to 0.9 mm in length. They are complex organisms that have five body segments, four pairs of legs ending in claws, complex mouth and pharynx systems, and no respiratory or circulatory systems (Kinchin 1997). These animals have been found worldwide in aquatic, marine, and terrestrial habitats. They are notable for their ability to tolerate extreme environments (Miller 1997,

Glime 2013). Terrestrial tardigrades (limno-terrestrial tardigrades) are commonly found in patches of lichen and moss and require a threshold of humidity as they are only active when surrounded by a film of water. Tardigrades are known for their ability to withstand a drying environment. When osmotically stressed, a tardigrade will decrease its surface area, and lose up to 98% of its water content, entering an anhydrobiotic state known as a "tun" (Wright 1989). Water bears in a tun state can tolerate an extreme range of environmental conditions for prolonged periods of time (Kinchin 1994). Different species of tardigrade show variation in the ability to enter the tun state, often limited by the rate of desiccation experienced in the environment (Wright 1991), thus species' response to seasonal humidity patterns create complex community dynamics (Schuster 2007).

Some tardigrade species are known to have broad geographic ranges (Glime 2013), however, little attention has been given to the study of small scale species distribution. Meyer (2006) reports high spatial variability in tardigrade community composition among seemingly identical habitats. Miller and Heatwole (1994) show significant evidence for non-random clustering of species within a patch of moss, with more than half of the specimens occurring within the top 1.76 cm. Wright (1991) documents different tardigrade species specifically found in regularly desiccated, high stress habitats (xerophillic) as compared to species with limited tolerance to desiccation requiring consistently higher humidity (hygrophyllic) (Wright 1989).

Over the last 75 years, 25 species of tardigrades have been reported by four papers from the state of Oregon (Meyer 2013). This project aimed at beginning to document the tardigrade diversity in Smith Rock State Park, Oregon by collecting saxicolous lichen and moss species from two elevations on the same rock cliff. The project tested a hypothesis of uniformity, i.e. there should be no differences in tardigrade populations between habitats (moss & lichen species), elevation, or sample weight. This is the first report of the phylum Tardigrada from the walls of Smith Rock State Park.

Methods

Field Collection

The exposed cliffs of Smith Rock State Park are located on the leeward side of the Cascade Range in a desert setting in central Oregon. The region receives an average of 21.6 cm of rain per year, with temperatures exceeding 37º C in summer and below -18º C in the winter (Watts 1992). The rock is primarily composed of tuff, a volcanic rock with silica rich magma intrusions. The sheer cliffs rise above 120 meters allowing for variation in humidity along a vertical gradient.

Patches of moss and lichen were collected from the base of the northwestern face of the Smith Rock Group formation and from 122 meters above, at the summit of the technical climbing route "Wherever I May Roam" (Watts 1992). A dull metal spatula was used to collect 5 by 5 cm patches of morphologically distinct lichen or moss. No rock was damaged. The resulting debris was collected in paper bags and dried at room temperature.

Oregon Parks Recreational Department Permit

Although no species of tardigrade is listed under either the State or Federal Endangered Species Acts, the cryptogamic communities they inhabit are sometimes considered rare or sensitive. It is therefore necessary that all rules, regulations, and guidelines regarding destructive sampling be followed. This project was conducted under Oregon Parks Recreational Department Permit No: 035-14

Processing Habitat for Tardigrades

Small habitat samples of unique lichen and moss morphologies were examined for traces of tardigrade communities including tardigrade eggs, cuticle bound eggs (exuvia), the remnants of adult and juvenile tardigrades (cuticles), and living tardigrades (Miller 1997). Prior to hydration, a dried sub sample of habitat (moss or lichen) was weighed using a Sartorius BP210 S scale. The sample was hydrated with 25 mL of commercial spring water for 24 hours. A broad mouthed pipette was used to vacuum the sediment from the bottom of the hydrating vessel and placed into a 2.5 x 5.0 x 0.5 cm trough. The

area of the trough was visually searched at 20x with a dissecting microscope. Specimens were selected and moved with the aid of an Irwin loop (Schram 2012). Specimens were deposited into a drop of PVA (polyvinyl alcohol) media (Salmon 1951) on a glass slide and covered with a glass cover slip.

Tardigrade Identification

Images were captured with a Zeiss AxioImager D2 compound microscope using DIC (Differential Interference Contrast) microscopy. Tardigrade identification was determined with the keys in Ramazzotti and Maucci (1983), Pilato and Binda (2010), and Michalczyk and Kaczmarek (2012). Nomenclature follows Guidetti and Bertolani (2005), Degma and Guidetti (2007), and Degma *et al.* (2009-2015).

Lichen Identification

Lichens were imaged in the field with an Olympus Tough tg-3 camera (Figure 1), and identified with *Lichens of North America* (Brodo *et al.* 2001). Dr. Bruce McCune, University of Oregon, kindly confirmed the identifications.

Statistical Tests

Analysis of Variance (ANOVA) in R (ver. 3.1.3) was used to analyze for the uniformity of tardigrade diversity for sample location, habitat, and weight (R Development Core Team 2008). The diversity of a sample was quantified using the Shannon index (Cain 2011). A *p*-value less than 0.05 would represent a significant difference between two variables.

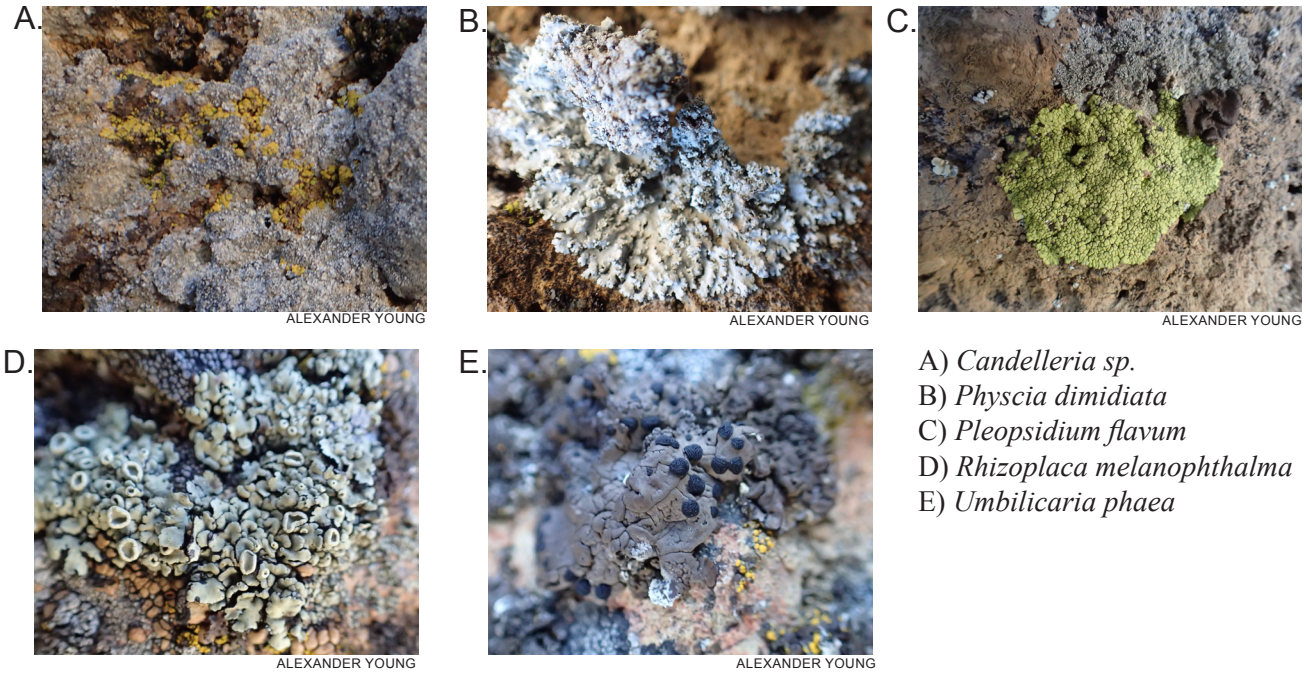
Results

Twelve patches of lichen and one patch of acrocarpous rock moss were collected. Samples contained five species of lichen (Table 1, Figure 1). One hundred seven tardigrades were recovered from seven of the lichen samples and 10 specimens were found in the moss sample (Table 1). Tardigrade abundance varied dramatically between samples: five samples did not contain tardigrades, whereas one sample contained 61 specimens (Table 1). All tardigrades were identified to genus and three were identified to species including *Echiniscus trisetosus* Cuenot, 1932, *Isohypsibius marcellinoi* Binda & Pilato, 1971, and *Ramazzottius oberhauseri* Doyere, 1840 (Figure 2). *Isohypsibius marcellinoi* is a new record for the state of Oregon having been recorded only from California, Tennessee and Greenland (Meyer 2013) and *Echiniscus trisetosus* was the most abundant species of tardigrade (Figure 2).

The mass of the samples ranged from 0.15 to 1.86 grams. A slight increase in tardigrade abundance was observed for samples above 1.5 grams (Figure 3) while the most abundant sample contained 61

Table 1. A summary of sample information including the abundance of each genus.

Lichen species	Weight (g)	Location	Total	*Echiniscus*	*Isohypsibius*	*Macrobiotus*	*Milnesium*	*Ramazzottius*
Candelleria	0.83	Top	0	0	0	0	0	0
Candelleria	0.15	Top	5	5	0	0	0	0
Moss	1.86	Top	10	0	7	0	3	0
Physcia dimidiata	1.07	Bottom	1	0	1	0	0	0
Physcia dimidiata	1.74	Top	0	0	0	0	0	0
Pleopsidium flavum	1.44	Top	2	0	0	2	0	0
Rhizoplaca melanophthalma	1.54	Bottom	4	0	1	0	1	2
Rhizoplaca melanophthalma	0.86	Top	61	47	0	0	12	2
Umbilicaria phaea	1.54	Bottom	0	0	0	0	0	0
Umbilicaria phaea	1.00	Top	0	0	0	0	0	0
Umbilicaria phaea	1.58	Top	0	0	0	0	0	0
Umbilicaria phaea	1.6	Top	1	0	0	0	0	1
Umbilicaria phaea	1.67	Top	33	32	0	0	1	0



A) *Candelleria sp.*
B) *Physcia dimidiata*
C) *Pleopsidium flavum*
D) *Rhizoplaca melanophthalma*
E) *Umbilicaria phaea*

Figure 1. Images of lichen collected at Smith Rock State Park, Oregon.

tardigrades and only weighed 0.86 grams.

The diversity of each sample was calculated as the Shannon index. No evidence was found that diversity was related to cryptogam species (moss or lichen), location of collection (top or bottom), or the mass of the sample.

Discussion

This report has added the phylum Tardigrada with five genera and three species to the Smith Rock State park diversity records, and the species *Isohypsibius marcellinoi* is new to the biodiversity of Oregon. This is not a complete view of composition of the tardigrade community in Smith Rock State Park or Oregon but is a snapshot in time of a small collection area. This report does not account for spatial or seasonal variation. It provides evidence of the presence of tardigrades in the park, an observation of some of the resident tardigrade species, and demonstrates the variation between patches of moss and lichen species.

Our data shows great differences in tardigrade population density and diversity (Table 1) thus the hypothesis of uniformity is rejected. Yet, the analysis of variance did not identify that differences in habitats (moss & lichen), location, or sample weight were sufficient to explain the differences. To explain the wide variation in tardigrade communities across the small collection site it is necessary to propose that random not uniform dispersal events into habitats with variable suitability result in the establishment of micro-populations (Glime 2013) that resemble islands.

Additionally, Miller and Heatwole (1994) showed that moisture may be the most important factor influencing community dynamics. If the distribution

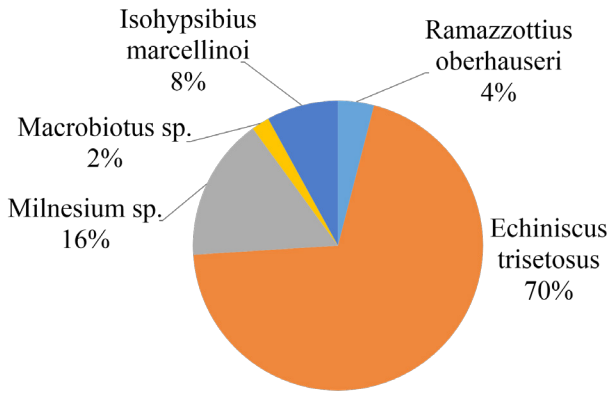


Figure 2. Proportional representation of Smith Rock State Park tardigrade genera.

of moss and lichen is also influenced by moisture, it is plausible that the variation in tardigrade communities is also a reflection of the suitability of the substrate that is the lichen or moss habitat. The ability of terrestrial tardigrades to exist in an anhydrobiotic state for a prolonged time does not supersede the need for a suitable environment.

Our experience suggests that future investigations into tardigrade communities must include a more rigorous investigative design and sampling technique to insure definitive analysis that will identify the contribution of each variable. Additionally, we recommend that field collections be greater than 1.5 grams. We conclude that the small size of the collection was not sufficient to identify the characteristics responsible for the disparity of tardigrade diversity and density.

Acknowledgements

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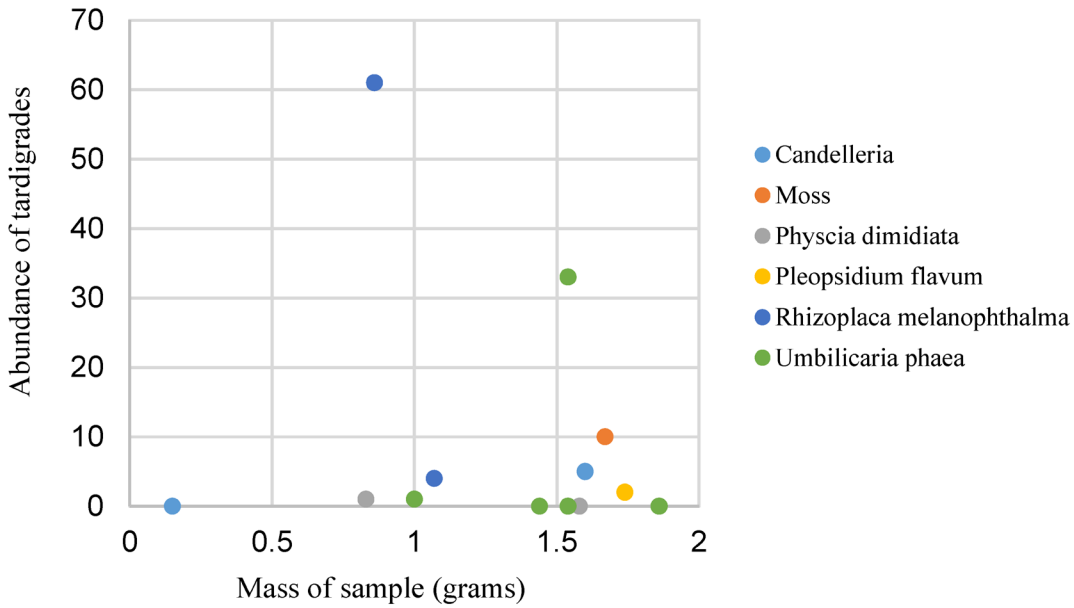

Figure 3. Sample mass versus the abundance of tardigrades in each sample.

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