Tardigrades of North America: Western Montana

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Abstract

Tardigrades are an under-studied phylum of microscopic aquatic animals that inhabit the interstitial moisture in mosses and lichens. This little-known group may at times be common and abundant, but because of the unique habitat, it is often overlooked in the biodiversity of an area. This is only the second report of tardigrades in Montana. The nine genera and 20 species are all new records for west of the Continental Divide in Montana. Each species collected represents a significant range extension, expands the known biodiversity of the state, and infers the diversity of the region is far greater than yet revealed. Five pairs of species were associated with each other statistically more frequently than expected. Four of the species were also statistically associated with the lichen habitat and three with the moss. Likewise, several species of tardigrade were found in habitats on soil and rock substrates more often than expected. These glimpses into habitat selection of tardigrades offer clues to the limits of tardigrade life.

Key words: tardigrades, association, biogeography, distribution, habitat, Montana
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Introduction

Tardigrades are a phylum of cuticle-shedding micro invertebrates that are related to Arthropods. They inhabit the interstitial space within the foliage of mosses and lichen. These free-living, aquatic animals are seldom > 0.5 mm in length, have five body segments, and look like miniature caterpillars (Miller 1997). Called water bears because of their slow, deliberate movement, they have four pair of telescopic legs, each ending in a set of claws (Fig. 1A and 1B). Water bears have a dorsal brain, a ventral nervous system, separate sexes, and lay eggs. They have a complete digestive system, and a complex feeding apparatus. They possess a simple excretory system but lack a circulatory or a respiratory system (Kinchin 1994). Over 1000 species have been described from marine, freshwater, and the terrestrial habitats. The phylum is divided into two major classes, Heterotardigrada with a dorsally armored cuticle, separated claws, and filamentous appendages or cirri (Fig. 1A) and Eutardigrada with a naked cuticle, paired claws, and no cirri. (Fig. 1B).

Tardigrades are known for cryptobiosis, i.e., their ability to survive long periods of dehydration and return to an active life. As their environment dries out, they shrivel to 1/3 adult size, desiccate, and wait for the environmental moisture to return.

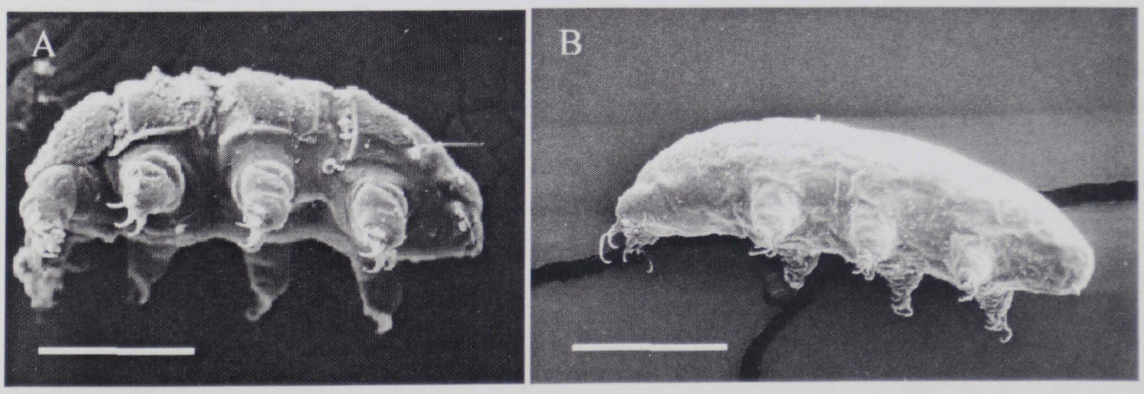

Figure 1. A. Heterotardigrada, B. Eutardigrada. Measure bars are 100 micrometers.

Cryptobiosis allows tardigrades to survive extreme environmental conditions and be distributed on the wind as flakes of dust (Pennak 1953).

As a cosmopolitan animal one would expect tardigrades to be to found everywhere. They are not, and distributional patterns are vague and incomplete (Miller 1997). It is not known if they are habitat selective, or what factors limit their distribution. Tardigrades do not cause any human problems, but there is increasing evidence that their presence or absence could be indicators of air pollution or environmental change (Hohl et al. 2001, Steiner 1994, 1995). But there are no reports of the response of tardigrade species to environmental chemicals.

In a zoogeographic review, McInnes (1994) credits Beasley (1988) with the only record of a tardigrade from Montana. In work on altitudinal distribution in New Mexico, Beasley (1988) described the new species *Hypsibius macrocalcaratus* collected it in several western states including Montana but provided no details. The next closest record is Maucci (1987) who reported on the tardigrades of Yellowstone National Park.

This project was undertaken to (1) survey tardigrades of Montana west of the Continental Divide and (2) test an hypothesis that there should be little difference in the density or diversity of cosmopolitan animals unless there is a habitat limitation.

Study Area

Western Montana is defined as that portion of the state west of the Continental Divide, from W116°, N49° to W112°, N45° and bounded on the west by the crest of the Bitterroot Range, and encompasses approximately 62,500 km^2 (Fig. 2). A series of mountain ranges and intervening valleys formed by two large river systems, the Kootenai and the Clark Fork of the Columbia, characterize the region. Valleys are typically 840 m deep and the mountains may extend above 3400 m. Vegetation varies from alpine on the higher peaks through coniferous forests to bunchgrass prairie along the rivers.

Materials and Methods

Samples of lichens and mosses (habitats) were collected from a variety of substrates (trees, rocks, soil, log) at 40 sites selected to provide coverage of the types of environs (streams, meadows, forest, alpine) in the study area (Fig 2). Field and laboratory procedures later described by Miller (1997) were developed. I collected samples by scraping a handful of plant material into a brown paper bag. Collection data was written directly on the bag. Approximately half of each sample was used as herbarium reference and half from which I extracted tardigrades. Each sample was soaked for 48 hrs in a bowl of distilled water and then squeezed and removed. Debris on the bottom of the bowl was examined with a dissecting microscope at 20X and reflected light. Tardigrades were transferred by micropipette to 70-percent alcohol tinted with iodine for preservation and mounting on glass slides in Hoyer's medium.

The keys of Schuster and Grigarick (1965), Pennak (1953), Marcus (1959), Riggin (1962) and Ramazzotti (1962) were used for identification of specimens. The taxonomy has been updated based on Ramazzotti and Maucci (1983) and Nelson (1991).

Chi-Square (χ^2) analysis of association was used to detect relationships between species of tardigrades and between tardigrades and their habitat. The expected value was calculated as the product of the two elements being compared divided by the total possibilities. This produced an estimate in proportion to the concentration of each within the collection. I calculated the χ^2 statistic as the summation of the squares of the difference between the expected and observed values divided by the expected value. For a *P*- value of 0.05 the critical value for χ^2 is 3.84 with 1 degree of freedom. A minimum expected value of 5 is required, or else the test is too sensitive to the shift of a single data point and the result was considered unreliable.

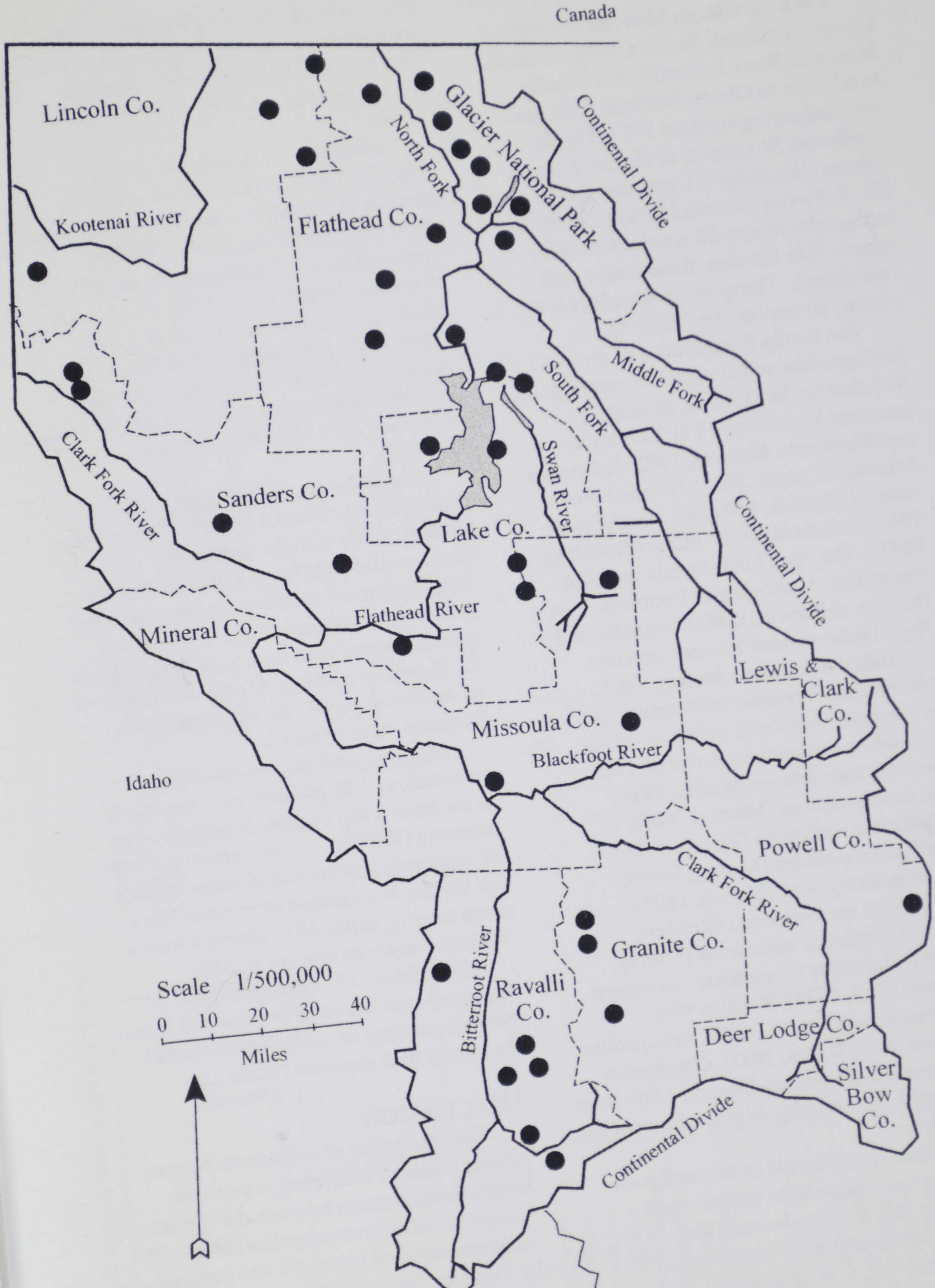

Figure 2. Montana west of the Continental Divide, showing the collection sites.

Results

The nine western Montana counties of Lincoln, Flathead, Sanders, Lake, Mineral, Missoula, Powell, Ravalli, and Granite in addition to Glacier National Park were sampled during summer 1967 (Fig. 2). I collected 88 samples of mosses (75), lichens (10), fungi (1), and vascular plants (2). Fifty-one samples (58%) yielded 794 tardigrades among 20 species from nine genera, four families, three orders, and two classes. Thirty-seven samples (42%) yielded no tardigrades (Table 1).

The family Echiniscidae of the order Echiniscoidae in the class Heterotardigrada included the five species: *Echiniscus arctomys* Ehrenberg, 1853; *Echiniscus quadrispinosus* Richters, 1902; *Echiniscus trisetosus* Cuenot, 1932; *Pseudechiniscius raneyi* Grigarick, Mihelcic, and Schuster, 1964; *Pseudechiniscius victor* (Ehrenberg, 1853). The class Eutardigrada included two orders, Apochela and Parachela. Two families of Parachela, Macrobiotidae and Hypsibiidae yielded fourteen species: *Macrobiotus areolatus* Murray, 1907; *Macrobiotus harmsworthi* Murray, 1907; *Macrobiotus hufelandi* Schultze, 1834; *Macrobiotus islandicus* Richters, 1903; *Macrobiotus richtersi* Murray, 1911; *Diphascon alpinus* (Murray, 1906); *Diphascon arduifrons* (Thulin, 1928); *Diphascon oculatus* (Murray, 1906); *Diphascon scoticus* (Murray, 1905); *Diphascon spitzbergensis* (Richters, 1903); *Platicrista angustatus* (Murray, 1905); *Hypsibius (Hypsibius) convergens* (Urbanowics, 1925); *Ramazzottius oberhaeuseri* (Doyere, 1840); *Isohypsibius prosostomus* (Thulin, 1928). One species, *Milnesium tardigradum* Doyere, 1840, from the family Milnesiidae of the order Apochela was found.

Three-quarters (15) of the tardigrade species were considered less common because they were recovered from < 10 percent of the samples. Five species (*H. convergens, R. oberhaeuseri, M. richtersi, M. harmsworthi, and M. hufelandi*) were more common and recovered from 14 to 48 percent of the samples (Table 1). Eight samples had only a single specimen of a more common species, whereas the less common species were always found in the presence of the more common species (Table 1).

Diversity within positive samples ranged from one to eight species with an average of 3.0. Mosses exhibited the greatest diversity range of one to eight tardigrade species with a 3.3 average/moss sample, lichens expressed a maximum diversity of seven species with an average of 2.2 and the vascular plant samples had four total species of tardigrades and an average of 3.5. Average diversity for substrates was trees, 2.16; soil, 2.85; rock, 3.53; and logs, 3.80 (Table 1).

Three species of tardigrade—*M. hufelandi*, *M. richtersi*, and *H. convergens*—each occurred with the species *M. harmsworthi* significantly more frequently than expected if each were independent and randomly distributed as expressed by a χ^2 statistic greater than the critical value (Table 2). *Hypsibius convergens* also occurred with *M. hufelandi* and *R. oberhaeuseri* more frequently than expected.

Macrobiotus harmsworthi, *M. hufelandi*, and *M. richtersi* each occurred in the moss samples more frequently than expected (Table 2). There were indications of significant association by these animals for lichens, but because the sample sizes were smaller, expected values < 5, so the statistical analysis was not reliable.

In addition, *M. harmswrothi* and *M. hufelandi* both occurred in moss and lichen habitats growing on soil substrate more frequently than expected (Table 2.)

Discussion

The limitations of collections became evident when we attempted to calculate associational patterns between the species of tardigrade and their moss species habitat. The minimum expected value of 5 was invoked by the moss diversity of our collection. We defaulted to pooling the mosses and lichens to compare their usage by the species of tardigrades. Still, three of the five most abundant species occurred significantly

Table 1. Tardigrade species habitat and substrate selection.

Habitat / Plant	Substrate	*E. arctomys*	*E. quadrispinosus*	*E. trisetosus*	*P. raneyi*	*P. victor*	*M. areolatus*	*M. harmsworthi*	*M. hufelandi*	*M. islandicus*	*M. richtersi*	*D. alpinus*	*D. arduifrons*	*D. oculatus*	*D. scoticus*	*D. spitzbergensis*	*P. angustata*	*H. convergens*	*R. oberheauseri*	*I. prosostomus*	*M. tardigradum*	# tardigrade species
Fungi	S								1													1
Lichen	R								1										1			2
Lichen	R							1	1									1	1			4
Lichen	S							1	1													2
Lichen	S				1			1	1									1				4
Lichen	T							1	1													2
Lichen	T							1														1
Lichen	T							1			1											2
Lichen	T							1										1				2
Lichen	T																		1			1
Brachythecium albicans	L							1	1		1											3
Brachythecium albicans	S							1	1													2
Brachythecium albicans	S								1												1	2
Brachythecium frigidum	L							1	1		1							1				4
Brachythecium velutinum	S							1	1						1			1				4
Claopodium bolanderi	R	1		1			1	1	1		1											6
Cratoneuron filicinum	R							1			1	1		1								4
Dicrananoweisia crispula	R								1										1			2
Dicranum sp.	S		1						1										1			3
Dicranum sp.	S				1	1		1	1				1	1				1	1			8
Dicranum fuscscens	S								1													1
Dicranum tauricum	L							1	1						1	1					1	5
Drepanocladus aduncus	S								1									1				2
Eurynchium pulchellum	L							1	1		1				1			1				5
Eurynchium pulchellum	R							1	1		1											3
Eurynchium pulchellum	S		1						1							1						3
Fissidens adianthoides	S								1													1
Homalothecium nevadense	R							1	1													2

Table 1. Tardigrade species habitat and substrate selection (cont.).

Habitat / Plant	Substrate	*E. arctomys*	*E. quadrispinosus*	*E. trisetosus*	*P. raneyi*	*P. victor*	*M. areolatus*	*M. harmsworthi*	*M. hufelandi*	*M. islandicus*	*M. richtersi*	*D. alpinus*	*D. arduifrons*	*D. oculatus*	*D. scoticus*	*D. spitzbergensis*	*P. angustata*	*H. convergens*	*R. oberheauseri*	*I. prosostomus*	*M. tardigradum*	# tardigrade species
Homalothecium nevadense	R							1	1		1										1	4
Homalothecium nevadense	S							1	1		1											3
Homalothecium nevadense	S								1									1				2
Homalothecium nevadense	S							1	1		1											3
Homalothecium occidentali	R						1	1	1		1			1				1	1			7
Hypnum revolutum	R							1	1				1		1			1	1			6
Leptodictyum riparium	S				1			1	1	1	1	1						1				7
Orthotrichum speciosum	S							1			1											2
Pleurozium schreberi	S								1									1				2
Pogonatum alpinum	S											1					1					2
Pohlia nutans	S								1	1												2
Polytrichum juniperinum	S								1		1											2
Polytrichum juniperinum	T							1	1						1			1	1			5
Pseudoleskea patens	S								1			1										2
Rhytidiopsis robusta	S							1	1			1										3
Rhytidiopsis triquetrus	S								1		1											2
Sphagnum sp.	S								1						1							2
Timmia austriaca	R								1									1	1			3
Moss	L																		1			1
Moss	R								1													1
Moss	R							1														1
Moss	S							1	1			1		1						1		5
Vascular Plant	R								1							1		1				3
Vascular Plant	R							1			1							1	1			4
Total Number of Samples		1	2	1	3	1	2	30	42	2	16	6	2	4	6	3	1	17	12	1	3	
Total Number of specimens	3	2	1	5	4	3	109	459	2	90	9	9	4	6	3	1	44	34	1	5	794	

1 = species present, blank = species not present. Substrate code: R = rock, S = soil, T = tree, L = log.

Table 2. Analysis of association between tardigrade species and tardigrades, habitats and substrates.

		M. harmsworthi	*M. hufelandi*	*M. richtersi*	*H. convergens*	*R. oberheauseri*
	n	χ^2	χ^2	χ^2	χ^2	χ^2
Animals						
M. harmsworthi	30	-	-	-		
M. hufelandi	42	5.26+	-	-	-	-
M. richtersi	16	13.40+	2.49	-	-	-
H. convergens	17	6.64+	5.84+	1.18	-	-
R. oberheauseri	12	0.89	1.87	0.39	9.46+	-
Habitats						
Moss	38	5.00+	13.90+	7.28+	1.82	0.64
Lichen	10	11.10+	12.10+	0.10	16.10+	5.10+
Substrates						
Soil	26	5.20+	10.83+	0.34	0.78	0.67
Rock	15	4.67+	3.27	3.93+	3.32	12.0+
Total Samples	88	χ^2 = Chi square, CV = 3.84 at P > 0.05, df = 1. + = significant association.				

more frequently in mosses than expected. Because the moss collection was diverse enough to prevent associational analysis at the species level, we suggest that the expressed association of tardigrade species to each other is rather an independent expression for the habitat, moss.

One of the general rules about tardigrades is that the wider the buccal tube the more predacious the species, and conversely, the narrower the buccal tube the more herbivorous. The three most common species of Macrobiotus (*M. harmsworthi*, *M. hufelandi*, and *M. richtersi*) were all large animals with wide buccal tubes that are known as carnivores (Kinchin 1994). The other animals, with the exception of *Milnesium tardigradum*, are smaller animals with narrow buccal tubes. We do not suggest that the bigger tardigrades prey exclusively on the smaller species, but the predators were greatest in number and may have been acting as a controlling agent for the populations of the less common species that always occurred in small numbers with the bigger animals.

Considering the cosmopolitan nature of the animal, given wind distribution, and their broad dispersion over the study area, it is difficult to think that the animals have not arrived at any specific sample. Rather, it is more likely they arrived, found the habitat unacceptable for active life and have not persisted. The fact that 37 of 88 samples yielded no tardigrades suggested there were habitat-induced limits for active tardigrade life. This suggested that a range of conditions can exist within the moss or lichen interstitial space. This was also supported by the fact that 30 out of 38 moss samples had different combinations of tardigrade species. All 20 species were found in moss samples, only seven were found in lichens, five in vascular plants, and one in a fungus.

Maucci (1987) reported 23 species of tardigrade from Yellowstone National Park, of which only 11 were also found in our Montana collection. The finding of *D. arduifrons* as new to North America and five other species (*D. spitzbergensis*, *E. quadrispinous*, *E. arctomys*, *P. raneyi*, and *P. victor*) on the continent for only the second or third time infers the diversity of the region is still far greater than yet revealed.

These clues into the natural history of the tardigrades should lead to investigations into the nature and limits for active life. Such information may shed light on the differential survival relative to man-made sources of environmental modification such as power plants (Hohl et al. 2001) or roads (Steiner 1994, 1995). Identification of tardigrades as vectors of botanical pathogens by Krantz et al. (1999) opens many questions about the impact of wind dispersal of micro invertebrates.

Tardigrades are a new phylum to be added to the known biodiversity of Montana. This addition to their known ranges only confirms our limited knowledge of their patterns and mechanisms of dispersal, survival, and sustainable existence.

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LITERATURE CITED

Beasley, C. W. 1988. Altitudinal Distribution of Tardigrada of New Mexico with the Description of a New Species. American Midland Naturalist 120:436-440.

Krantz, S., T. G. Benoit, and C. W. Beasley 1999. Phytopathogenic bacteria associated with Tardigrada. Zoologischer Anzeiger 238:259-260.

Hohl, A. W. R. Miller, and D. R. Neslon. 2001. The Distribution of tardigrades upwind and downwind of a Missouri coal-burning power plant. Zoologischer Anzeiger, 240:395-401.

Kinchin, I. M. 1994. The Biology of Tardigrades. Portland Press, London, UK. 184 pp.

Maucci, W. 1987. A Contribution to the Knowledge of the North American Tardigrada with Emphasis on the Fauna of Yellowstone National Park (Wyoming). Pp. 187-210 *in* Bertolani ed.. Biology of Tardigrades. Selected Symposia and Monographs U.Z.I. R.

McInnes, S. J. 1994. Zoogeographic distribution of terrestrial/freshwater tardigrades from current literature. Journal of Natural History 28:257-352.

Miller, W. R.1997. Tardigrades: Bears of the Moss. The Kansas School Naturalist 3-4:1-16.

Marcus, E. 1959. Tardigrada. Pp. 509-521 in W. T. Edmonson, ed. Freshwater Biology, Wiley, New York.

Nelson, D. R. 1991. Tardigrada. Pp. 501-521 in J. H. Throp, and A.P.Crovich, eds. Ecology and Classification of North American Freshwater Invertebrates. San Diego Academy Press.

Pennak, R. W. 1953. Tardigrada. Pp. 240-256 in Freshwater Invertebrates of the United States. Ronald Press, New York.

Ramazzotti, G. 1962. Il Phylum Tardigrada. Memorie dell'Istituto Italiano di Idrobiologia 16:1-529.

Ramazzotti, G., and W. Maucci. 1983. Il Phylum Tardigrada. III. Memorie dell'Istituto Italiano di Idrobiologia 41:1-1011.

Riggin, G. T. 1962. Tardigrada of Southwest Virginia: With the addition of a description of a new species from Florida. Virginia. Agricultural Experiment Station Technical Bulletin 152:1-145.

Schuster, R. O., and A. A. Grigarick. 1965. Tardigradea from western North America with emphasis on the fauna of California. UC publications in Zoology No.76, Uiversity of California Press, Berkeley, CA. 87 pp.

Steiner, W. A. 1994. The Influence of Air Pollution on Moss-Dwelling Animals: 2. Aquatic Fauna with Emphasis on Nematoda and Tardigrada. Revue Suisse de Zoologie 101: 533-556.

Steiner, W. A. 1995. The Influence of Air Pollution on Moss-dwelling Animals: 5. Fumigation Experiments with SO2 and Exposure Experiments, Revue Suisse de Zoologie 102: 13-40.

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