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## Notes on the Tardigrades of Southwestern Michigan

James J. Sawtell

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NOTES ON THE TARDIGRADES OF SOUTHWESTERN MICHIGAN

A Thesis  
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of the requirement for  
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in Biology

by  
James J. Sawtell  
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## Table of Contents

Introduction	1
Systematic Position of the Tardigrada	3
Methods	4
Accounts of Species	7
Key to species found in Michigan	11
Summary	12
Table 1	5

## Introduction

Tardigrades, comprising fewer than 350 species, are microscopic animals which inhabit mosses, lichens, and algae. Tardigrades or "water bears" are distributed throughout the world. These invertebrates are found in fresh-water substrates (Cooke, 1959; Marcus, 1939, Moore, 1939; Pennak, 1940), marine substrates (Chitwood, 1951; Green, 1950; Jeannel et al, 1940; Marcus, 1927, 1946; Pennak, 1953:240; Wieser, 1959) and terrestrial habitats (Cuenot, 1929; Marcus, 1960; Rodriguez-Roda, 1948; Pennak, 1953:240). The "water bears" have also been found in the psammolittoral zone where they live between grains of sand, (Pennak, 1940) and there feed on algal cells (Marcus, 1929). Tardigrades puncture the cell wall of mosses and other cryptogams and suck out the cell contents (Higgins, 1959). Only one species, Milnesium tardigradum Doyere, is considered to be carnivorous (Pennak, 1953:243). The first tardigrades were described as the "little bears of water" by J. Goeze in 1773 (Marcus, 1960). The name "tardigrades" was applied in L. Doyere's fundamental memoirs on the class (Marcus, 1960).

Although tardigrades have long been a subject of investigation in Europe (Bartos, 1934, 1935, 1936, 1941; Bertelani, 1941; Cuenot, 1932; Cunha, 1943, 1944a, 1944b, 1947, 1948; Felfoldy, 1947; Grell, 1937; Grimp and Wagler, 1928; Iharos, 1947; Marcus, 1929; Mihelcic, 1938a, 1938b, 1949; Monard, 1928;

Ramazzotti, 1943, 1945, 1954, 1956, 1958; Rodriguez-Roda, 1952; Schulz, 1935; Teodaro, 1926 and Thulin, 1911), they have received little attention from American investigators. The first report of a tardigrade from the United States was furnished by Packard in 1873. Higgins (1959) listed 14 states from which tardigrades have been recorded: California (Mathews, 1938); Colorado (Higgins, op.cit.); District of Columbia (Marcus, 1926; Mathews, op.cit.); Illinois (Newhaus, cited by Mathews, op.cit.); Maine (Beal, 1880; Packard, 1873); Massachusetts (Marcus, 1946); Maryland (Curtin, 1957); Michigan (Mathews, op.cit.; Moore, 1939); New York (Marcus, 1929); North Carolina (Hay, 1917); Vermont (Mathews, op.cit.); Washington (Mathew, op.cit.; Wiesser, 1959; Whittaker and Fairbanks, 1958); Wisconsin (Mathews, op.cit.; Pennak, 1940); Texas (Mathews, op.cit.; Chitwood, 1951). With the addition of New Jersey (Hausman, 1923; Moul, 1960, personal communication) the total number of states reporting tardigrades is 15.

Tardigrades occur in other parts of the world besides Europe and the United States. Murray (1907, 1910) found tardigrades in both the Arctic and Antarctic. Peterson (1951) offers some ecological remarks on the tardigrade fauna of Greenland. The mosses from Krakatau (East Indies) have produced specimens of "water bears" (Heinis, 1928). Marcus (1933) and Teunissen (1938) discovered tardigrades in Africa. The fresh

water fauna including tardigrades of New Zealand were investigated by Brehm (1928). South America has had many active investigators in recent years: Marcus (1939, 1946); du Bois-Reymond Marcus (1944); de Barros (1939a, 1939b, 1942a, 1943); and Rahm (1932).

Marcus (1929, 1936, 1960) has summarized most of the literature on tardigrades. Higgins (1959) investigated the number of ecdyses in the life history of Macrobiotus islandicus Richters. Green (1950) suggested facultative commensalism for Echiniscoides sigismundi as is the case for some other tardigrades. Anabiosis of tardigrades has been the point of investigation of such authors as Baumann (1929), Rahm (1926), Pigon and Weglarska (1955a, 1956) and Labunets (1950). The possibility of tardigrades being transmitters of virus diseases in bryophytes was investigated by Blattny, Pilous and Vosvald (1949). Metabolic rate was investigated by Pigon and Weglarska (1955b) and Rahm (1928, 1929). May (1946) investigated the coelomic corpuscles in the intact animals. Overgaard (1948) and Marcus (1928, 1929) have contributed to our knowledge of the ecology of tardigrades.

#### Systematic Position of the Tardigrada

There is some question as to whether tardigrades are to

be regarded as more closely related to the Arthropoda or to the Annelida. Class status in the phylum Arthropoda has been assigned to them by Marcus (1960), and Borradaile et al. (1958) considered the tardigrades as arachnoids. They were placed in the Onychophora by de Barros (1942a). Mayr et al., (1953) Pearse (1949), Curtin (1957) and Higgins (1959) have considered the Tardigrada as a separate and distinct phylum.

On the basis of the work by Higgins (op.cit.) and Curtin (op.cit.) I consider the Tardigrada to be a phylum, and I also concur with the theory of Higgins that the tardigrades probably arose as an isolated group from the annelid stock which also gave rise to the primitive arthropod groups.

#### Methods

Collections were made from various areas in the lower peninsula of Michigan with special attention being given to localities in Kalamazoo County. The investigations were made during the months of June, July and August in 1959, and during June and July in 1960. Materials were obtained from a variety of habitats (Table 1). From habitats characterized by the presence of mosses and liverworts, samples were obtained by squeezing out and saving the water in the mosses or liverworts. Lichens were soaked in tap water for a short time, then teased apart, and the detritus, consisting of sediment and small



Table 1. Occurrence of tardigrade species by habitat (Total numbers collected).

Habitat or vegetation	Number of samples	Species			
		<u>Macrobiotus hufelandi</u>	<u>M. intermedius</u>	<u>Hypsibius dujardini</u>	<u>Milnesium tardigradum</u>
Bog mat	155	31	22	0	0
Rain gutters	10	0	0	0	0
Mosses of forest floor	200	23	0	0	0
Mosses on tree trunks	150	0	0	0	0
Lichens	20	0	0	0	0
Beach sands	20	0	0	0	0
Detritus, lake area	80	0	0	2	1
Totals	653	54	22	2	1

particles of lichens, was examined in a watch glass with the aid of a binocular dissecting microscope. In addition to the aquatic forms, terrestrial species also occur in the bottom sediment and algae of standing water. It is possible that these normally terrestrial forms have been washed into streams or ponds following a rain. This possibility also has been mentioned by Marcus (1960) and thus both forms were sought for in aquatic habitats. The psammolittoral area was examined by washing the sand into a small container. A total of 635 samples was collected and examined.

Localities from which collections were made in Kalamazoo County were as follows: in the Schoolcraft Quadrangle, Austin Lake (Sec. 26, T.3S., R.11W.), Long Lake (Sec. 25, T.3S., R.11W.), West Lake (Sec. 26, T.3S., R.11W.), Limeklin Lakes (Sec. 11, T.2S., R.11W.), Little Sugar Loaf Lake (including the associated mat, bog and wooded areas) (Sec. 6, T.4S., R.11W.), West Fork of Portage Creek (Sec. 4, T.3S., R.12W.), Hampton Lake and adjacent State Game Area (Sec. 19, T.3S., R.12W.), Portage Bog and the wooded area around the bog (Sec. 28, T.3S., R.11W.), in the Kalamazoo Quadrangle, Asylum Lake (Sec. 30, T.2S., R.11W.), Bonnie Castle Lake (Sec. 16, T.2S., R.12W.), Woods Lake (Sec. 29, T.2S., R.11W.), Twin Lakes (Sec. 36, T.1S., R.12W.), Kleinstuck Preserve (Sec. 28, T.2S., R.11W.), Coopers

Glen (Sec. 34, T.1S., R.11W.), in the Galesburg Quadrangle, Gull Lake (Sec. 12, T.1S., R.9W.), Potter Lake and Blue Lake (Sec. 34, T.2S., R.9W.), in the Leonidas Quadrangle, McGinnis Lake (Sec. 3, T.2S., R.9W.). Other sites examined were Crooke Lake, Barry County (Galesburg Quadrangle, Sec. 7, T.1N., R.10W.), and in the Gobles Quadrangle, Wolf Lake and surrounding wooded area (Sec. 14, T.1N., R.10W.), and Campbell Creek, Van Buren County (Sec. 11, T.1N., R.10W.).

#### Accounts of Species

Representatives of three genera, Hypsibius, Milnesium, Macrobiotus, were collected in the course of this study (Table 1). All belong to the Class Eutardigrada (considered the Order Eutardigrada by Richters, 1927). The lack of any members of the Class Heterotardigrada (considered the Order Heterotardigrada by Marcus, 1960) is consistent with the predominantly arctic distribution of this group (Richter, 1927).

Tardigrades were most frequently encountered in the mosses of bog mats and of the forest floor (Table 1). Detritus of lakes also yielded specimens of two species. No tardigrades were found in mosses of tree trunks, lichens, or material from rain gutters.

Macrobiotus hufelandi S. Schultze

This ubiquitous organism has been reported from the following localities in the United States, Washington: San Juan Island (Mathews, 1938:635), Wisconsin: Ephraim (loc. cit.), Colorado: Boulder Canyon (Higgins, 1959:158), Washington, D.C. (Mathews, loc. cit.), Michigan: Isle Royale (loc. cit.). It also has been reported from Vancouver Island, British Columbia, Canada (Richters, 1908:205). The specimens collected at the Portage Bog wooded area were in the following mosses: Grimmia calyptrata, Hypnum imponens and Dicranum sp. Specimens taken at Wolf Lake were scooped up with surface mud and debris. The water in which these tardigrades were taken was at a temperature of 22°C and a pH of 7.1 on the morning of July 5, 1960. Liverworts at Little Sugar Loaf Bog (mat area) which were examined on the morning of June 20, 1960, also provided specimens of this species. The water squeezed from these liverworts was at 18°C and had a pH of 7.0.

A total of 23 specimens was collected from bryophytes, 10 were found in the material at Wolf Lake and 31 were found in the liverworts at Little Sugar Loaf Lake. White pigmented areas, as noted by Higgins (1959), were present on all specimens collected. The processes on the periphery of the eggs varied from 22 to 29 in numbers but such differences in

in egg types have been noted by Higgins (1959) and Peterson (1951). The total length of the 23 specimens varied from 235 to 550 $\mu$ .

Macrobiotus intermedius Plate

Twenty two specimens of M. intermedius were collected at Little Sugar Loaf Bog (mat area). These specimens measured from 211 to 310 $\mu$  in total length. The ovoid eggs measured 40.1 to 47.5 $\mu$  at greatest diameter. Both eggs and adults were found in the water which was squeezed from the liverwort, Cephalozia connivens. The temperature of this water was 17.5°C and had a pH of 7.0. This collection was made on the morning of June 24, 1960. The eggs were ornamented and, in as much as this ornamentation varies among different species, it is a reliable means of identification. The first reported occurrence of this species for the United States was from Maryland in 1957 (Curtin, 1957:144). Murray (1910:171) reported its occurrence in Canada. This is the first time it has been reported from Michigan.

Hypsibius dufardini Doyere

The first published record of occurrence of this species in the United States was that by Curtin in 1957. H. dufardin

is an aquatic animal with a highly translucent body. Only two specimens were found in this study and they were from the detritus collected at McGinnis Lake. The temperature of the water on the morning of June 28, 1960, when the collections were made, was 22°C; the pH was 7.0. The specimens measured 235 and 228  $\mu$  in total length. This is the first report of the occurrence of this tardigrade in Michigan.

Milnesium tardigradum Doyere

M. tardigradum has been reported from Canada, (Murray, 1910:166) and in the United States, from Colorado: Boulder Canyon (Higgins, 1959:151), Maryland: Emmitsburg quadrangle (Curtin, 1957:142), Wisconsin: Friday Harbor (Mathews, 1938:626), Washington: San Juan Island (Mathews, 1938:626), and Illinois: Techney (Mathews, 1938:262). This tardigrade is easily distinguished by the presence of sensory papillae around the mouth, with two additional papillae placed slightly posteriorad to these. The pear-shaped pharynx contains no placoids. No stylet is present and the pharyngeal bulb is elongated. The genus Milnesium is monotypic. The single species is unique among tardigrades in that it is carnivorous (Pennak, 1953:243). Only one specimen was collected and it was deep red-brown in color. The total length was 385  $\mu$ .

This specimen was collected at McGinnis Lake along with H. dujardini on the morning of June 28, 1960. The temperature of the water at that time was 23°C. The pH was 7.0. Higgins (1959) reported finding M. tardigradum in the moss, Grimmia calyptrata, but in this study none was found in samples of this moss. This is the first reported occurrence of this tardigrade in Michigan.

#### Key to species found in Michigan

1. Head with anterior cirri (rarely absent) and lateral filaments; with four separate but similar claws on each leg; semiaquatic.  
 Class Heterotardigrada (has not been reported in Michigan)  
 Head without anterior cirri and lateral filaments; each leg with two double claws or two unlike pairs of claws; semiaquatic and aquatic.  
 Class Eutardigrada, Order Macrobiotidea.....2
2. Sucking pharynx with macroplacoids; eyes present or absent; each leg with two double claws.  
 Macrobiotidae.....3  
 Sucking pharynx elongated and without macroplacoids; mouth surrounded by prominent papillae; eyes present; each leg with two long, slender claws and two short, heavy claws; up to 1200 long; one cosmopolitan species.  
 Arctiscidae...Milnesium tardigradum Doyere
3. The two double claws of each leg similar in shape; the two larger claws of each leg more or less turned toward each other, the two smaller claws more or less turned away from each other; cuticle smooth; eggs mostly free and sculptured; up to 1,100 long.  
 Microbiotus.....4

The two double claws of each leg usually dissimilar; the two larger claws of each leg more or less parallel; the two smaller claws more or less parallel; cuticle smooth or sculptured; eggs smooth or sculptured and deposited in the shed exoskeleton; up to 700  $\mu$  long.

Hypsibius.....5

4. Three macroplacoids.....6

Two macroplacoids.....7

5. The secondary branch of the outer claw continues the common base of the claw evenly arched; gullet not longer than half the length of the pharynx; pharynx distinctly longer than broad; macroplacoids thin rods; the branches of both claws united at the bases only, and much longer than the common bases; aquatic.

Hypsibius dujardini (Doyere) 1840

6. Placoids as long as broad; mouth tube narrow.

Macrobiotus intermedius Plate 1888

7. Placoids longer than broad; mouth tube wide, processes of egg shell end with discs.

Macrobiotus hufelandi S. Schultze 1883

### Summary

1. During the months of June, July and August of 1959, and June and July of 1960, a search for tardigrades was undertaken in southwestern Michigan.

2. The four species found in this area were Macrobiotus hufelandi S. Schultze, M. intermedius Plate, Hypsibius dujardini (Doyere) and Milnesium tardigradum Doyere. The last three were heretofore unknown from the state of Michigan.



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