A STUDY OF THE TARDIGRADA FROM A FARM IN MONTGOMERY COUNTY, TENNESSEE

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A STUDY OF THE TARDIGRADA FROM A FARM IN MONTGOMERY COUNTY, TENNESSEE

An Abstract

Presented to

the Graduate Council of

Austin Peay State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Martha Elizabeth Hunter

December, 1977

ABSTRACT

Eight species of tardigrades representing five genera (Echiniscus mauccii, Echiniscus virginicus, Itaquascon bartosi, Macrobiotus hufelandii, Macrobiotus intermedius, Macrobiotus tonollii, Milnesium tardigradum and Pseudechiniscus suillus) were collected from epiphyte samples on Juniperus virginiana (cedar) and Cornus florida (dogwood) trees, the phorophyte species, from two sample areas on a farm in Montgomery County, Tennessee. The two sample areas were both located on north-facing slopes.

The distributions of the tardigrades are discussed with respect to epiphyte species and phorophyte species. There was no apparent relationship between the species of tardigrades and the species of epiphytes on the trees. One species of tardigrade was significantly different with respect to presence or absence on the phorophytes. Echiniscus virginicus was observed to be significantly predominant on dogwood trees. Some possible factors that limit tardigrade distributions are also discussed.

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To the Graduate Council:

I am submitting herewith a Thesis written by Martha Elizabeth Hunter entitled "A Study of the Tardigrada from a Farm in Montgomery County, Tennessee." I recommend that it be accepted in partial fulfillment of the requirement for the degree of Master of Science, with a major in Biology.

Diane S. Findley
Major Professor

We have read this thesis and recommend its acceptance:

David W. Bath
Second Committee Member

R. J. McCay
Third Committee Member

Accepted for the Council:

William N. Ellis
Dean of the Graduate School

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Dr. Diane I. Findley, my major professor, for her advice, guidance and encouragement. Thanks are extended to the other members of my committee, Dr. Daniel W. Bath and Dr. Ralph H. McCoy, for their advice and careful editing of the manuscript.

Appreciation is expressed to numerous individuals at Austin Peay State University for their assistance and encouragement. I especially thank my fellow graduate students, William Atkinson, Larry Carpenter, Michael Davis, Fred Keyes, and Jackie Stack, for their companionship and help during the study.

I am greatly indebted to Dr. Diane R. Nelson, Department of General Science, East Tennessee State University. Her knowledge and enthusiasm intrigued me to pursue this study. The assistance and advice she has given me has been invaluable. I also want to thank her husband, Jack, and her friends for their hospitality during my visit to Johnson City, Tennessee.

I also wish to express my deepest thanks to my parents, Mr. and Mrs. John A. Hunter, for their patience and encouragement during the study.

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Chapter I

INTRODUCTION

Tardigrades are minute invertebrates which are generally referred to as "water bears". These organisms can be found in various marine, freshwater, and terrestrial habitats. Tardigrades were once considered to belong in a class of the Phylum Arthropoda; however, they are now regarded as a separate phylum (Tardigrada) which has similarities to arthropods and to the aschelminthes complex (Riggin, 1962).

Studies of tardigrades have been conducted in various regions of North, Central and South America. Argue (1971, 1972, 1974) collected and described tardigrades from Canada. Dougherty and Harris (1963) and Murray (1907a) conducted investigations on tardigrades in the Antarctic and Arctic respectively. Studies have been done in Central America by Beasley (1972) who sampled in Mexico. Also, Mehlen (1969a) and Riggin (1963) made collections from Costa Rica. Schuster and Grigarick (1966a) investigated tardigrades from cryptogams on soil, rocks, and vegetation collected in the Galapagos and Cocos Islands.

The majority of the research on tardigrades has been conducted in Europe. Murray (1907b) did extensive research on Scottish

tardigrades collected throughout the country. General distributions and descriptions were presented for the tardigrades found in Scotland. Twenty-four moss samples were taken throughout Switzerland from which tardigrades were collected by Bartosi (1949). He found 19 different species representing six genera. Welgarska (1959) studied tardigrades from Poland where she made the initial description of Itaquascon bartosi. Species from the genera Pseudechiniscus, Macrobiotus and Hypsibius were also observed. The monograph by Ramazzotti (1972) and the supplement (1974) discussed systematics, morphology, ecology, methods of preparation, and included extensive bibliographies. This is the definitive work on the tardigrades. It is written in Italian, but is essential to anyone seriously studying tardigrades.

The amount of research conducted on tardigrades in the United States has been rather sparse when compared with that of Europe. A thorough review of the literature of Europe and North America was reported by Riggin (1962). Pennak (1953) provided general descriptions of tardigrade reproduction, body systems, ecology, and characteristics. A key with some general information on morphology, distribution, and identification was presented by Marcus (1959). Higgins (1975) edited a volume considering various aspects of tardigrades such as physiology, speciation, systematics, cytogenetics, and ecology. In the first comprehensive study of tardigrades in North America,

Mathews (1938) stated that there were 32 species known from North America, 12 of which were from the United States.

During the past two decades many researchers have collected and described tardigrades from various areas of the United States. Other species of tardigrades have been reported from several states by the following authors: Curtin (1957) from Maryland; Higgins (1959) from Colorado and (1960) from North Carolina; Riggin (1962) from Southwest Virginia, South Carolina, Florida and Tennessee and (1964) from North and South Carolina; Beasley (1968) from Kansas; and Mehlen (1969b) from Texas. Schuster and Grigarick (1965, 1966b, 1970) have done extensive studies of the tardigrades in Western North America, particularly California.

Three studies on tardigrades have been previously reported from Tennessee. Barnes (1974) did a taxonomic study of the tardigrades from Rutherford County, Tennessee. A study was done on the tardigrades from Roan Mountain in East Tennessee (Nelson, 1975) This investigation examined the distributions of tardigrades with respect to slope exposure, height of the epiphytes above the ground, epiphyte species and exposure of the epiphytes on the tree. Riggin (1962) collected from Carter and Sevier Counties in East Tennessee, although the bulk of the material obtained and identified was derived from Southwestern Virginia. He found 26 species representing eight genera.

Objectives of the Investigation

Since there has been no published research on the tardigrade fauna in Montgomery County, Tennessee, this study was undertaken and the objectives were outlined as follows:

1. to collect and identify tardigrades present in epiphytes on a farm in Montgomery County, Tennessee;
2. to determine the distribution of tardigrades present;
3. to determine certain population parameters for the tardigrades found;
4. to determine which, if any, of the ecological factors considered may have a significant effect on the distribution of the tardigrades.

Description of the Study Area

The two stands of trees were located approximately 21 kilometers south of Clarksville, Tennessee, on the Martha's Chapel Road. The elevation of the study area was 168 meters above sea level. It was located at 87°20' longitude and 36°23' latitude (U. S. Defense Mapping Agency, 1964).

Montgomery County is underlain by limestone of Mississippian age. The southern portion of the county is underlain by St. Louis limestone formations which is underlain by cherty limestone that weathers slowly. A mantle of loess about three tenths to one meter

thick covers most of the rolling or sloping soils. Three types of soil are found to compose the study area. They are as follows:

BaC Baxter cherty silt loam, 12 to 20% slope

BgE3 Baxter soils, 12 to 25% slopes, severely eroded

BrC Brandon silt loam, 5 to 12% slopes.

The topography of the southern part of the county is characterized by deep hollows, steep hillsides, and winding ridgetops (United States Department of Agriculture, 1975). A map of the study area is shown in Figure 1.

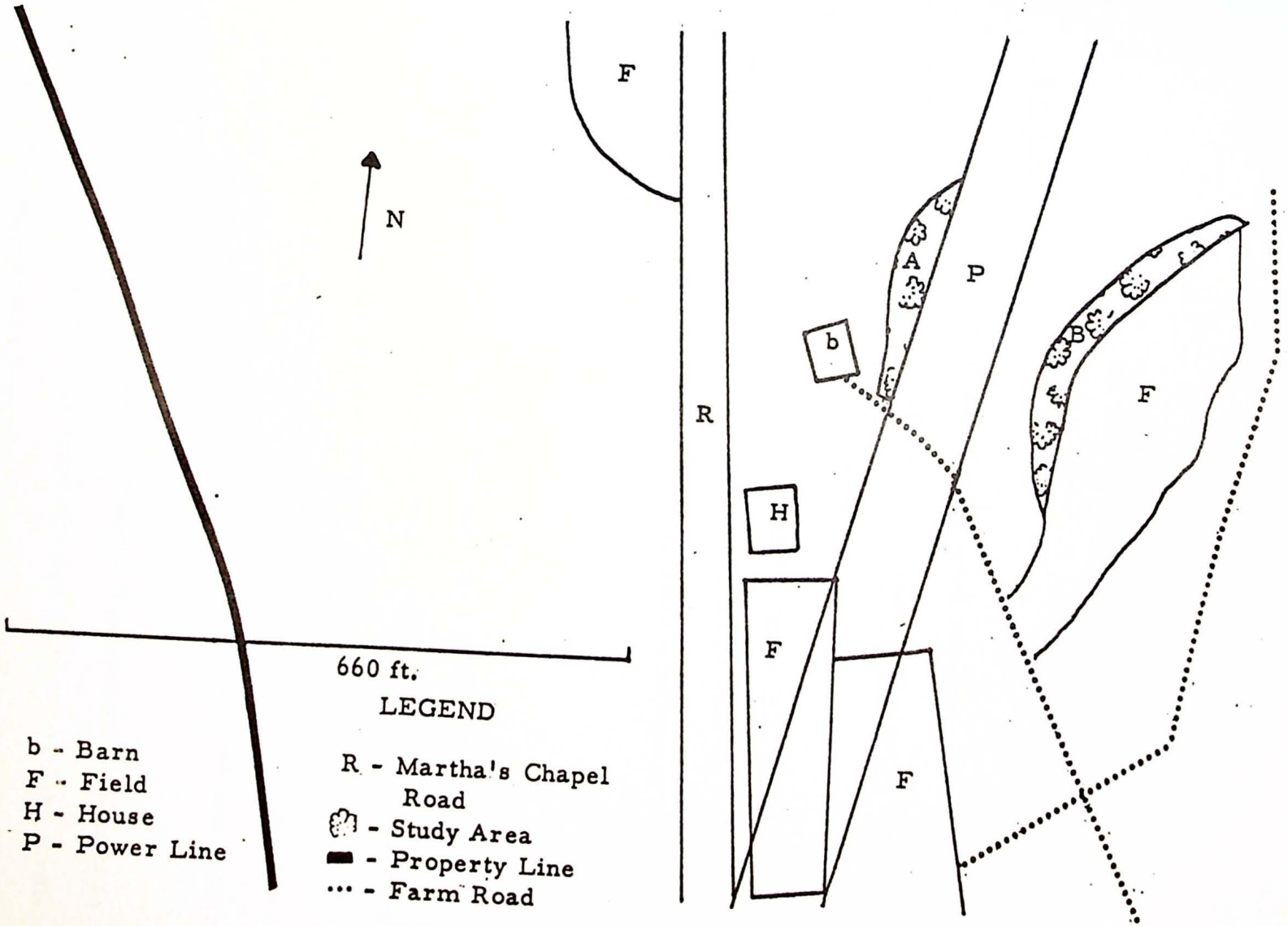

Figure 1. Map of Study Areas.

Chapter II

METHODS AND MATERIALS

Collection

Two disjunct stands of trees were selected on the basis of the following criteria: accessibility, presence of Juniperus virginiana and Cornus florida trees, and presence of epiphytes on the phorophytes. Epiphytes from fifteen Juniperus virginiana [Cedar (C)] and Cornus florida [Dogwood (D)] trees, all located on north-facing slopes at an elevation of approximately 168 meters were chosen for comparison.

Collection of Epiphytes

A single sample was taken from each tree. The epiphytes were scraped from the bark with a knife, placed in an individual paper sack, marked with the sample number 1-15, and the tree type (C, D). The top of the sack was folded and paper clipped and then placed in a large collecting bag. Each of the two tree types sampled was numbered consecutively 1-15.

Samples weighing between .5 and 1.5 grams were brought to the lab for identification of the epiphytes and extraction of the tardigrades. The mosses and liverwort were identified by Dr. David K. Smith, Botany Department, University of Tennessee, Knoxville,

with reference to Crum, Steere, and Anderson (1965). The samples have been deposited in the herbarium at the University of Tennessee, Knoxville. The author identified the lichens according to Hale (1969) with the assistance of Dr. Haskell C. Phillips, Professor Emeritus, Austin Peay State University, Clarksville, Tennessee.

Treatment of Samples

The samples were allowed to air dry in the lab for one week. After this time a subsample was removed for identification, and the remainder of the sample was scraped from the bark that was taken with each sample. These raw samples were weighed on a Mettler Gram-atic analytical balance.

Apparatus

Extraction of the tardigrades from the epiphytes was accomplished by bear traps (Figure 2). Each trap consisted of a 1.42 l glass funnel with a cork stopper at the base. The funnel was placed in a ring stand for support. A wire basket to contain the epiphytes was made of cloth 49 meshes per square centimeter, which was cut into 22.9 cm diameter circles and folded in quarters. Two or three bent paper clips were used to suspend the basket in the funnel.

Procedure

Five bear traps allowed five samples to be processed each day. One sample was placed in each of the five funnels. Each of the

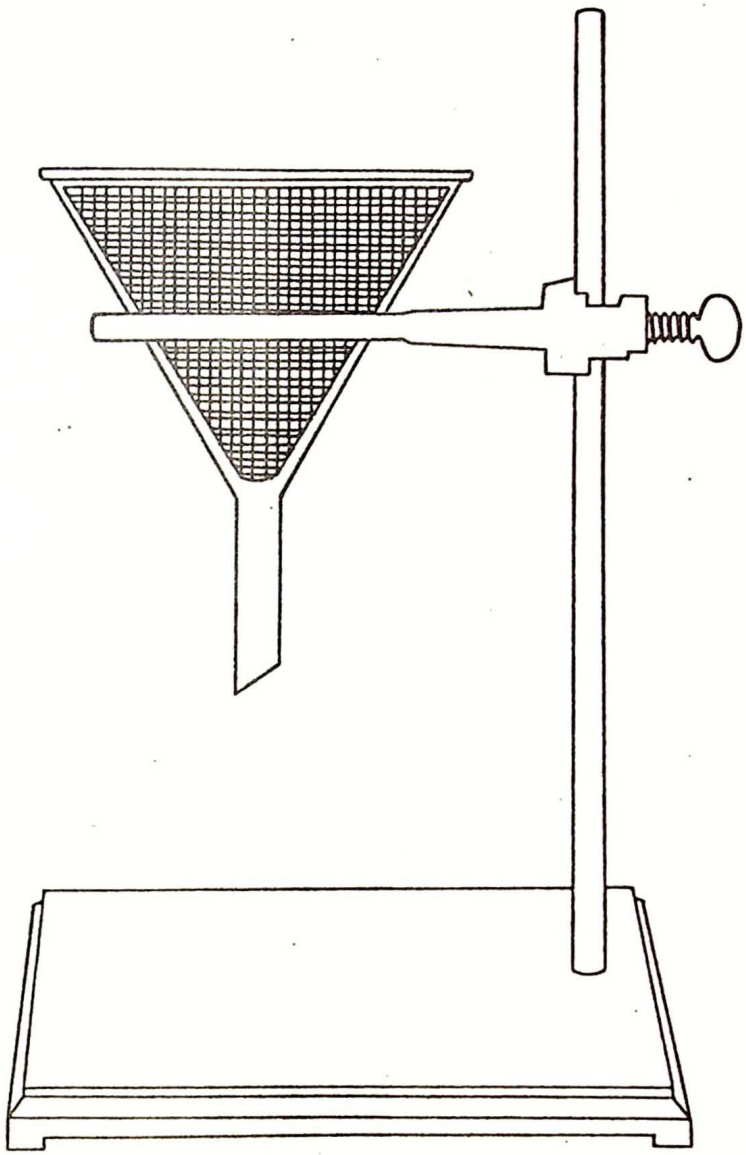

Figure 2. Diagram of a "Bear Trap".

funnels was marked with the corresponding sample number. Approximately 1.0 l of distilled water was added until the funnels were three-quarters full. Floating epiphytes were swirled until they were thoroughly moistened. The epiphytes were allowed to soak at least four hours at ambient temperature.

Individually, each wire basket was agitated and gently lifted above the funnel. The sample was squeezed over the funnel. Approximately 0.4 l of distilled water was washed through the epiphytes and the basket was again squeezed. The sample and basket were placed in numbered dishes to dry. The contents of the funnel were agitated with a clean stirring rod and allowed to settle. The upper 0.7 l of the water was removed from the funnel with a squeeze bottle. This water was discarded after initial checks proved that few, if any, tardigrades were lost in this manner. The contents of the funnel were allowed to run into a numbered finger bowl. The cork and funnel were rinsed twice with distilled water. These rinsings were then allowed to run into the corresponding finger bowl. The sample was swirled, allowed to settle, and run through a "Mini-Sieve"^{TM} micro sieve set, which was obtained from the Lab Apparatus Company. This apparatus consisted of two coupled sieves 5.13 cm in diameter with a number 60 mesh on top and a number 325 mesh on the bottom. The top sieve caught debris which was discarded. The mesh of the lower sieve was 40 μm which was small enough to retain the tardigrades. The number

325 sieve was backwashed into a clean, labeled finger bowl. Hot water was poured onto each of the samples. This killed the tardigrades by coagulating the protein and rendering them opaque against a dark background. The squeeze bottle was used to remove all but approximately 10 ml of the water from the finger bowl. The remaining water and the detritus containing the tardigrades were poured into a liquid scintillation vial. The finger bowl was rinsed with approximately 15 ml of 85% ethyl alcohol. These rinsings were then poured into the vial. The vials were labeled, capped with screw-top lids, and stored until the contents could be examined.

The dry epiphyte samples were placed into the corresponding paper sacks. The funnels, corks, baskets, and dishes were thoroughly washed in tap water. This procedure was repeated for all samples from all trees.

Isolation and Slide Preparation

The contents of the vials were examined in a Petri dish with a Bausch and Lomb dissecting microscope. The entire area was examined on high-power (45x). Each tardigrade was removed with a pipette and dropped on a clean glass slide. A drop of Hoyer's modified Berlese mounting media (Table I) was placed on the tardigrade. The ingredients in Table I should be dissolved in order with addition of heat and then filtered. Each tardigrade was positioned near the center of the drop of mounting media with a probe. A number one

Table I

Hoyer's Modified Berlese Mounting Media

Substance	Amount
Distilled water	50 cc
Gum arabic, crude	30 gm
Chloral hydrate	200 gm
Glycerine	20 gm
Potassium iodide	1 gm
Iodine	2 gm

18 mm square coverslip was placed on the slide. Each slide was labeled with the date, sample number, and type of tree. The slides were dried in an oven at 30°C for one month.

Identification

The specimens were first divided into genera and species, if known. If more than one type of tardigrade was present each genus was then subdivided into similar kinds which generally represented species.

Verifications of species identifications were made with the personal assistance of Dr. Diane R. Nelson of East Tennessee State University, Johnson City, Tennessee, and Mr. Robert O. Schuster of the University of California at Davis. A major portion of the identifications were made with reference to Ramazzotti (1972). In one instance Riggin (1962) was used to verify a species.

Statistical Analysis

Two nonparametric statistical tests suited for nominal data were used in this study to determine the significance of differences at the 0.05 level between two independent groups. The Chi-Square test for two independent groups, corrected for continuity, was calculated according to Siegel (1956). Significance levels for the Fisher test were determined from a table of critical values of D by methods described by Siegel (1956).

Contingency tables were set up for the eight species of tardigrades, 27 species of epiphytes, and four epiphyte combinations found in the study areas. Rows represented the phorophytes. Column headings were: (1) the number of samples in which the species was present; and (2) the number of samples in which the species was absent. All tests were done at the 0.05 level of significance.

Size of Sample Areas

The size of area within the study areas was determined by the use of a compensating polar planimeter obtained from the Gelman Instrument Company. This was done by measuring the area of an enlarged scale map of the sample plots with the polar planimeter and converting square inches to square meters.

Chapter III

SYSTEMATICS

Presented below are definitions of morphological terms which apply to the tardigrades of this study. The abbreviations of the terms used in the figures are in parentheses. The structure can be found in the respective figure following the definition.

Terms for Eutardigrada:

Annulation (a) - a thin, linear cuticular thickening in the pharynx (Figure 5).

Apophysis (ap) - cuticular thickenings at the junction of the mouth tube and the pharynx (Figure 6).

Furcae (f) - enlarged, posterior portions of the stylets which serves as the location of protractor and retractor muscles of the stylets (Figure 8).

Inner claw (i) - the innermost or hind claw (Figure 5).

Macroplacoid (ma) - large, cuticular thickenings in the pharynx that occur in two or three transverse rows (Figure 7).

Microplacoids (mi) - small, cuticular thickenings located posterior to the macroplacoids (Figure 6).

Mouth ring (mr) - ringlike structure surrounding the mouth opening (Figure 7).

Mouth tube (mt) - structure extending posteriorly from the mouth openings to the stylet supports (Figure 8).

Mouth tube supports (ms) - a small support that extends ventrally from the mouth ring to the middle of the mouth tube (Figure 8).

Oral papillae (op) - short, rounded appendages that surround the mouth opening (Figure 9).

Outer claw (o) - outermost or fore claw (Figure 5).

Pharyngeal tube (pt) - structure that extends from the stylet supports to the pharynx (Figure 7).

Pharynx (p) - the somewhat rounded cuticular structure that is the end-point of the buccal apparatus (Figure 9).

Primary branch (pb) - the longer branch of the inner and outer claws (Figure 5).

Secondary branch (sb) - the shorter branch of the inner and outer claws (Figure 5).

Stylet (s) - structures situated laterally to the mouth tube that are anteriorly sharply pointed (Figure 9).

Stylet support (ss) - structures that attach the stylets to the mouth tube (Figure 6).

Terms for Heterotardigrada:

Cephalic papillae (CP) - short, rounded appendages that occur on either side of the mouth opening (Figure 3).

Clava (C) - short, rounded appendages that occur at or near the junction of the head plate and the first segmental plate (Figure 3).

Dentate collar (DC) - a row of short spines located on the fourth pair of legs (Figure 4).

Dorsal spines (DS) - short appendages that are located on the dorsal posterior edges of the first, second, and third segmental plates (Figure 4).

External cirri (EC) - short, filamentous appendages that are located external to the cephalic papillae (Figure 10).

Internal cirri (IC) - short, filamentous appendages that are located internal to the cephalic papillae (Figure 10).

Lateral cirri (LC) - elongate, filamentous appendages that occur at or near the junction of the head plate and the first segmental plate (Figure 3).

Lateral spines (LS) - short appendages that are located on the lateral posterior edges of the first, second, and third segmental plates (Figure 4).

End plate (E) - the most posterior cuticular plate (Figure 3).

Head plate (H) - the most anterior cuticular plate that bears the cephalic appendages (Figure 3).

First segmental plate (I) - the plate immediately behind the head plate located in the region of the first pair of legs (Figure 10).

Second segmental plate (II) - the first row of paired plates located in the region of the second pair of legs (Figure 10).

Third segmental plate (III) - the second row of paired plates located in the region of the third pair of legs (Figure 10).

Pseudosegmental plate (P) - a single plate that is located immediately anterior to the end plate (Figure 10).

First intersegmental plate (1) - the plate that is located between the first and second segmental plates (Figure 3).

Second intersegmental plate (2) - the plate that is located between the second and third segmental plates (Figure 3).

Third intersegmental plate (3) - the plate that is located between the third segmental plates and the end or pseudosegmental plate (Figure 3).

Taxonomic Key to Tardigrades from this Study

The key is adapted from Schuster and Grigarick (1965) and Ramazzotti (1972).

Key to the Tardigrades of This Study

1a		Head with lateral cirri. Order Heterotardigrada	2
1b		Head without lateral cirri. Order Eutardigrada	4
2a	(1a)	Dorsal body cuticle clearly divided into head plate, segmentals I, II, III, intersegmentals 1, 2, 3, and end plates. Family Echiniscidae	3
2b		Dorsal body cuticle clearly divided into head plate, segmentals I, II, III, P (pseudosegmental plate), intersegmentals 1, 2, 3, and end plate. *Pseudechiniscus suillus*	p. 30
3a	(2a)	Lateral and dorsal spines present. *Echiniscus (E.) virginicus*	p. 21
3b		Lateral and dorsal spines absent, two pairs of hemispherical protrusions between segmentals II and III and segmental III and end plate. *Echiniscus (E.) mauccii*	p. 21
4a	(1b)	Oral papillae and lateral cephalic appendages present pharynx without cuticular thickenings, claws with branches completely separated. Family Milnesiidae, *Milnesium tardigradum*	p. 30
4b		Oral papillae and lateral cephalic appendages absent, pharynx with cuticular thickenings, claws with branches partially separated. Family Macrobiotidae	5
5a	(4b)	Pharynx with placoids, mouth tube support present, claws similar in size and structure. *Macrobiotus*	6

5b Pharynx without placoids, with annulations, mouth tube support absent, claws dissimilar in size and structure. Itaquascon bartosi p. 24

6a (5a) Microplacoids present 7

6b Microplacoids absent, third macroplacoid longer than second, and sometimes with bulbous satelite on posterior end. Macrobiotus tonollii p. 27

7a (6a) Three separate macroplacoids present, round or oval in shape, microplacoid small, if present. Macrobiotus intermedius p. 27

7b Two separate macroplacoids present, first with deep constriction, second shorter, microplacoid elongated. Macrobiotus hufelandii p. 24

Descriptions of the Species Found in the Epiphytes from the Study Areas

Eight species of tardigrades were identified from the study areas. The descriptions of these tardigrades are as follows. Measurements that are included represent averages for values obtained for respective structures.

Echiniscus (Echiniscus) mauccii Ramazzotti, 1956 (Figure 3)

The cuticle has large, irregular granulations that appear to have a hexagonal arrangement because of the arrangement of cuticular pores. The presence of hemispherical projections between the second and third segmental plates (II and III) and the third segmental plate and end plate (III and E) are the distinguishing characteristics for the species. Dorsal leg spines are present on the fourth pair of legs. Short spines are present on the inner claws of the fourth pair of legs. There is a short spine present on the first pair of legs. The total body length of the organism is small, up to 211 μm. There are no eyespots present.

Echinsicus (Echiniscus) virginicus Riggin, 1962 (Figure 4)

The cuticle exhibits heavy granulation. The first segmental plate (I) is broad and the second and third segmental plates (II and III) are paired. Intersegmental plate 1 is triangular with its apex directed anteriorly. Short, broad dorsal spines are sometimes present at

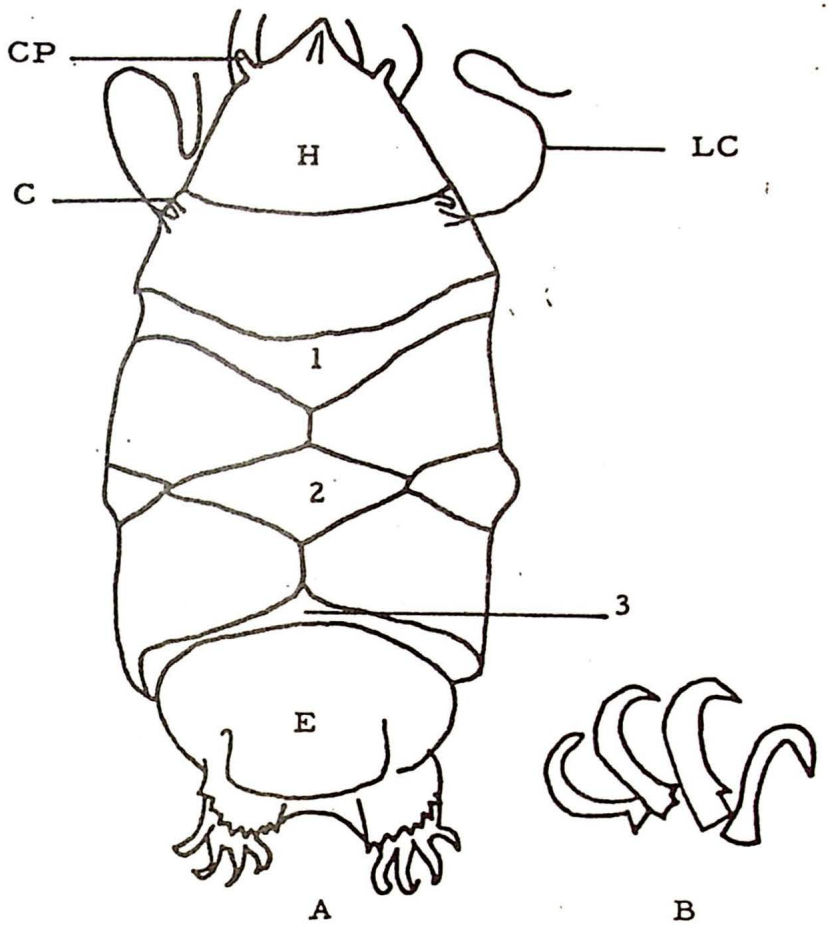

Figure 3. Echiniscus (E.) mauccii from Nelson, 1975. A. Dorsal view. B. Claws of fourth leg.

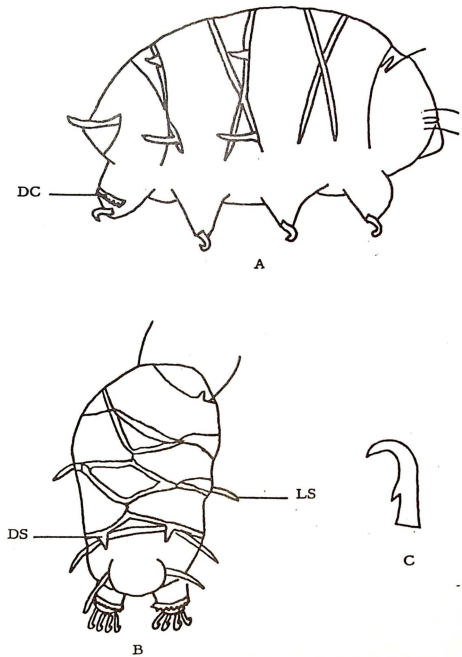

Figure 4. Echiniscus (E.) virginicus from Riggin, 1962.
A. Lateral view. B. Dorsal view.
C. Inner claw from fourth leg.

c_2 and d_2. Long lateral spines are present at c, d and e. The end plate (E) is partially divided. The fourth pair of legs possess dorsal spines. On each leg the inner claws have strongly developed secondary branches which are directed proximally. Total body length is up to 167 µm. No eyespots are present.

Itaquascon bartosi Welgarska, 1959
(Figure 5)

The cuticle is smooth. The mouth tube is 3.7 µm wide by 22.0 µm long. Annulatations, which are difficult to detect, are present on the posterior portion of the pharyngeal tube. Recurved and divergent stylets are present. The pharynx is long and cylindrical; the length is approximately double the width. There are no placoids or apophyses present. Each leg has two claws that are dissimilar in size and shape. The primary branch of the fore claw is thin and very long, 13.5 µm with small accessory spines. The primary branch of the hind claw is much shorter, 7.3 µm, and has accessory spines. Total body length is up to 307 µm. There are no eyespots present. The identification of this species is questionable due to the quality of the slides and the small number of specimens collected.

Macrobiotus hufelandii Schultze, 1833
(Figure 6)

The cuticle is smooth. A mouth ring with lamellae is present. The mouth tube is 3.2 um wide by 24.7 µm long. There is a well

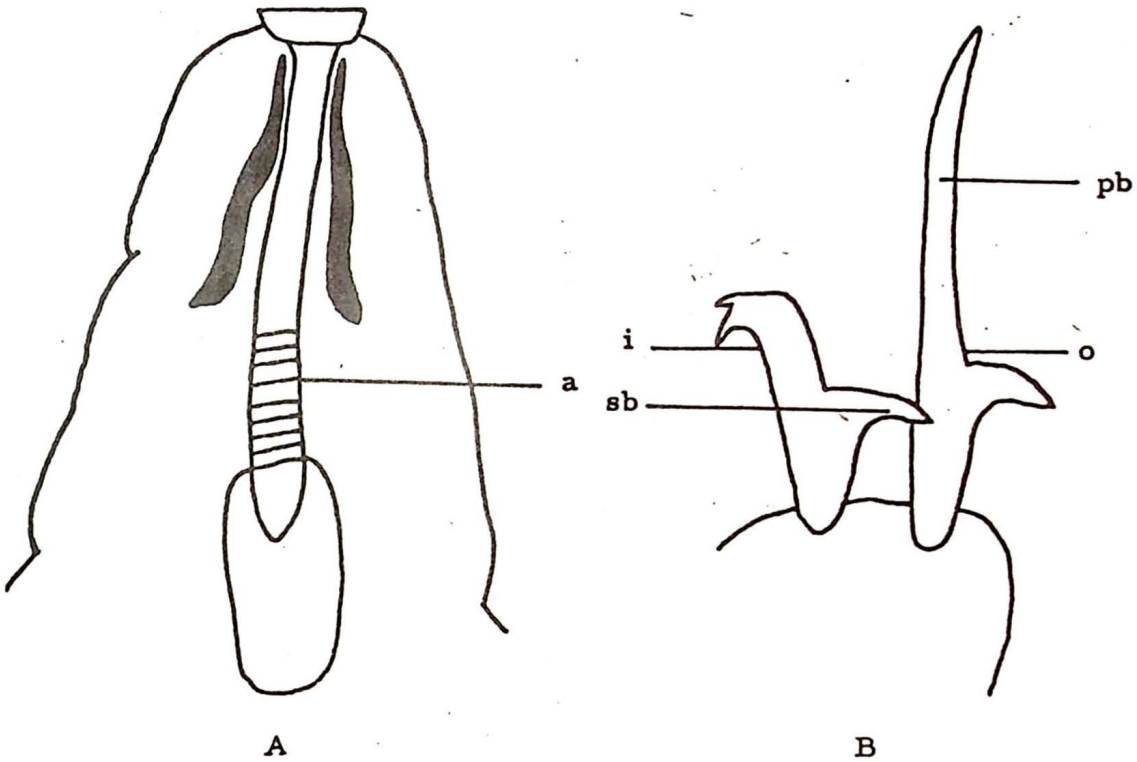

Figure 5. Itaquascon bartosi from Ramazzotti, 1972.
A. Buccal apparatus. B. Claws from fourth leg.

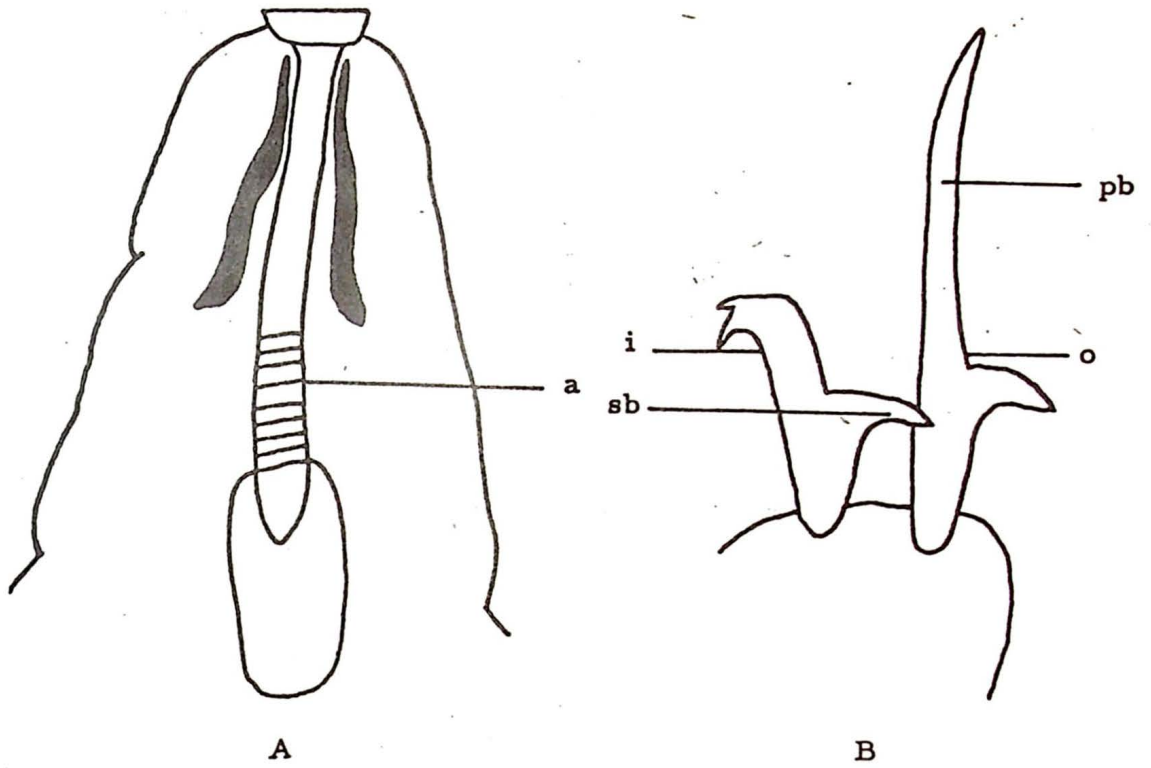

Figure 5. Itaquascon bartosi from Ramazzotti, 1972.
A. Buccal apparatus. B. Claws from fourth leg.

developed mouth tube support present. The pharynx is round to oval in shape, and it contains well developed apophyses. There are also two rod-shaped macroplacoids, the most anterior having a deep constriction (sometimes appearing separate, forming three sets of macroplacoids); the second is shorter, sometimes with an enlarged posterior end in the pharynx. One set of elongated microplacoids are present. The claws are paired with complete lunules that are smooth or toothed. Total body length is up to 297 µm. Eyespots are present, near the level of the stylet supports.

Macrobiotus intermedius Plate, 1888
(Figure 7)

The cuticle is slightly granulated. The mouth tube is narrow, 1.0 µm by 15.1 µm long. It is dorsally curved; therefore, the mouth is subterminal. There is a small mouth tube support present. A single mouth ring is present but the lamellae are absent. The pharynx is spherical in shape. It contains apophyses and three oval shaped macroplacoids. The first set of macroplacoids are partially hidden by the apophyses. If present, the microplacoids are very small. The total body length is up to 231 µm. These are small organisms with eyespots.

Macrobiotus tonollii Ramazzotti, 1956
(Figure 8)

The cuticle is smooth. The mouth tube is wide, 4.8 µm by 32.0 µm long. A mouth ring is present and has lamellae. There is

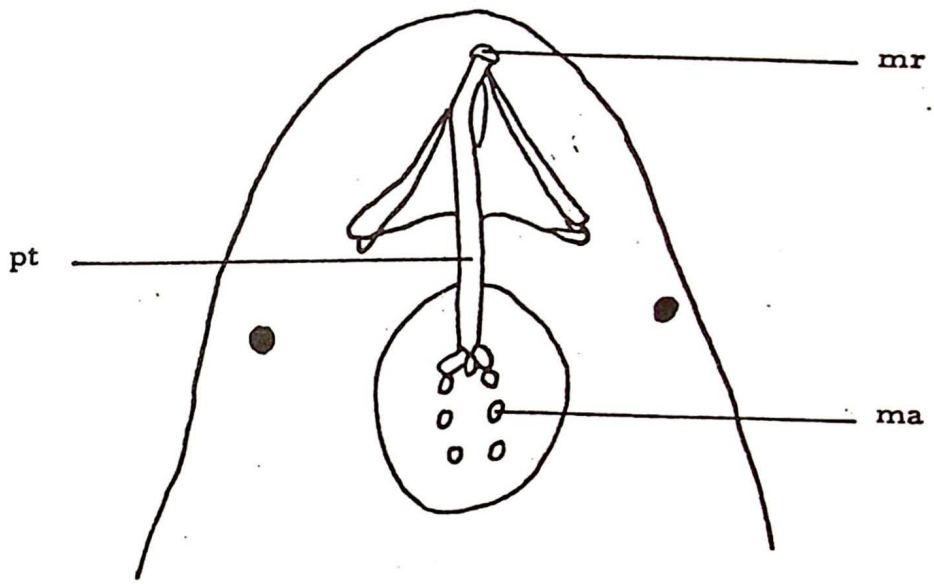

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Figure 7. Macrobiotus intermedius from Nelson, 1975. A. Buccal apparatus.

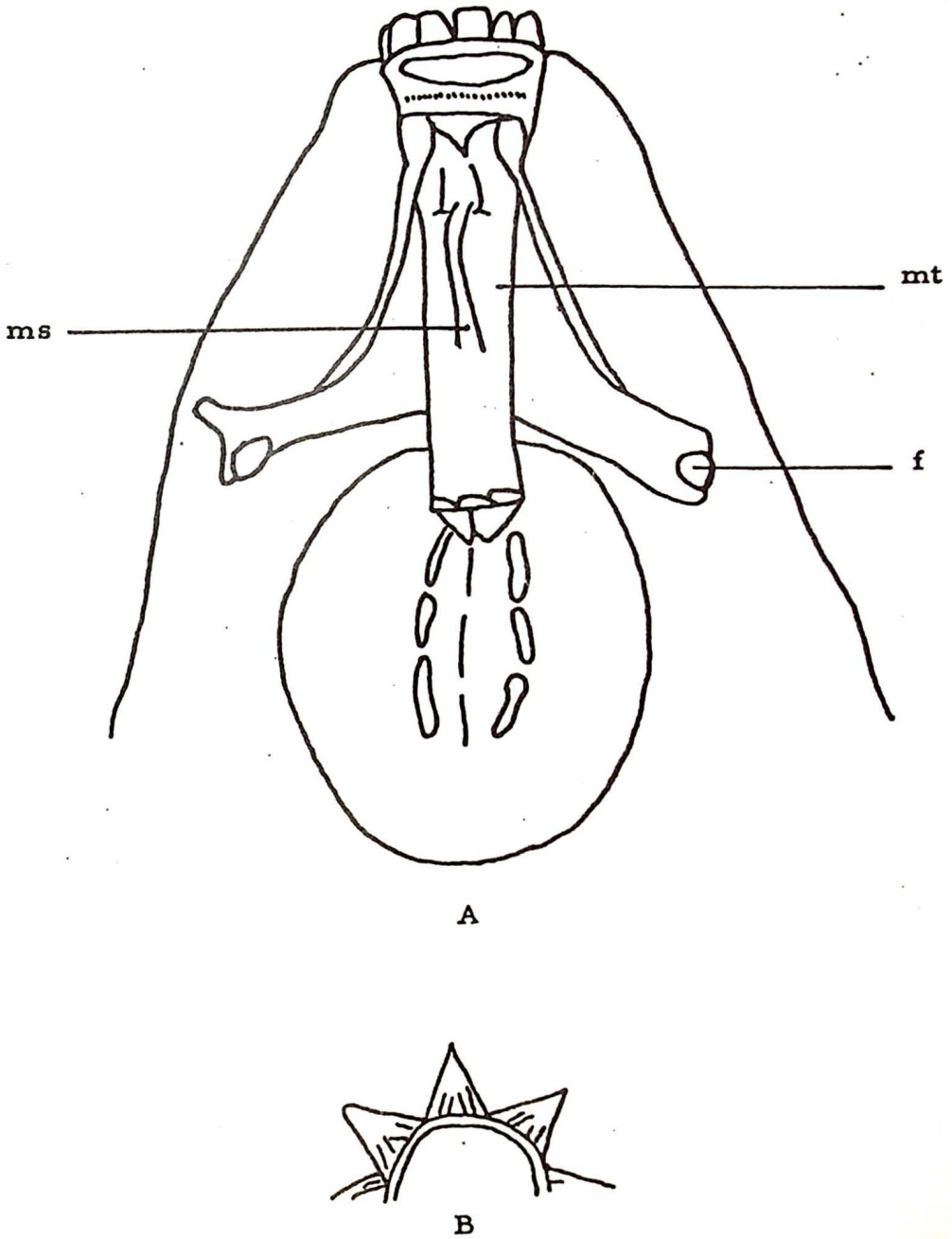


Figure 8. Macrobiotus tonollii from Nelson, 1975.
A. Buccal apparatus. B. Egg.

a well developed mouth tube support present. The pharynx is oval in shape, containing three macroplacoids and one set of apophyses. The median macroplacoid is the shortest, and the posterior macroplacoid is slightly longer than the anterior macroplacoid. Both the anterior and median macroplacoids are very close together and appear attached. There are no microplacoids present. Claws are paired and have smooth lunules. These organisms are large; the total body length is up to 515 µm. Eyespots are not present. The eggs have characteristic cone-shaped processes.

Milnesium tardigradum Doyere, 1840
(Figure 9)

The cuticle is very smooth. The mouth tube is long, 40.1 µm and very wide, 10.9 µm. There is an elongated pharynx that has no placoids. Claws are located on toe-like extremities. Both the primary and secondary branches of the double claws are completely separated. The primary branch is long and thin and the secondary branch is stout and forked. These individuals are large with a total body length of up to 545 µm. Eyespots are present.

Pseudechiniscus suillus Ehrenberg, 1853
(Figure 10)

The cuticle has regular fine granulations that are more prominent on the segmental plates but are present on the head plate and legs. The head plate (H) has a zigzag patterned suture. Dorsal leg spines

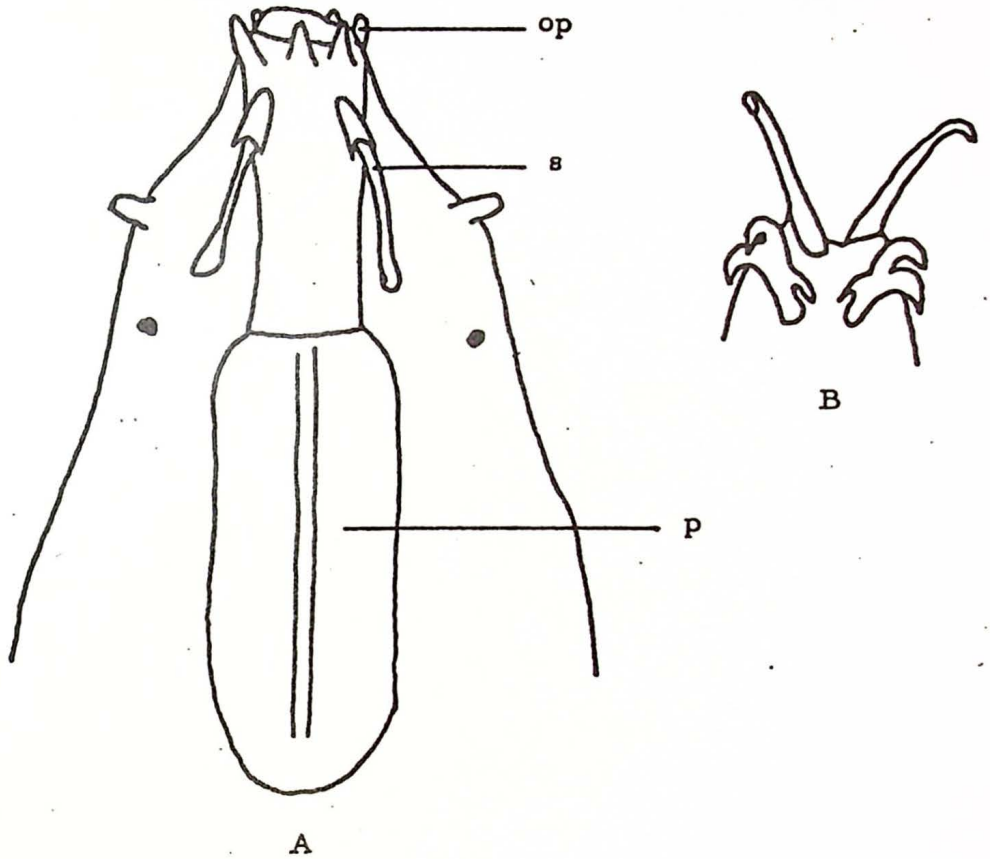


Figure 9. Milnesium tardigradum from Nelson, 1975.
A. Buccal apparatus. B. Claws from fourth leg.

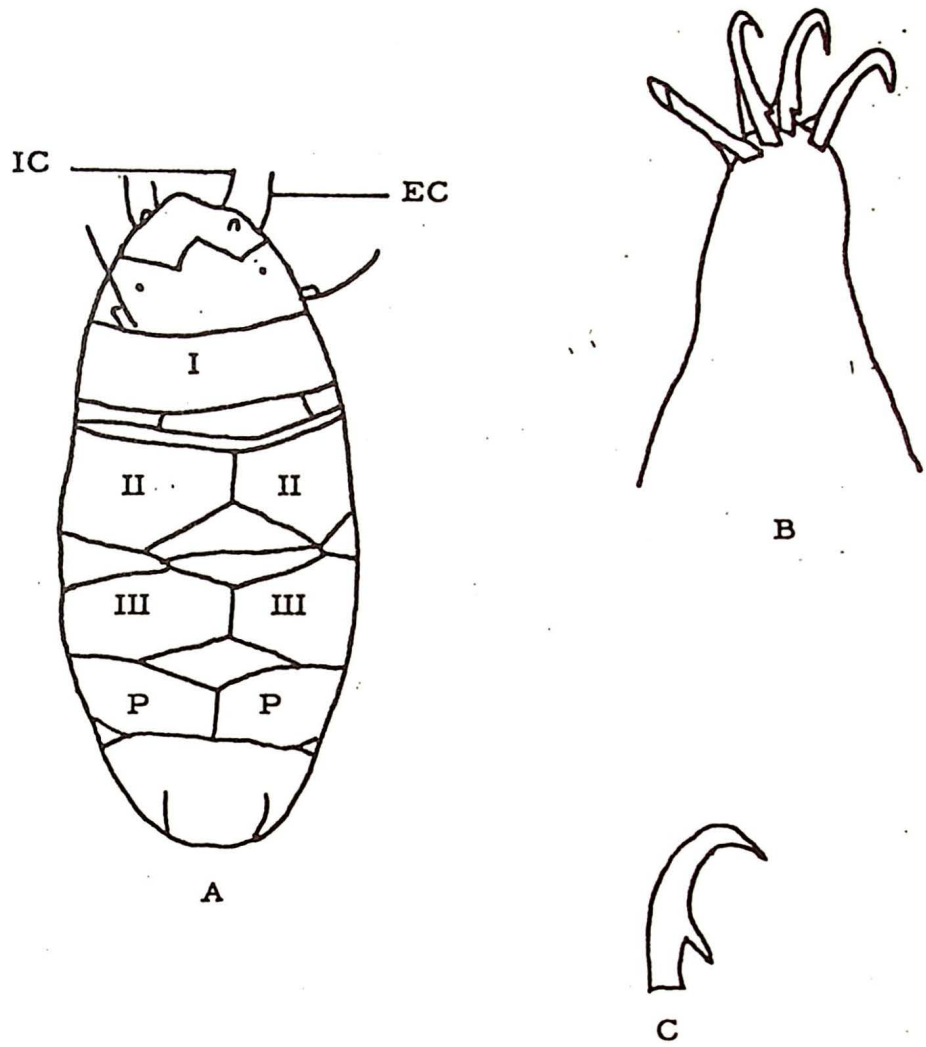


Figure 10. Pseudechiniscus suillus from Riggin, 1962. A. Dorsal view. B. Fourth leg with claws. C. Inner claw from fourth leg.

are absent. The inner claws of the fourth pair of legs have recurved basal spurs. These organisms are small with a total body length of up to 143 μm. There are small eyespots present.

Chapter IV

RESULTS

Statistical analyses consisted of two nonparametric statistical tests that were suited for nominal data. These tests were utilized to determine the significance of differences at the 0.05 level between two independent groups.

The Chi-Square test is applicable to data in a contingency table only if the expected frequencies are sufficiently large. The expected frequencies must be greater than five for the test to be properly used or meaningful. When the above criteria are met and the calculated Chi-Square value is equal to or greater than the observed value for the appropriate number of degrees of freedom (1) and level of significance (0.05), the null hypothesis can be rejected. A table of Chi-Square values was used from Siegel (1956).

The Fisher test is useful in analyzing data represented by frequencies in a 2 x 2 contingency table (Table II) when the sample size is small (less than 30).

Table II

2 x 2 Contingency Table

	+	-	Total
Group I	A	B	A + B
Group II	C	D	C + D
Total	A + C	B + D	N

If the observed value of D is equal to or less than the critical value for D in the table under the 0.05 level of significance, then the observed data are significant at that level and the null hypothesis can be rejected. The use of the word significant in the text refers to a significant difference at the 0.05 level.

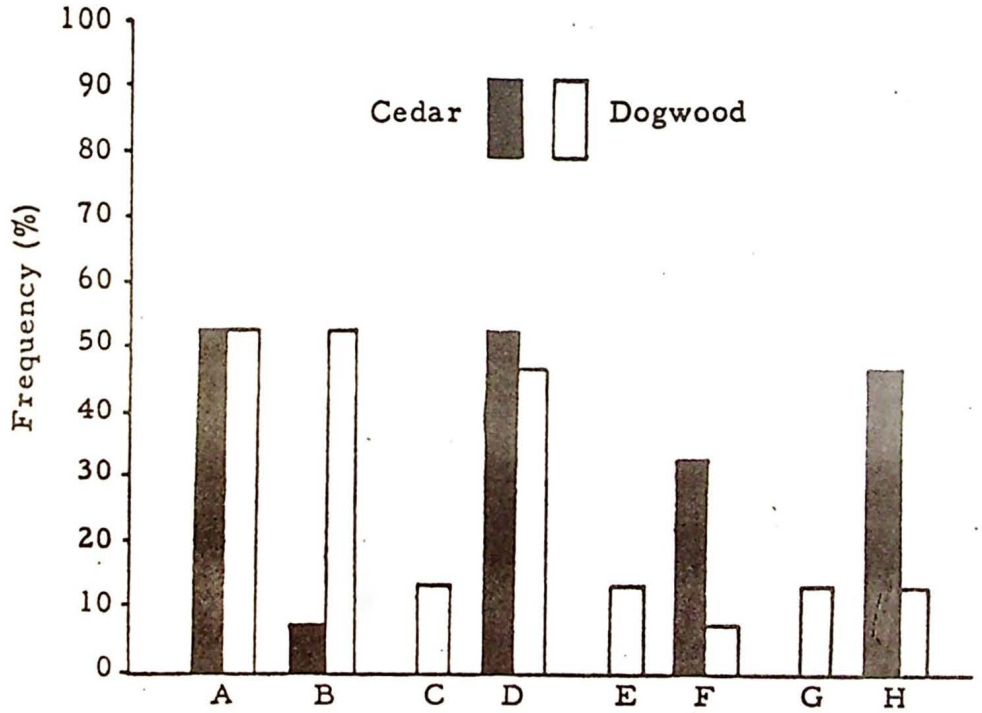
Echiniscus virginicus was the only tardigrade species, of the eight found, that showed a significant difference with respect to presence or absence of the species on the cedar and dogwood trees. It was found predominantly on dogwood trees. Epiphytes representing the following three plant groups were identified from the phorophytes: liverworts, mosses, and lichens. The single species of liverwort, Frullania inflata, was found to be significantly predominant on cedar trees. Of the nine species of mosses found, Clasmatodon parvulus an Leucodon brachypus var. andrewsianus were both observed to be significantly predominant on cedar trees. Sixteen species of lichens were identified; of these, four were found to exhibit significant differences between presence and absence of the species on cedar and dogwood trees. Parmelia rudecta was found on significantly more dogwo trees. The three species of lichens predominantly observed on cedar trees were Candelaria concolor, Crocynia membranaceae, and Physcia tribacoides.

Since the present study dealt with three groups of epiphytes, the following combinations were observed: (1) liverwort, moss,

lichen; (2) liverwort, lichen; (3) moss, lichen; and (4) lichen. Two of these four groups were significantly different. The liverwort, moss, lichen combination was predominant on cedars, while lichens alone were predominant on dogwoods. No apparent relationship existed between the epiphyte species and the tardigrade species.

Frequency is defined as the number of samples in which the tardigrade, liverwort, moss, and lichen occurred divided by the appropriate number of samples involved. The number of samples was as follows: total 30; from cedars, 15; from dogwoods, 15. If a species is present on both types of trees, but not equally abundant, then the frequency values are useful in that they represent a more valid picture of the species' distribution. Therefore, the frequency depicts the relative distribution of a species among the phorophytes and provides some degree of probability of finding a particular species. Frequency values for the eight species of tardigrades are given in Table III and Figure 11. Tardigrade and epiphyte species were grouped according to frequency as follows: 70-100%, abundant; 20-70%, common; 0-20%, rare. Table IV lists the number of samples containing an epiphyte in which each tardigrade species was found. Figure 11 and Tables III and IV were used to compile the distributions for each tardigrade species.

Echiniscus mauccii was common on both of the phorophytes sampled. The single liverwort, eight moss, and thirteen lichen



Tardigrades

A = Echiniscus mauccii
B = E. virginicus
C = Itaquascon bartosi
D = Macrobiotus hufelandii
E = M. intermedius
F = M. tonollii
G = Milnesium tardigradum
H = Pseudechiniscus suillus

Figure 11. Frequency Values of Tardigrades Found on Cedars and Dogwoods from the Study Areas.

Table III

Frequency Values of Tardigrades from Cedars and Dogwoods in the Study Areas

Tardigrade Species	Frequency (%) C*	D**
Echiniscus mauccii	53.33	53.33
E. virginicus	6.66	53.33
Itaquascon bartosi	0.00	13.33
Macrobiotus hufelandii	53.33	46.66
M. intermedius	0.00	13.33
M. tonollii	33.33	6.66
Milnesium tardigradum	0.00	13.33
Pseudechiniscus suillus	46.66	13.33

*C = Cedar
**D = Dogwood

Table IV

Number of Samples and Species of Epiphytes Inhabited by Tardigrades

Tardigrade Species	Epiphytes																									T'
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	
Echiniscus mauccii	9	4		2	1	2	1	1	1	1	3	1	4	1	2	3	4		2	10		1		4	4	1	22
E. virginicus	2								1		1		1				2	1	2	6	1			3		1	11
Itaquascon bartosi	1						1						1				1			2				2			6
Macrobiotus hufelandii	10	5		2	4	2	2	1		1	5	1	3		5	4	1		1	10		1		5	3	2	20
M. intermedius													1				1		1	2				1		1	6
M. tonollii	4	3	1		2	1	1	1			2		4		2	2	2			3		1	1	4		1	17
Milnesium tardigradum																				2							1
Pseudechiniscus suillus	7	2			2	1	2	2	1	1	8	1	1	1	4	3	3			6		1		3	3	1	20

Where, all figures = Number of samples containing a specific epiphyte that were inhabited by each tardigrade species.
T' = Total number of epiphytic species inhabited by a tardigrade species.

Liverwort

A = Frullania inflata

Mosses

B = Clasmatodon parvulus
C = Cryhaea glomerata
D = Homalotheceilla subcapillata
E = Leucodon brachypus var. andrewsianus
F = L. julaceus
G = Leskea obscura
H = Orthotrichum pusillum
I = Platygyrium repens
J = Pylaisiella selwynii

Lichens

K = Anaptychia obscurata
L = A. ravenellii
M = A. speciosa
N = A. squamulosa
O = Candelaria concolor
P = Crocynia membranaceae
Q = Parmelia aurulenta
R = P. caperata
S = P. reticulata
T = P. rudecta
U = P. subcrinita
V = P. subrudecta
W = Physcia orbicularis
X = P. orbicularis forma rubropulchra
Y = P. tribacoides
Z = Pyxine caesiopruinosa

contained E. mauccii.

Echiniscus virginicus was found on both cedars and dogwoods. However, it was rare on cedars and common on dogwoods. E. virginicus was observed on the one liverwort, one moss, and nine lichen species.

A single specimen of Itaquascon bartosi was found in each of two epiphyte samples from dogwood trees. It was absent from cedar trees. One liverwort, one moss, and four lichen species held I. bartosi.

Macrobiotus hufelandii was common on both cedars and dogwoods. It was contained in the single liverwort, seven moss, and twelve lichen species.

Macrobiotus intermedius was rare on dogwoods and absent on cedars. This tardigrade was observed in six species of lichens.

Macrobiotus tonollii was present in samples from both cedars and dogwoods. However, this species was not equally present on both types of trees. It was found to be common on cedars and rare on dogwoods. M. tonollii was observed in the one liverwort, six moss, and ten lichen species.

Milnesium tardigradum was rare in samples from dogwood trees and absent on cedars. It was found in one moss and one lichen sample.

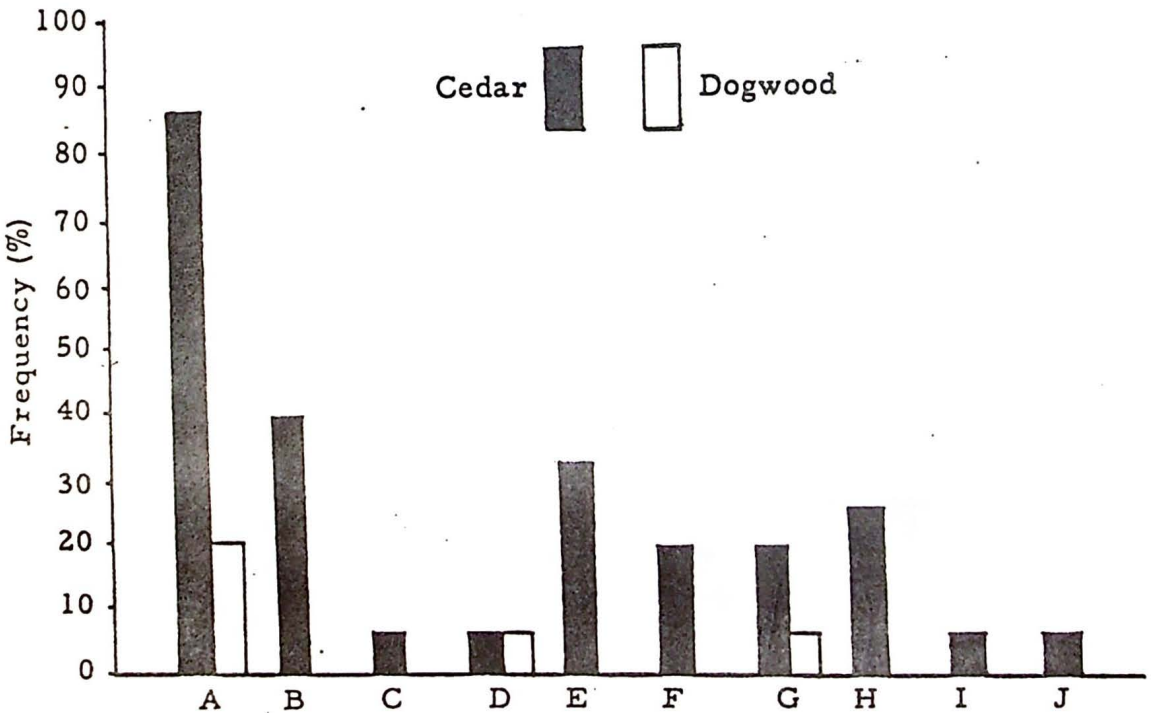
Pseudechiniscus suillus was present in samples from both

phorophytes. It was not equally abundant on cedars and dogwoods. P. suillus was common on cedars and rare on dogwoods. The single liverwort, seven moss, and twelve lichen species contained this tardigrade.

The frequencies for the one liverwort and nine moss species can be found in Figure 12. The lone liverwort, Frullania inflata, was abundant on cedars and rare on dogwoods. The two predominant moss species on cedars were Clasmatodon parvulus and Leucodon brachypus var. andrewsianus. There were only two species of mosses present on dogwoods. Homalotheceilla subcapillata and Leskea obscura were each present in one sample from a dogwood tree.

Frequency values for the 16 lichens can be seen in Figure 13. The two predominant lichen species on cedars were Anaptychia speciosa and Candelaria concolor. Parmelia rudecta was by far the most frequent lichen species on dogwoods and was also common on cedars.

Several differences were noted between samples from cedar and dogwood trees (Table V). The mean number of each of the three types of epiphytes per sample was greater for cedars than dogwoods. Cedar trees had a mean value of 0.87 liverwort per sample, while dogwood trees had a mean value of 0.20 liverwort per sample. The mean number of mosses per sample was 2.53 for cedars and 0.13 for dogwoods. Cedar trees had a mean value of 3.06 species of lichens per sample while, the mean value for dogwoods was 1.73.



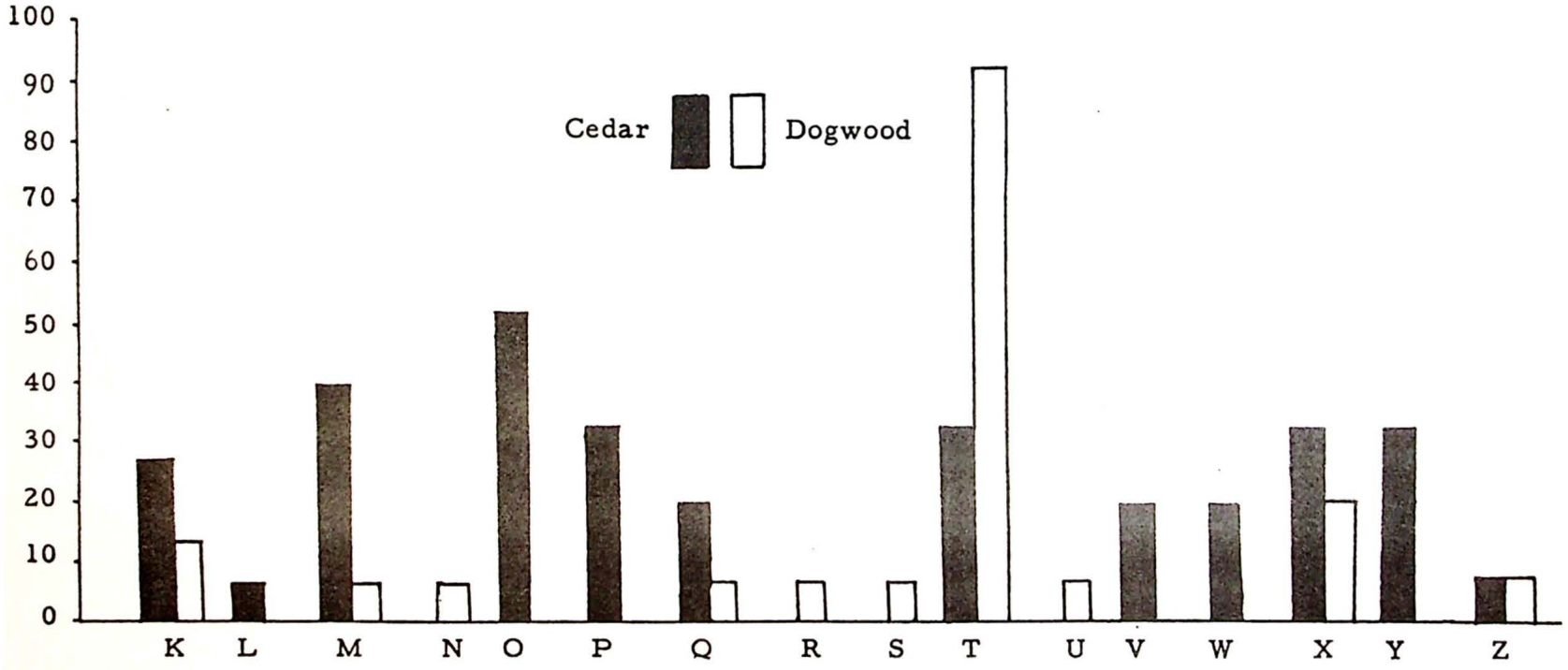
Liverwort

A = Frullania inflata

Mosses

B = Clasmatodon parvulus
C = Cryphaea glomerata
D = Homalotheciella subcapillata
E = Leucodon brachypus var. andrewsianus
F = L. julaceus
G = Leskea obscura
H = Orthotrichium pusillum
I = Platygyrium repens
J = Pylaisiella selwynii

Figure 12. Frequency Values for Liverwort and Mosses Found on Cedars and Dogwoods from the Study Areas.



Lichens

K = Anaptychia obscurata
L = A. ravenelii
M = A. speciosa
N = A. squamulosa
O = Candelaria concolor
P = Crocynia membranaceae
Q = Parmelia aurulenta
R = P. caperata
S = P. reticulata
T = P. rudecta
U = P. subcrinita
V = P. subrudecta
W = Physcia orbicularis
X = P. orbicularis forma rubropulchra
Y = P. tribacoides
Z = Pyxine caesiopruinosa

Figure 13. Frequency Values for Lichens Found on Cedars and Dogwoods from the Study Area.

Table V

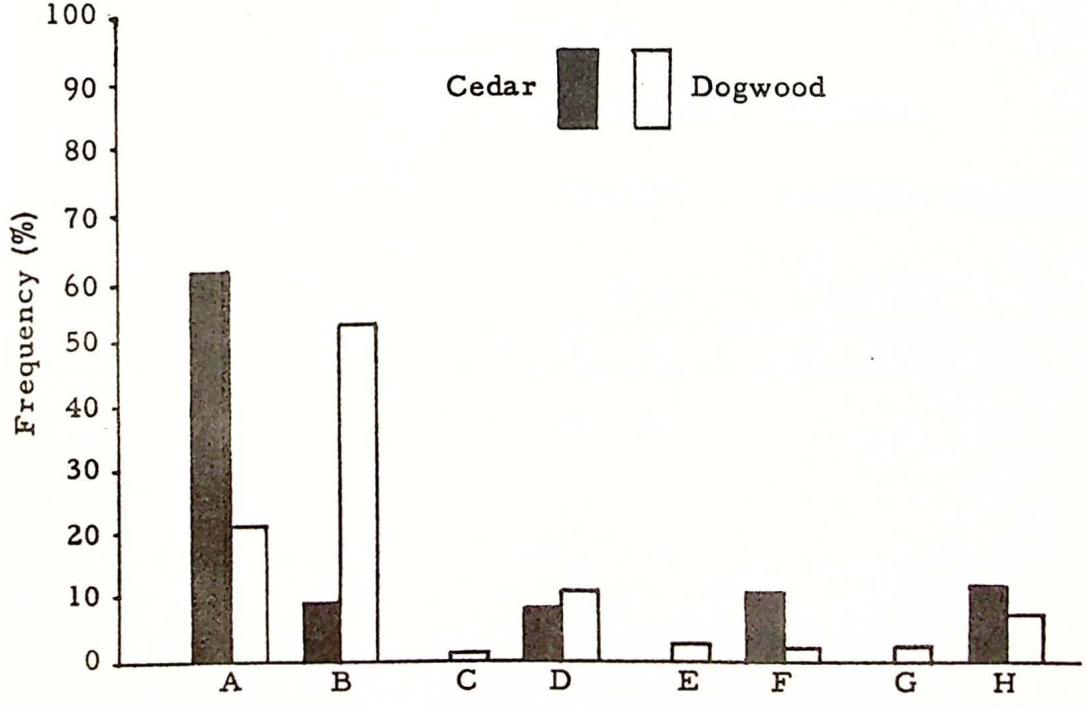
Comparison of Total and Mean Number of Epiphytes and Tardigrades for Cedars and Dogwoods from the Study Areas

	Total Number of Samples	Total Number of Tardigrade Species	Total Number of Tardigrades	Mean Number of Species Per Sample			Mean Number of Tardigrade Species Per Sample	Mean Number of Tardigrades Per Sample
				Liverwort	Mosses	Lichens		
Cedars	15	5	221	0.87	2.53	3.06	1.93	14.73
Dogwoods	15	8	198	0.20	0.13	1.73	2.13	13.20

Species diversity of tardigrades was also included in Table V. The samples from dogwood trees contained a slightly larger number of tardigrade species per sample (2.13) than those on cedar trees (1.93). A larger total number of different tardigrade species was observed on dogwood trees (8) than on cedars (5). However, the mean number of tardigrades per sample was slightly larger for cedars (14.73) than that of dogwoods (13.20). The total number of tardigrades on cedars was larger (221) than for dogwoods (198).

Since every tardigrade found in each sample was mounted, the percentages of the total number of tardigrades represented by each species found on cedars and dogwoods are presented in Figure 14. Two species of tardigrades comprised by far the bulk of the numbers of tardigrades in this study. Echiniscus mauccii made up 63% of the total number of tardigrades found in epiphyte samples from cedar trees. Echiniscus virginicus composed 52% of the total number of tardigrades from epiphyte samples on dogwood trees. Three species of tardigrades, Itaquascon bartosi, Macrobiotus intermedius, and Milnesium tardigradum, were observed only on dogwoods.

The number of tardigrades of each species per sample is shown in Tables VI and VII for cedars and dogwoods respectively. The largest sample (7) with a total of 82 tardigrades, was obtained from cedars. There are also two samples (13 and 15) that contained no tardigrades. All samples from dogwood trees contained tardi-



Tardigrades

A = Echiniscus mauccii
B = E. virginicus
C = Itaquascon bartosi
D = Macrobiotus hufelandii
E = M. intermedius
F = M. tonollii
G = Milnesium tardigradum
H = Pseudechiniscus suillus

Figure 14. Percentages of the Total Number of Tardigrades Represented by Each Species Found on Cedars and Dogwoods.

Table VI

Numbers of Tardigrades/Sample from Cedars

	Cedar Tardigrades/Sample														
Tardigrade species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Echiniscus mauccii	1	2	26	1		21	79	4			1				
E. virginicus	20														
Itaquascon bartosi															
Macrobiotus hufelandii		1		3	2	7	1		1		1			2	
M. intermedius															
M. tonollii					5			2	7	2	7				
Milnesium tardigradum															
Pseudechiniscus suillus	4			1		9	2		1			7		1	
Totals	25	3	26	5	7	37	82	6	9	2	9	7	0	3	0

Table VII

Numbers of Tardigrades/Sample from Dogwoods

Tardigrade species	Dogwood Tardigrades/Sample 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Echiniscus mauccii			1	1	3	2	18		4				1		11
E. virginicus	32	25		14	8				2	9	10				
Itaquascon bartosi		1						1						4	
Macrobiotus hufelandii		2		2		6	2		4	3					3
M. intermedius		1			5										
M. tonollii															5
Milnesium tardigradum	3											1			
Pseudechiniscus suillus		13	1												
Totals	35	42	2	17	16	8	20	1	10	12	10	1	1	4	19

grades (Table VII). Sample number 2 contained the largest number of tardigrades for the dogwoods. Three samples (8, 12, and 13) had only one tardigrade from each dogwood tree.

Tables VIII and IX show the number of tardigrades of each species per dry weight of sample for cedars and dogwoods respectively. The values on Table VIII for cedars range from 77.87 for sample 8 which contained 82 tardigrades to 0 for samples 13 and 15 where no tardigrades were found. Samples from dogwood trees range from 55.04 for sample 2 which contained 42 specimens to 0.85 for sample 13 with one tardigrade. There appears to be no apparent relationship between the dry weight of the sample and the number of tardigrades of each species per sample.

The area of the two sample plots (Figure 1) was found to be 6,072.5 square meters for plot A and 10,120.8 square meters for plot B.

Table VIII

Numbers of Tardigrades/Dry Weight of Sample from Cedars

Tardigrade species	Cedar Tardigrades/Dry Weight of Sample														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Echiniscus mauccii	1.43	2.71	23.30	1.17		25.42	75.02	5.30			1.36				
E. virginicus	28.53														
Itaquascon bartosi															
Macrobiotus hufelandii		1.35		3.51	1.74	8.47	.95		.91		1.36			2.13	
M. intermedius															
M. tonollii					4.35			2.65	6.36	2.07	9.54				
Milnesium tardigradum															
Pseudechiniscus suillus	5.71			1.17		10.89	1.90		.91			7.45		1.07	
Totals	35.67	4.06	23.30	5.85	6.09	44.78	77.87	7.95	8.18	2.07	12.26	7.45	0	3.20	0

Table IX

Numbers of Tardigrades/Dry Weight of Sample from Dogwoods

	Dogwood Tardigrades/Dry Weight of Sample														
Tardigrade species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Echiniscus mauccii			1.51	.88	2.98	1.89	19.89		6.68				0.85		16.90
E. virginicus	27.33	32.76		12.36	7.95				3.34	9.05	13.26			4.44	
Itaquascon bartosi		1.31						1.33							
Macrobiotus hufelandii		2.62		1.76		5.68	2.21		6.68	3.02					4.61
M. intermedius		1.31			4.97										
M. tonollii															7.68
Milnesium tardigradum	2.56											1.08			
Pseudechiniscus suillus		17.04	1.51												
Totals	29.89	55.04	3.02	15.00	15.90	7.57	22.10	1.33	16.70	12.07	13.26	1.08	0.85	4.44	29.19

Chapter V

DISCUSSION

From the beginning the bulk of the research done on tardigrades was concerned with the taxonomy of the group. Therefore, many descriptions and lists of species have been published from various parts of the world. More recently, cryptobiosis and physiology became areas of interest to tardigradologists. Since the availability of scanning electron microscopes has increased, ultrastructure is presently one of the major topics of investigation. Little research has been conducted on the ecology and on the populations of tardigrades. Riggin (1962) stated, "Although several voluminous monographs concerning them have been written, the tardigrades, as a group, are as yet poorly known and still await extensive treatment of their embryology, ecology, and life cycles."

In this study all of the samples were taken during one month to eliminate any seasonal variation in the tardigrades that might occur. Measurement of the dry weight of the epiphyte sample in grams was found to be the best means of quantifying samples. There was also no apparent relationship between the dry weight of the sample and the number of species or individuals per sample.

All of the individuals were mounted since the numbers of specimens per sample were small when compared to other studies (Nelson, 1975 and Ramazzotti, 1972) where thousands of tardigrades per gram of dry sample weight were found. However, according to Ramazzotti (1972) some samples contained variable distributions of tardigrades in the same clump of moss; very dense concentrations in one part and zero population in another. This might explain the one large sample with 82 tardigrades and the two samples with no tardigrades that came from cedar trees. He also found that lichens supported extremely sparse populations of tardigrades. The epiphyte samples from dogwoods contained mainly lichens alone (12 of 15 samples), while cedar samples were composed of mainly liverwort, mosses, and lichens (12 of 15 samples). The total number of tardigrades for dogwoods was 198 and cedars contained 221.

The two major factors considered in this study were the epiphyte species and the phorophyte species. These factors, plus others not measured in the present investigation but which undoubtedly have an influence on the distributions of tardigrades, are discussed below.

No relationship existed between the epiphyte species and the species of tardigrades found in the epiphytes. Generally, the more frequent tardigrade species inhabited a greater number of different epiphytes. If a tardigrade was rare, then it usually was found in a

small number of epiphyte samples.

There was a significant difference between phorophytes with respect to the frequency of one tardigrade species. Echiniscus virginicus was significantly present on dogwood trees. This could be a result of the small number of samples and the similarities between the two sample plots.

Moisture is by far the most important limiting factor concerning the distribution of tardigrades (Pennak, 1953). If water is not present, then the tardigrades will either die or enter the cryptobiotic state. Various classifications of mosses based on moisture conditions have been published. Ramazzotti (1972) divides mosses into the three following groups: aquatic (wet or submerged); damp (humid); and dry. The mosses from the present study could be placed in one of two groups, either in the damp (humid) group or in the dry group. The samples from the thickly wooded interior of the sample areas would fall into the damp group because little direct sunlight reached them. Those samples taken from the edges of the plots (Figure 1) that paralleled the powerline right of way (sample area A) and the field (sample area B) would be considered dry because they were exposed to direct sunlight during the morning hours.

The following eight species were observed in the present study: Echiniscus mauccii, E. virginicus, Itaquascon bartosi, Macrobiotus hufelandii, M. intermedius, M. tonollii, Milnesium tardigradum, and

Pseuchiniscus suillus. The identification of I. bartosi was questionable. It was confirmed by an external reviewer, but there were only two specimens and the slides made observations of distinguishing characteristics difficult. However, if the identification is correct then this is the first time I. bartosi has been identified in the United States.

Undoubtedly moisture content of the epiphytes is affected by numerous other factors such as precipitation, relative humidity, evaporation, wind, temperature, and solar radiation. No measurements of these parameters were taken in the present study. However, it is reasonable to assume that since these factors affect moisture they could also have some effect on the distribution of tardigrades.

Tardigrades are also very sensitive to low oxygen concentrations in the water surrounding them. Little is known about the minimal oxygen requirements for tardigrades, but they cannot exist in the low oxygen tensions that some small aquatic metazoans can (Pennak, 1953). Two factors affecting oxygen concentrations in epiphytes are wind and photosynthesis. Wind movements facilitate gaseous exchange and affect evaporation of moisture from the epiphytes, while photosynthesis, in the epiphyte samples, would have an effect on the amount of oxygen present in the film of water around the tardigrades.

Two other limiting factors affecting the distribution of tardigrades are species associations and eating habits. Since most tardi-

grades feed on the cell sap of the epiphytes they inhabit, their food is generally available in abundance (Pennak, 1953). Tardigrades are often found in association with other organisms which may either have a predatory or a prey relationship with them (Nelson, 1975). Nematodes, predaceous tardigrades, and parasites such as fungi have predatory relationships with tardigrades (Nelson, 1975 and Pennak, 1953). Milnesium tardigradum has been known to prey on nematodes, rotifers, and other tardigrades (Pennak, 1953). Some species of tardigrades feed on algae, bacteria, and fungi. In these cases sufficient quantities of the preferred food may predetermine the occurrence of a species depending upon the requirements of tardigrade.

A vast amount of research is yet to be done in the area of tardigrade ecology. Tardigrades can survive when moisture, oxygen, food, and other undetermined factors are present in sufficient quantities, but different species undoubtedly have unique tolerances and requirements for a variety of environmental limiting factors which remain to be delineated.

Chapter VI

SUMMARY

This study was undertaken because of the paucity of literature from Tennessee, and because of the general lack of ecological studies of tardigrades. The objectives of the study were to determine the species of tardigrades present on the study areas, their distributions, certain population parameters, and environmental-limiting factors influencing their distributions.

Epiphyte samples were collected from the bark of Juniperus virginiana (cedar) and Cornus florida (dogwood) trees, from north-facing slopes at an elevation of 168 meters, to compare the tardigrades found on the two phorophyte species. The epiphyte samples were brought to the laboratory, and all of the tardigrades found were mounted on slides. A total number of 419 individuals were identified.

From the present investigation the following eight species of tardigrades were observed: Echiniscus mauccii, E. virginicus, Itaquascon bartosi, Macrobiotus hufelandii, M. intermedius, M. tonollii, Milnesium tardigradum, and Pseudechiniscus suillus. The identification of I. bartosi was confirmed but remains questionable due to the small number of specimens and the quality of the slides containing them.

The Chi-Square and Fisher tests were utilized to determine the significant differences at the 0.05 level between phorophytes with respect to the presence or absence of a particular tardigrade or epiphyte species and epiphyte combinations. Echiniscus virginicus was the only species of tardigrade that was significantly predominant on either phorophyte. It was found mainly on dogwood trees. Of the twenty-six species of epiphytes, the presence of the liverwort, Frullania inflata, was significant on cedars; two mosses, Clasmatodon parvulus and Leucodon brachypus var. andrewsianus, were predominantly present on cedars; three lichens, Candelaria concolor, Crocynia membranaceae, and Physcia tribacoides, were significantly present on cedars and one lichen, Parmelia rudecta, was predominant on dogwoods Two epiphyte combinations were significantly different. The liverwort, moss, lichen combination was predominant on cedars, while lichens alone predominated on dogwoods. No relationship between epiphyte species and tardigrade species was noted. The more abundant tardigrades were present on a greater variety of epiphytes. Rare tardigrades were found on fewer epiphytes. Frequency values were determined for the eight tardigrade and 26 epiphyte species. Each species was discussed with respect to its frequency on the respective phorophyte and epiphytes.

The diversity of the three types of epiphytes (liverworts, mosses, and lichens) was greater on cedar trees. The species

diversity of tardigrades was greater on dogwoods even though the mean number of tardigrades per sample and the total number of tardigrades was slightly greater on cedar trees.

Ecological limiting factors such as sufficient quantities of moisture, oxygen, and food are known to be prerequisites for the occurrence of tardigrades. However, the tolerances and requirements for numerous other limiting factors remain to be delineated for each tardigrade species.

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