TARDIGRADES OF NORTH AMERICA:
A PRELIMINARY SURVEY OF NEW JERSEY, U.S.A.

Michael W. Shaw, PO Box 742, Midlothian, VA 23113

William R. Miller, Department of Biology, Baker University, Baldwin City, KS 66006

ABSTRACT

Terrestrial tardigrade habitat samples were collected from fifty locations throughout the twenty-one counties of New Jersey, USA. Four species from four genera, within three families are reported. *Milnesium tardigradum, Macrobiotus hufelandi, Minibiotus intermedius, and Ramazzottius oberhaeuseri* are all considered cosmopolitan and found on most other continents. Tardigrades were found in all counties, in all types of habitats throughout the state.

Key Words: tardigrade, biodiversity, distribution

INTRODUCTION

Tardigrades (phylum *Tardigrada*) or water bears are microscopic aquatic animals found in the interstitial water of terrestrial habitats such as moss, lichen, leaf litter, and algae on tree bark. These habitats cycle between dry and moist, retaining moisture long enough to allow tardigrades to be active, grow and reproduce (Miller, 1997). When the habitat desiccates, the animal loses 97% of its body water, and shrivels into a structure called a "tun" until moisture returns. In this state, called "cryptobiosis," the water bear can survive extreme temperatures (-200C, +160C), high pressures (6,000 ATM), vacuum and excessive radiation (Kinchin, 1994). With their extreme survival capability, cryptobiotic tardigrades have recently become the first multi-celled animal to survive exposure to outer space (Jonsson et. al., 2007).

Tardigrades have five body segments, four pairs of legs, with claws on each leg. They have a dorsal brain and a ventral nervous system. They have a complex pharyngeal structure, complete digestive system, but lack a circulatory or respiratory system (Miller, 1997). Tardigrades have a single gonad and separate sexes but some genera are hermaphroditic. Water bears are ecdyzoia and shed their chitinous cuticle to grow; some lay their eggs in the cuticle as they shed, while others deposit their eggs free in the environment. They range in size from 0.3mm to about 1.2 mm (Kinchin, 1994). Despite more than 900 described taxa (Guidetti & Bertolani, 2005; Degma and Guidetti, 2007) there have been few systematic surveys of large (country sized) areas to document distributional patterns such as England (Morgan & King 1976) or Poland (Dastych, 1978). In the United States, few state sized surveys such as Illinois (Pugilla, 1964), California (Schuster & Gragrick, 1965), or western Montana (Miller, 2007) exist. Many states such as New Jersey have not been surveyed, thus the distributional patterns, environmental affinities, and habitat data is non-existent (McInnes, 1994; Miller 1997).

This project is a model for naturalist or citizen science exploration and demonstrates how true collaboration can lead to discovery and the expansion of knowledge. This is the first report of the existence of four species of the animals of the phylum *Tardigrada* in New Jersey. It expands

the known range and distribution of each species, makes observations on habitat requirements, and adds to the known biodiversity of the region and state of New Jersey.

MATERIALS AND METHODS

Tardigrades were surveyed by sampling in all twenty-one New Jersey counties, between 2001 and 2009. Rural and urban sites ranging from parking lots, roadside trees, urban office complexes, nature preserves, interstate highway rest stops, and residential neighborhoods were selected (Table 1). A Magellan Global Positioning System (GPS) was used to fix sample locations. Most collection sites were photographed (68%).

COLLECT DATE	SPECIMEN No.	LATITUDE	LONGITUDE	ELEVATION (meters)	CITY/ TOWN NAME	COUNTY	SAMPLE TYPE	SUBSTRATE
4/18/2001	8	40 DEG 50.455 N	074 DEG 28.541 W	195.12	Morris Plains	Morris	bark	Dawn Redwood *Metasequoia glyptostroboides*
7/2/2002	9	40 DEG 54.244 N	074 DEG 49.382 W	401.10	Hackettstown	Warren	moss	rock
4/14/2003	1	40 DEG 38.856 N	074 DEG 16.142 W	28.82	Roselle	Union	moss	dirt
4/19/2003	2	40 DEG 38.856 N	074 DEG 16.142 W	28.82	Roselle	Union	moss	dirt
4/24/2003	3	40 DEG 20.356 N	074 DEG 28.958 W	60.94	Cranbury	Middlesex	moss	dirt
5/1/2003	4	40 DEG 50.455 N	074 DEG 28.541 W	195.12	Morris Plains	Morris	bark	Dawn Redwood *Metasequoia glyptostroboides*
5/9/2003	5	40 DEG 56.956 N	074 DEG 523 W	43.94	Fair Lawn	Bergen	moss	dirt

5/28/2003	6	40 DEG 1	74 DEG 20	35.43	Lakehurst	Ocean	bark	Silver Maple *Acer Saccharinum*
5/30/2003	7	40 DEG 37	74 DEG 34	195.12	Somerset	Somerset	bark, lichen	Norway Maple *Acer platanoides*
7/9/2003	10	41 DEG 09.939 N	74 DEG 33.435 W	224.88	Hamburg	Sussex	moss	rock
7/9/2003	11	40 DEG 54.244 N	074 DEG 49.382 W	409.13	Hackettstown	Warren	lichen	rock
7/17/2003	12	41 DEG 00.991 N	073 DEG 56.794 W	30.24	Northvale	Bergen	bark	September Elm *Ulmus serotina*
7/17/2003	13	41 DEG 00.799 N	073 DEG 56.798 W	19.37	Northvale	Bergen	bark	Black Mulberry *Morus nigra*
7/18/2003	14	40 DEG 51.077 N	074 DEG 20.110 W	86.93	Pinebrook	Essex	bark	Paper Birch *Betula papyrifera*
7/23/2003	16	41 DEG 04.490 N	074 DEG 08.311 W	86.93	Ramsey	Bergen	bark	American Sycamore *Platanus occidentalis*
9/3/2003	15	40 DEG 03.591 N	074 DEG 37.874	83.62	Cookstown	Burlington	bark	American Linden *Tilia americana*
9/26/2003	17	40 DEG 34.916 N	074 DEG 40.676 W	69.92	Bridgewater	Somerset	bark	Osage Orange (Bodark) *Maclura pomifera*
10/27/2003	18	39 DEG 41.910 N	075 DEG 23.746 W	19.37	Auburn	Salem	bark, lichen	Pin Oak *Quercus palustris*
11/4/2003	19	40 DEG 26.951 N	074 DEG 40.261 W	65.20	Harlingen	Somerset	bark	Scarlet Oak *Quercus coccinea*
11/5/2003	20	40 DEG 52.178 N	074 DEG 26.690 W	184.25	Parsippany	Morris	bark, lichen	Hybrid Crab Apple *Malus hybrids*
11/12/2003	21	40 DEG 16.493 N	074 DEG 03.562 W	85.98	Eatontown	Monmouth	bark, lichen	Little leaf Linden *Tilia cordata*

11/12/2003	22	40 DEG 16.493 N	74 DEG 03.562 W	86.46	Eatontown	Monmouth	moss	dirt
11/14/2003	23	41 DEG 02.866 N	074 DEG 44.200 W	294.33	Newton	Sussex	moss	rock
11/18/2003	24	40 DEG 58.556 N	074 DEG 59.398 W	167.24	Sycamore Park	Warren	bark	American Sycamore *Platanus occidentalis*
11/19/2003	25	40 DEG 26.881 N	074 DEG 26.643 W	33.54	Brunswick	Middlesex	moss	dirt
11/21/2003	26	40 DEG 40.432 N	074 DEG 26.454 W	130.87	Berkeley Heights	Union	moss	dirt, pavement
5/25/2004	27	40 DEG 11.328 N	074 DEG 09.838 W	34.49	Farmingdale	Monmouth	bark, lichen, tree moss	Sweet Gum Liquid *Ambar styraciflua*
5/25/2004	28	40 DEG 04.644 N	074 DEG 09.931 W	20.31	Lakewood	Ocean	bark, lichen	American Linden (Basswood) *Tilia americana*
5/25/2004	29	39 DEG 57.435 N	074 DEG 10.296 W	18.90	Toms River	Ocean	bark, lichen	Norway Maple *Acer platanoides*
6/3/2004	30	40 DEG 17.447 N	074 DEG 04.687 W	29.76	Ft. Monmouth	Monmouth	bark, lichen	Northern Red Oak *Quercus rubra*
6/3/2004	31	40 DEG 53.307 N	074 DEG 43.392 W	435.59	Budd Lake	Morris	moss	dirt
6/4/2004	32	41 DEG 00.150 N	074 DEG 14.638 W	197.48	Oakland	Bergen	bark, lichen	tree
6/4/2004	33	40 DEG46.728 N	074 DEG 05.039 W	5.20	Secaucus	Hudson	Bark	Black Locust *Robinia pseudoacacia*
6/13/2004	34	40 DEG 42.650 N	074 DEG 45.436 W	263.62	Tewksbury	Hunterdon	bark, lichen	Green Ash *Fraxinus pennsylvanica*
8/5/2004	35	40 DEG 46.072 N	074 DEG 15.122W	102.05	West Orange	Essex	bark, lichen	Scarlet Oak *Quercus coccinea*

4/20/2005	36	40 DEG 31.62 N	075 DEG 03.560 W	61.89	Frenchtown	Hunterdon	moss	American Sycamore *Platanus occidentalis*
4/20/2005	37	40 DEG 30.125 N	074 DEG 51.295 W	87.40	Flemington	Hunterdon	lichen	Common Pear Tree *Pyrus Communis*
7/7/2005	38	40 DEG 38.138 N	074 DEG 54.732 W	103.94	Clinton	Hunterdon	moss	Black Locust *Robinia pseudoacacia*
7/28/2005	39	40 DEG 09.513 N	074 DEG 25.704 W	77.01	Jackson	Ocean	lichen	Weeping Willow *Salix babylonica*
7/28/2005	40	40 DEG 10.537 N	074 DEG 35.218 W	39.68	Allentown	Monmouth	moss	American Hornbeam
9/1/2005	41	40 DEG 13.703 N	074 DEG 37.183 W	31.65	Robbinsville	Mercer	bark	Sweet Gum Liquid *Ambar styraciflua*
1/11/2006	42	40 DEG 01.118 N	074 DEG 43.930 W	39.68	Unionville	Burlington	lichen	Maple
3/4/2006	43	39 DEG 29.999 N	074 DEG 31.813 W	35.43	Pomona	Atlantic	bark	White Oak *Quercus alba*
3/4/2006	45	39 DEG 10.893 N	074 DEG 43.400 W	1.89	Sea Isle City	Cape May	bark	American Holly *Ilex opaca*
3/5/2006	44	39 DEG 29.999 N	74 DEG 31.813 W	35.43	Pomona	Atlantic	moss	Sand
8/8/2007	46	39 DEG 39.604N	074 DEG 52.708W	65.67	Blue Anchor	Camden	moss	White Oak *Quercus alba*
8/8/2007	47	39 DEG 41.349 N	075 DEG 00.845W	42.99	Downer	Gloucester	moss	White Oak *Quercus alba*
8/20/2007	48	40 DEG 55.154N	074 DEG 13.755W	70.39	Totowa	Passaic	lichen	Red Maple *Acer rubrum*
8/20/2007	49	41 DEG 00.067N	074 DEG 16.516W	76.06	Wayne	Passaic	moss, lichen	White Oak Quercus alba
9/6/2007	50	40 DEG 41.655 N	074 DEG 03.514W	0.94	Jersey City	Hudson	lichen	London Plane *Platanus x acerifolia*

9/25/2007	51	39 DEG 25.826 N	075 DEG 14.642 W	47.72	Bridgeton	Cumberland	lichen	White Oak *Quercus alba*
9/25/2007	52	39 DEG 25.736 N	075 DEG 14.824 W	40.63	Bridgeton	Cumberland	lichen	stone
9/25/2007	53	39 DEG 26.117 N	075 DEG 15.476 W	38.27	Hopewell	Cumberland	bark	Oak
9/25/2007	54	39 DEG 34.209 N	075 DEG 27.864 W	10.39	Salem	Salem	bark, lichen	American Sycamore *Platanus occidentalis*
3/7/2008	55	40 DEG 38.03 N	074 DEG 16.17 W	29.29	Linden	Union	bark, lichen	tree
6/25/2009	56	39 DEG 30.821 N	74 DEG 55.541 W	47.72	Buena	Atlantic	lichen	Brick
6/26/2009	57	40 DEG 32.287 N	74 DEG 17.673 W	72.28	Woodbridge	Middlesex	lichen	stone
6/26/2009	58	40 DEG 33.888 N	074 DEG 19.159 W	55.28	Woodbridge	Middlesex	lichen	stone

Table 1. Tardigrades of New Jersey Collection Sites Sorted by Date.

Samples were collected in paper "lunch bags" or small manila "coin envelopes." Moss samples were taken from dirt, rock, trees, stone (including asphalt), and sand substrata. Lichen, bark and algae was scraped from a tree, rock, brick, or stone substrate with a paring knife or a single edged razor blade. Scrapings went directly into a sample bag or envelope and were allowed to dry (Shaw, 2012). In some cases, 50mm deep plugs were collected by using a 62mm diameter soil corer, and the dirt substrate was retained (Figure No. 1).

Trees were photographed for height, crown shape, bark type, leaf characteristics, and branch pattern (Figure Nos. 4 through 7). When possible, leaf and seed pods were collected in season to aid in identification. No collections were made during December or February due to lack of foliage, however January was used as a collection month to represent winter for the survey (Table No. 2). Leaves were dried in a leaf press for one week using acid free paper, then color photocopied for detail preservation. Pressings were later photographed with centimeter ruler to aid botanical reference (Figure Nos. 5 and 6). Trees were identified to species with the keys of Brockman (1986), Coombes (1992), and Little (1980).

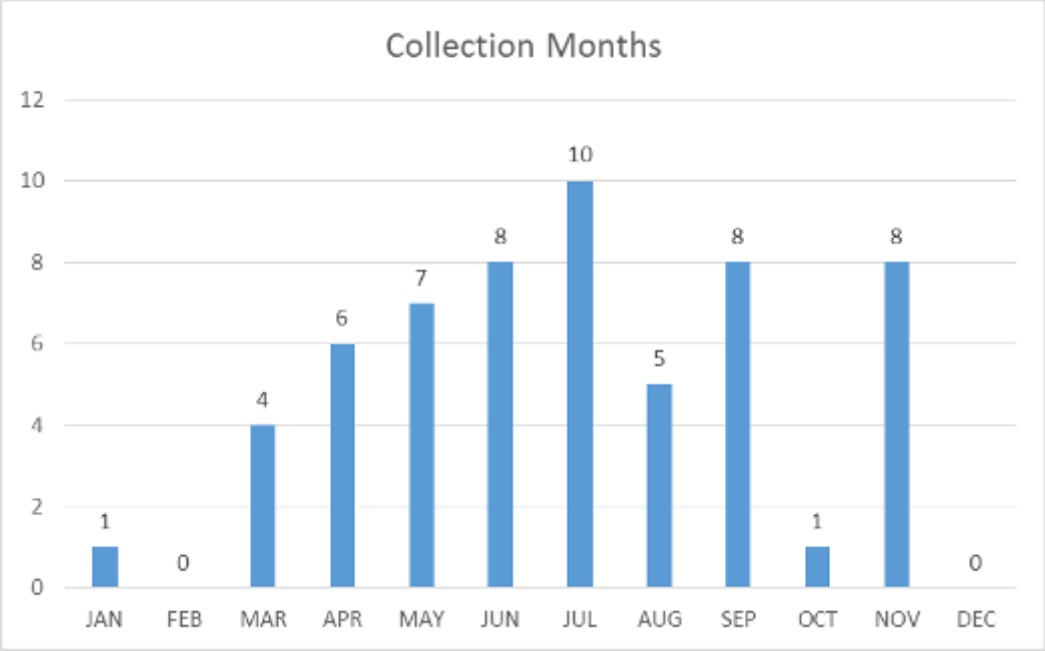

Table 2. Collection Months.

Samples of several grams each were placed in either plastic petri dishes or 2 oz. plastic cups and hydrated with spring water to a depth of 0.5 to 1.5cm. Each container was labeled with date, site name and specimen number. Samples were maintained at room temperature and examined for tardigrades after 6 to 48 hours, using a B&L Stereo-Zoom dissecting microscope, in a range between 20x and 30x magnification. Cross contamination was avoided when working with multiple samples by using a disposable pipette for each sample.

Individual tardigrades were transferred into a drop of media on a 25 x 75mm glass slide with an Irwin loop or eye-dropper (Shaw, 2012). Glass coverslips were applied, and live tardigrades were studied while on slides, observations recorded in notebooks. Permanent slides were prepared by replacing water with alcohol and/or solvents in stages, and finally with Hoyer's or PVA (Polyvinyl Alcohol) as final mounting media. Glass cover slips were applied and sealed with Cytoseal™ 60.

Tardigrades were examined and photographed with a Lomo Multiscope using bright-field, dark-field, incident lighting, phase contrast, polarized light, and Rheinberg illumination at various magnifications. Live tardigrades in vitro and fixed slide mounted tardigrades were photographed. Incident lighting shows the tardigrade as it appears most naturally in daylight, if one were able to observe it in nature with the naked eye (Figure No. 3). Cross polarization brightly illuminates only the stylets and certain minor internal structures (Figure No. 1). Phase contrast allows better determination of claw type when differentiating between species (Figure No. 8) Final species determinations presented here were made using a Differential Interference Contrast (DIC) microscope at various magnifications. Species identification was determined using the keys of Ramazzotti and Maucci (1983), and Nelson (2000).

Figure No. 1 Specimen No. 25. Background is site location with moss in foreground. Inserts (clockwise) show in vitro tardigrade close ups (brightfield lighting) of mouthparts, pharynx, macroplacoids and claws (indicating possibly *M. harmsworthi*); same in polarized light; central portion with claws in polarized light; moss close up showing where round core samples were removed.

RESULTS

New Jersey, a state in the United States of America, lies on the Atlantic Ocean to its east, and is bordered on the west by the Delaware River. The state at its center is positioned on the North American continent at approximately 40 degrees North Latitude. It has a temperate climate, with monthly average temperatures ranging from about 29.4 C (85 F) in summer to about 4.4 C (24 F) in the winter of most years. Spring and autumn are very mild. The mean rainfall for the last decade was recorded at about 124cm (49 in.), up about 5cm (2 in.) from the previous decade. New Jersey encompasses 22,610 square kilometers (8,729 sq mi.), of which 14% is water. Mean elevation of the state is 76.2 meters (250 ft.) above sea level.

New Jersey geography is varied, comprising four major types of landscape. The Atlantic Coastal Plains covers 3/5ths of the state, characterized by marshlands and meadows. The industrialized Piedmont region includes New Jersey's major rivers: The Hudson River, Passaic River, Ramapo River, and Raritan River. The Highlands have flat-topped rocky ridges with many lakes interspersed throughout the region. The Appalachian Ridge and Valley Region consists of mountains in the northwest, with valleys of shale and limestone.

Because of this wide diversity in geography and climate, New Jersey seems to provide an excellent place to survey for the presence of tardigrades when seeking to understand more about habitat and conditions that might contribute to ubiquity in this or any species.

Fifty-eight habitat site samples were collected from fifty locations in the twenty-one counties of New Jersey. Locations included forests, wetlands, urban areas, residential neighborhoods, river side and ocean side locales, business and commercial districts, at a variety of elevations (Table Nos. 1 and 3).

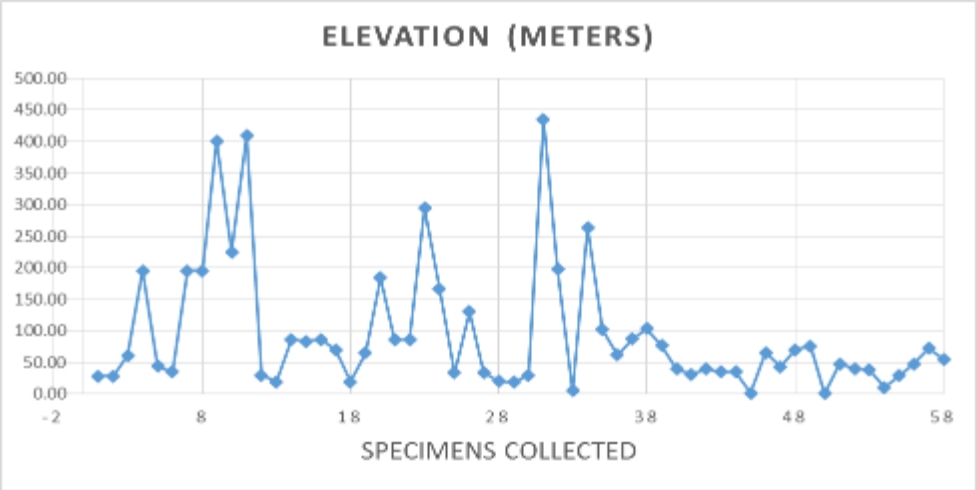

Table No. 3 Elevation Range.

GPS data from site locations (Table No. 1) was entered into Garmin MapSource (c) software to generate a population grid. The grid points were transferred into Google Earth (Google, 2013). A county map image from Mapwatch.com (c) was then layered into Google Earth and aligned with the terrain and boundaries. This new composite map was then made opaque, and is presented in Figure No. 2.

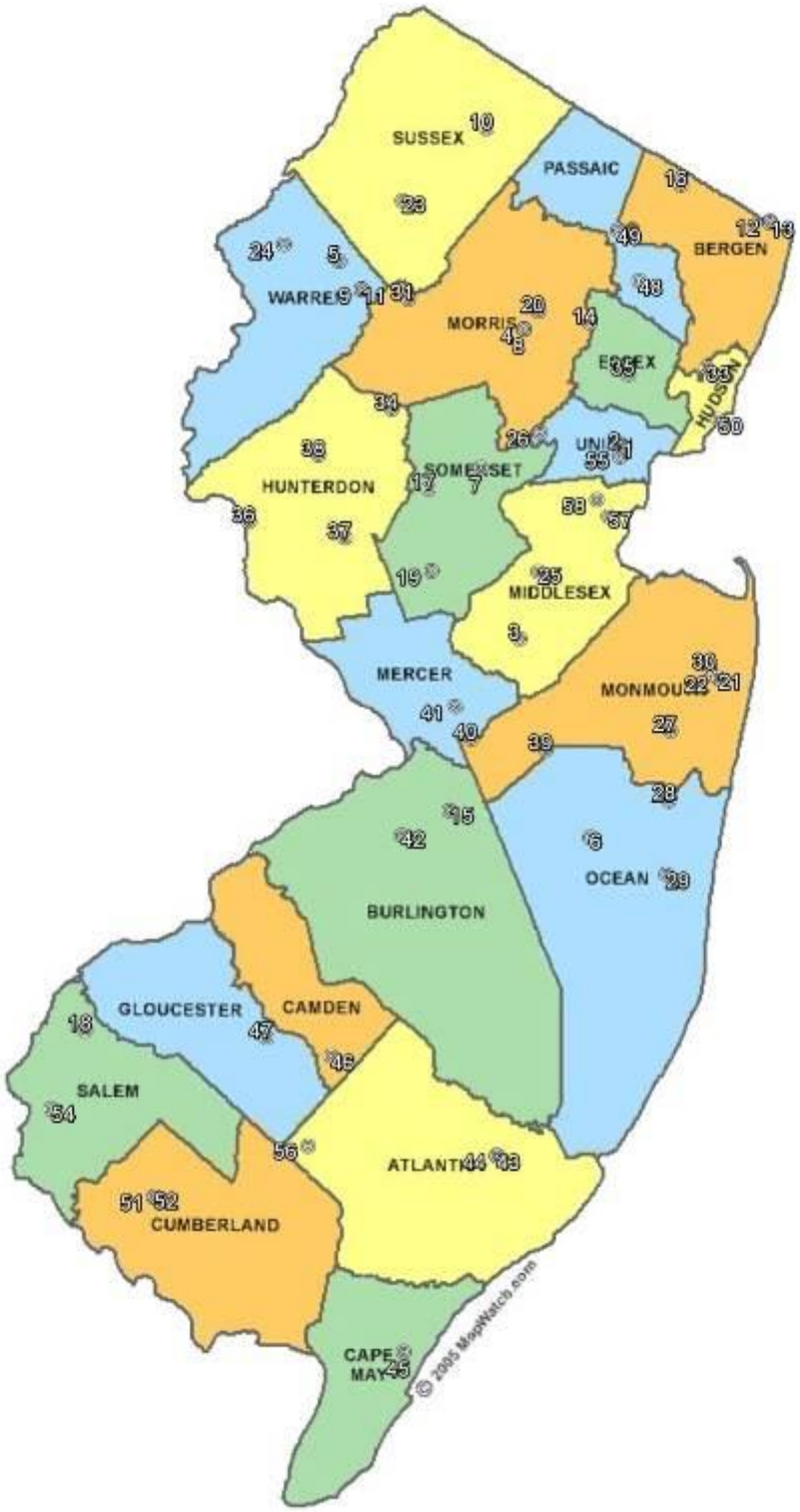

Figure 2. Map of New Jersey showing locations of study sites within their respective counties.

Collections took place over a period from 2001 through 2009 (Table Nos. 1 and 4). In four cases, samples were taken on the same day from different substrates at the same site; two locations were sampled on different days in the same month; two locations were sampled in different years, as shown in Table 1.

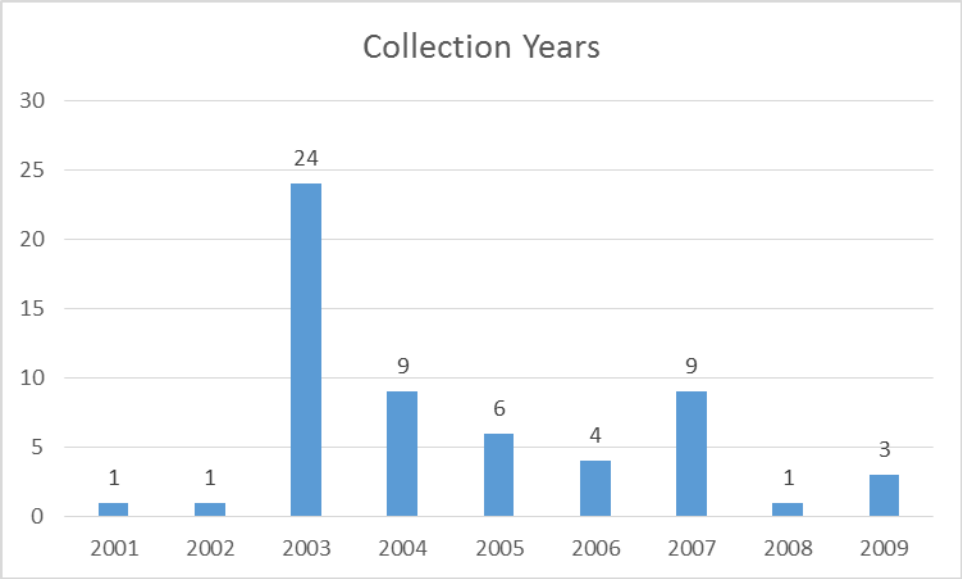

Table 4. Collection Years.

Average rehydration time for a dried sample was 4.9 days. Rehydration time to detect tardigrades ranged from 0.4 to 32 days (Table. 5), averaging 2.2 days. Specimen suspensions, 88 total, were discarded on an average of 10.3 days after no detection. Tardigrades were found in 50 of 58 (86%) site samples but in 7 cases tardigrades were lost before identification could be made, thus the number of positive identifications is only 43.

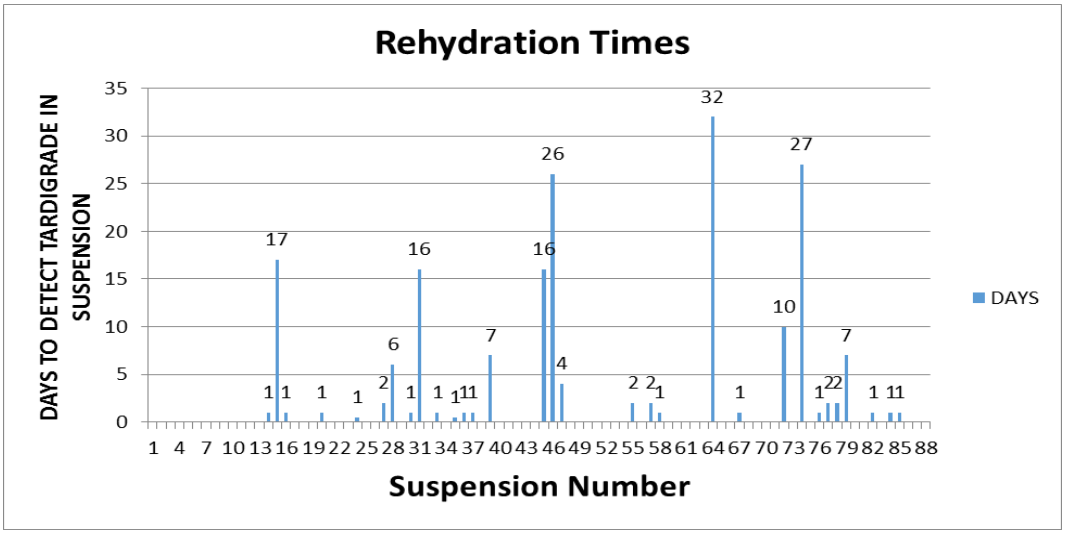

Table No. 5 Time from start of suspension to detection of tardigrade.

A total of 39 permanent slides were made, representing all New Jersey counties. Although observations over the course of the study indicate five or more species*, we report thirty-three tardigrades and six eggs which represent four species: *Milnesium tardigradum, Macrobiotus hufelandi, Minibiotus intermedius, and Ramazzottius oberhaeuseri*.

Identification is based upon cuticle, claw type, lunules, buccal apparatus, and egg observations when eggs were present. Distribution data is presented in Table 6.

New Jersey Counties	Specimen No.	*Macrobitus hufelandi*	*Minibiotus intermedius*	*Ramazzottius oberheauseri*	*Milnesium tardigradum*
Atlantic	43, 44, 56				X
Bergen	5, 2, 13, 16, 32	X			X
Burlington	15, 42	X		X	X
Camden	46	X			
Cape May	45	X			
Cumberland	51, 52, 53				X
Essex	14, 35		X		X
Gloucester	47*	X			
Hudson	33, 50	X		X	
Hunterdon	34, 36, 37	X			X
Mercer	41*	X			
Middlesex	3, 25*, 57, 58	X	X	X	
Morris	4, 20, 31	X			
Monmouth	21,22 27,30,40	X	X		X
Ocean	6, 28, 29, 39				X
Passaic	48, 49*	X		X	
Salem	54		X		X
Somerset	7, 17, 19	X		X	
Sussex	10, 23	X			
Union	1, 2, 26, 55	X			X

Warren	11, 24	X

Table 6 . Tardigrades of New Jersey, showing distribution by county.

*In the case of specimen No. 41 (examined on a permanent slide), this may be *Macrobiotus harmsworthi* due to macroplacoid appearance and claw patterns. Eggs would be required for a more positive identification. Supporting that possibility is specimen No. 25, which in vitro appears to be *M. Harmsworthi* as well (Figure No. 1). Specimen No. 37 in vitro provided both an egg of *M.harmsworthi* as well as a tardigrade that could be *M. harmsworthi* (Fig. No. 3). Specimen No. 49 provided an egg appearing to be *Macrobiotus areolatus* along with a tardigrade that appears to be *M. areolatus* (Figure No. 4).

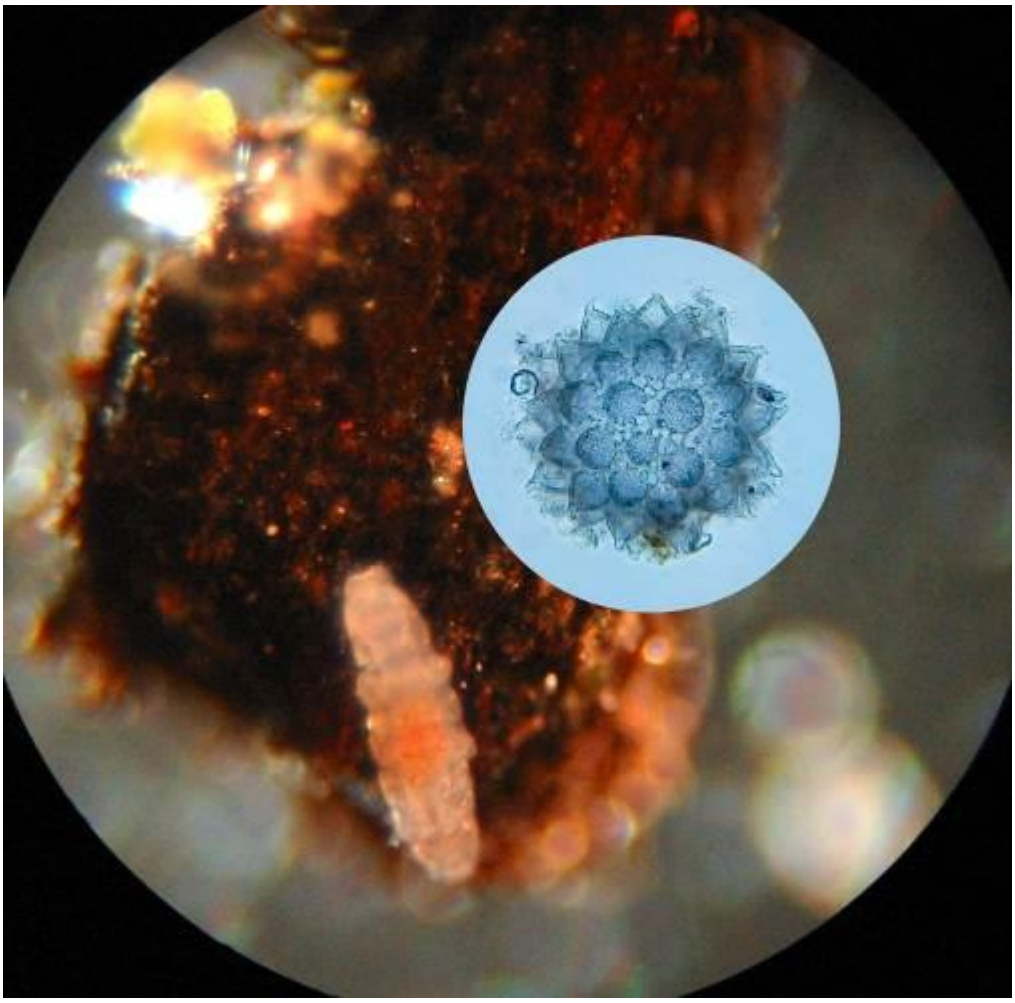

Figure 3. Specimen No. 37. Background shows in vitro tardigrade under incident lighting. Insert shows Brightfield photo of in vitro egg of *M. Harmsworthi* found with this specimen.

Figure No. 4 Specimen No. 49. Background is site location. Inserts (clockwise) show leaf close up; Brightfield photo of in vitro tardigrade close ups of mouthparts, pharynx and macroplacoids; claws; body (all indicating *Macrobiotus areolatus*); tree trunk detail; egg.

DISCUSSION

The only record of a tardigrade from New Jersey is a fossil, *Milnesium swolenski* (Bertolani and Grimaldi, 2000) found in Turonian amber near Sayerville in 1998. The specimen is estimated to be more than 90 million years old and documents that tardigrades in their existing form have been present in New Jersey since Upper Cretaceous (Turonian) (Bertolani and Grimaldi, 2000).

Although tardigrades were found in all counties within New Jersey, diversity was limited. This distinct lack of diversity across many types of rural, urban, coastal and central sites, and upon a variety of substrata, does not allow any major conclusions regarding how tardigrade species are distributed or dispersed. Blaxter, et. al. (2003) suggested that tardigrades in the size range under 1mm may be among the taxa that are ubiquitous and lack biogeography, as supported by Finlay (2002).

Substrata in this study were divided into five categories:

- Brick or Stone, man-made, from which a moss, lichen or algae sample was taken,
- Dirt, from which a moss sample was taken
- Rock, natural, from which a moss, lichen or algae sample was taken,
- Sand, from which a moss sample was taken
- Tree, from which a moss, lichen or algae sample was taken.

Tardigrades were found to be present on all five substrata (Table No. 6).

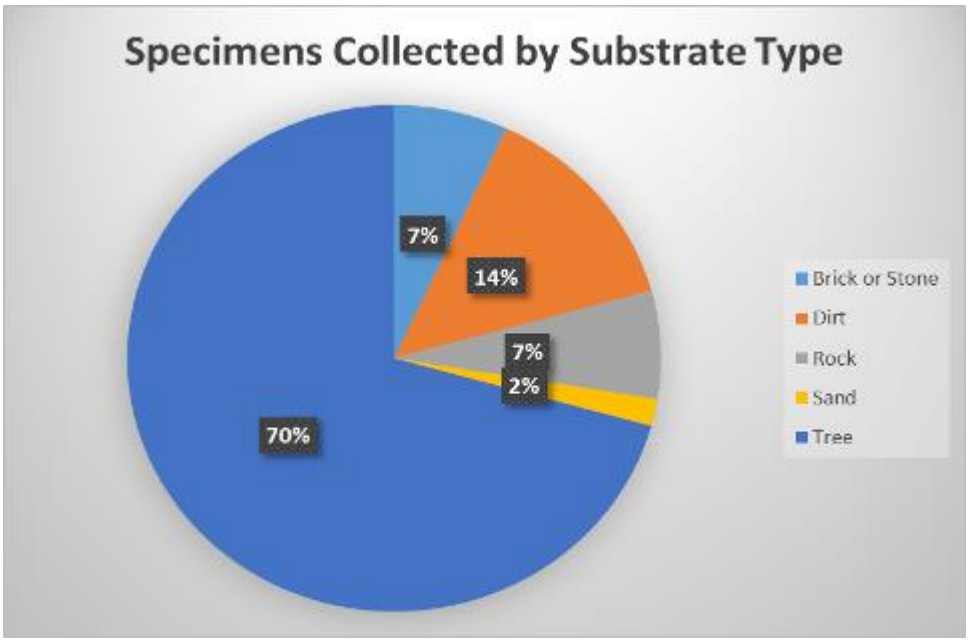

Table No. 6 Percentage of specimens collected by substrate type.

A further observation can be made about tardigrade distribution related to tree bark substrate. Trees with thin or smooth bark such as the Paper Birch (*Betula papyrifera*), and the Black Locust (*Robinia pseudoacacia*) tended not to support tardigrade presence despite multiple samplings. By contrast, the deeply furrowed bark of the Dawn Redwood (*Metasequoia glyptostroboides*), yielded tardigrade rehydration two years after specimen collection.

Figure No. 5 Specimen No. 20. Background is site location. Inserts (clockwise) show leaf pressing; fruit detail; Brightfield photo of vitro tardigrade mouthparts; posterior claws; pharynx and macroplacoids; body (indicating *Macrobiotus hufelandi*); tree trunk detail.

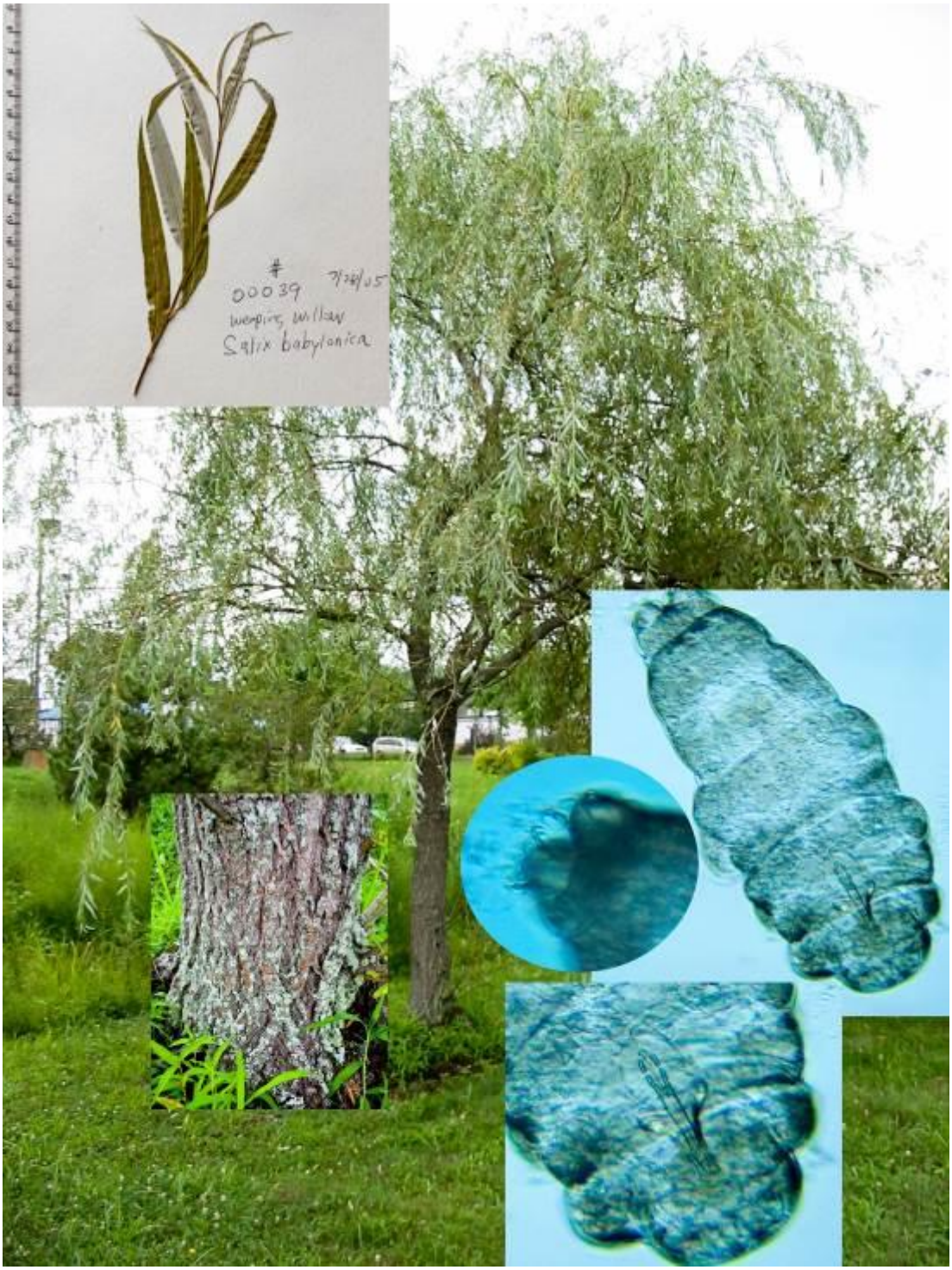

Figure No. 6 Specimen No. 39. Background is site location. Inserts (clockwise) show leaf pressing; Brightfield photo of in vitro tardigrade body; posterior claws; mouthparts, pharynx and macroplacoids (indicating *Minibiotus intermedius*); tree trunk detail.

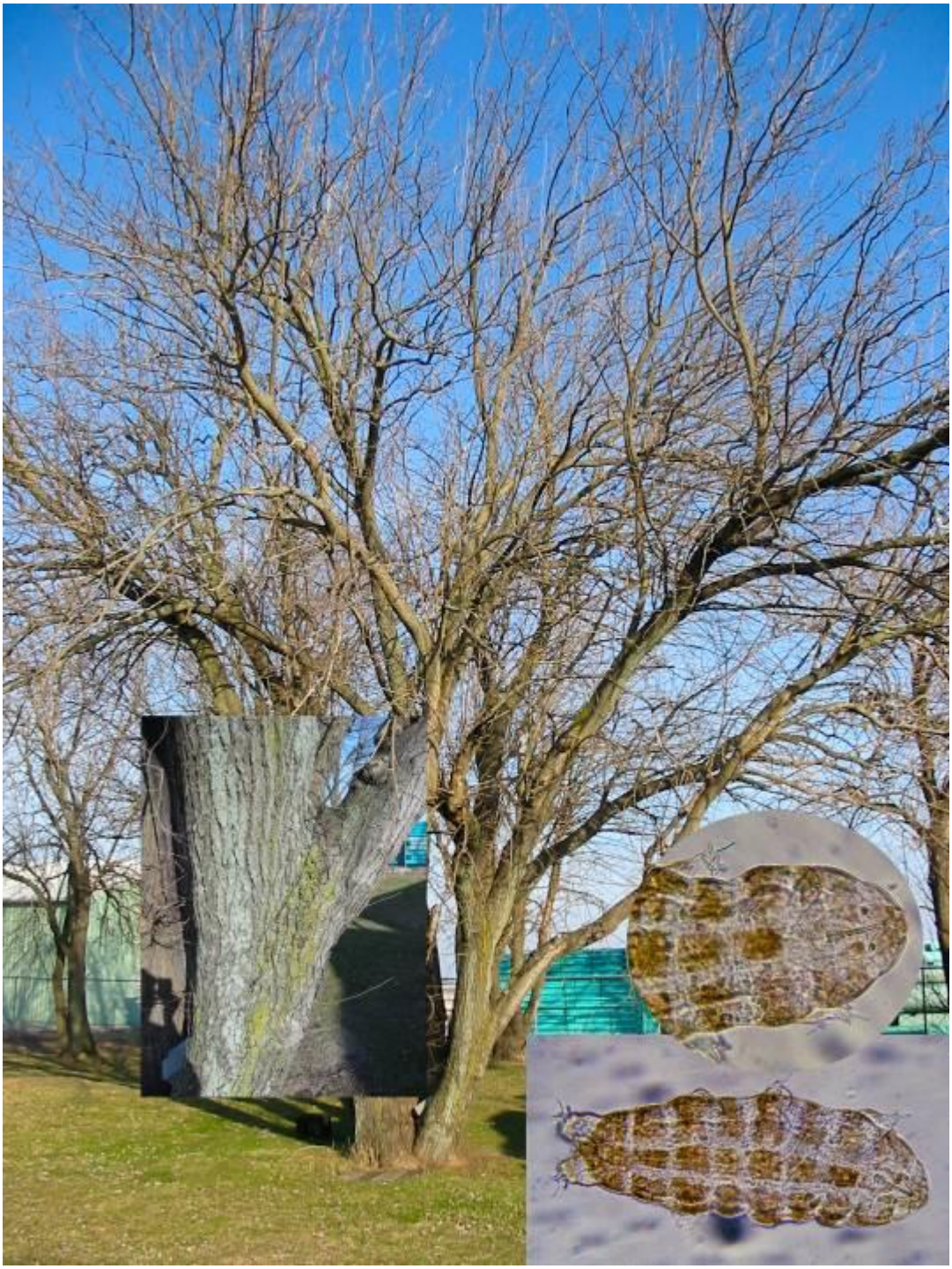

Figure No. 7 Specimen No. 42. Background is site location. Inserts (clockwise) show Brightfield photo of permanent slide mounted tardigrade claws, pharynx and macroplacoids; full body showing distinctive brown color markings (indicating *Ramazzottius oberhaeuseri*); tree trunk detail.

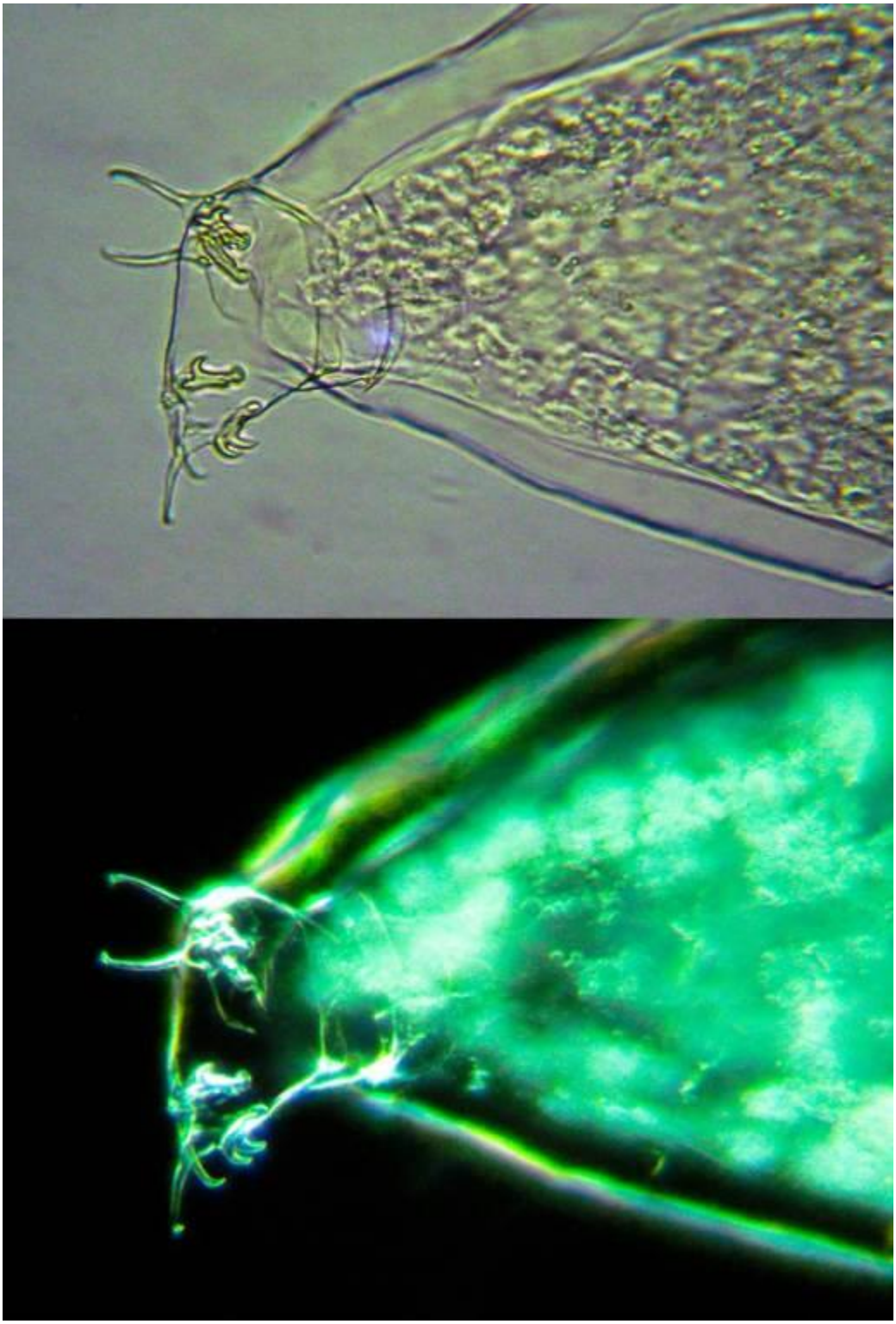

Figure No. 8 Specimen No. 35. Phase Contrast photo (above) of posterior claws (indicating *Milnesium tardigradum*) and Darkfield photo (below) of same.

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