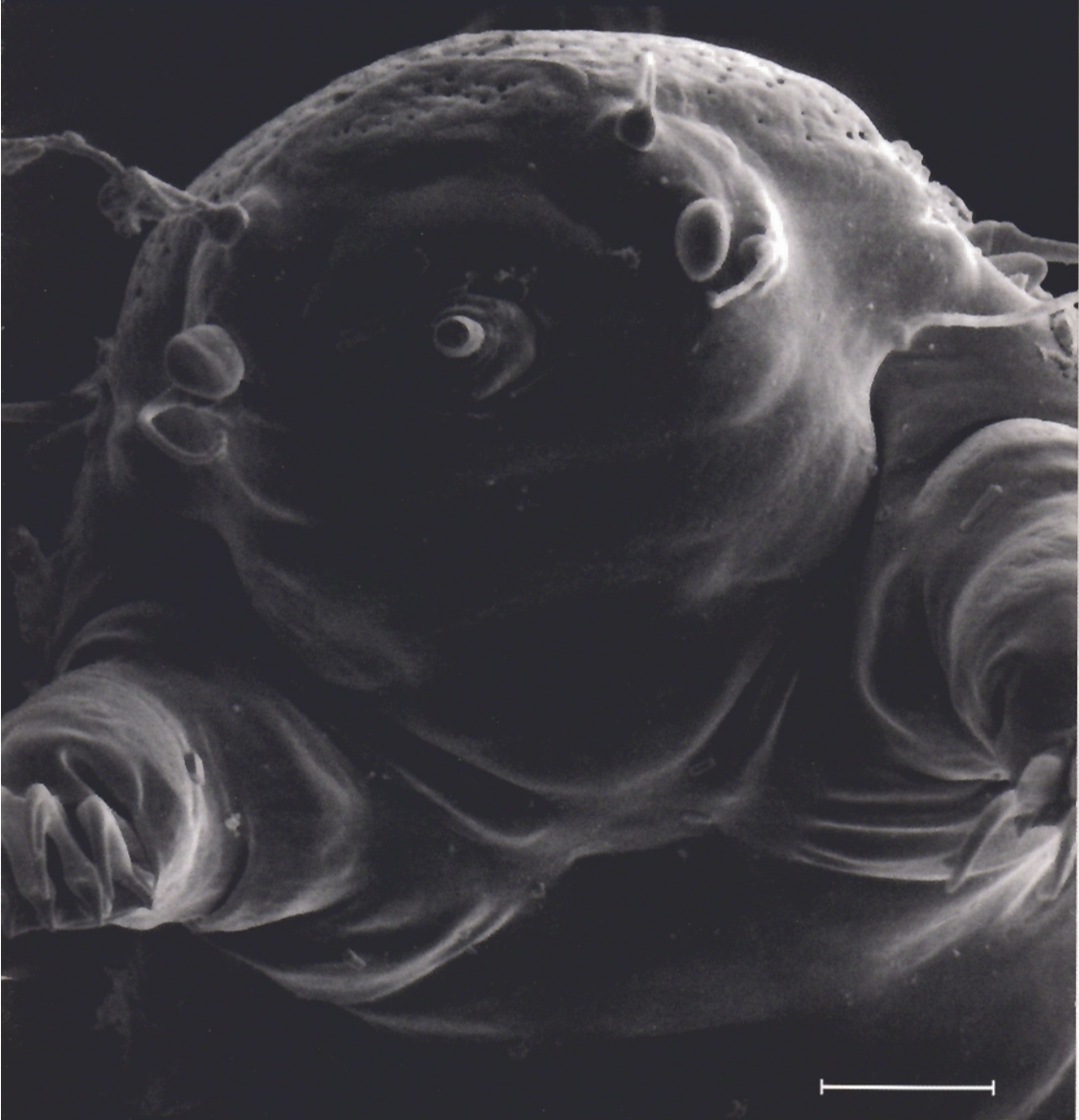


# TARDIGRADES

BEARS OF THE MOSS

WILLIAM R. MILLER



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**Cover Photo:** A Scanning Electron Microscope (SEM) photograph of *Echiniscus darienae* Miller, Horning and Dastych 1995, a new species of Heterotardigrade from the Australian Antarctic. The picture was taken at the Center for Electron Microscopy, University of Illinois, Urbana-Champaign. The scale bar equals 10  $\mu$ m (0.010 mm). The photograph clearly shows two of the characteristics that distinguish this as a new species. One, the "accessory spine" on each of the interior claws of each leg and two, the position of the primary clava closer to the external cirri than the internal cirri.

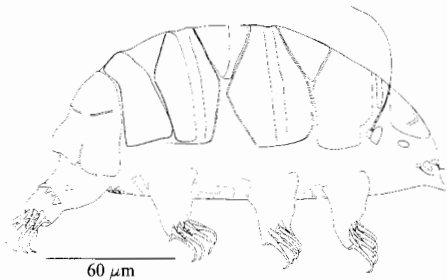
Dr. William R. Miller is a Research Associate of the Department of Biology at Southwestern College in Winfield, Kansas. For thirty years, he has studied tardigrades from Montana, Greenland, the Great Barrier Reef, and Antarctica.

# TARDIGRADES: BEARS OF THE MOSS

by William R. Miller

"Tardi what?" A question I am often asked, generally elicited by the SEM pictures of tardigrades on my office walls. With that opening, I get to explain an animal that you cannot see, has never been heard of, and causes you no harm. My story is about the adventure of discovery, the excitement of working with animals that few humans have ever seen. You fell into my trap when you picked up this booklet, so take a few minutes, read what follows, and maybe you too will be intrigued by Tardigrades, Bears of the Moss.

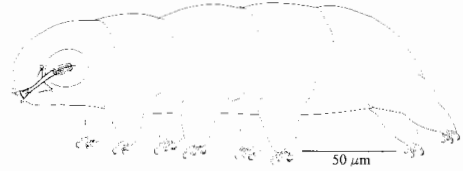
Tardigrada is one of several little-known phyla of invertebrates located between the nematodes (roundworms) and the arthropods (crustacea, insects, ticks and mites). They are small, 0.2-0.5 mm in length, about the size of a dot made with a "fine" mechanical pencil. A big tardigrade can be seen with the naked eye, but the light must be just right.



**Figure 1.** Heterotardigrada, *Pseudechiniscus suillus*.

Tardigrades look like miniature caterpillars with five body segments and four pairs of clawed legs (Figures 1 & 2). Like "higher" animals they have digestive, excretory, and nervous systems; separate sexes; and well-developed muscles. Like "lower"

animals, they lack respiratory and circulatory systems. Instead, they breathe through their skin or cuticle and the whole body acts as a pump to circulate fluids.



**Figure 2.** Eutardigrada, *Hypsibius antarcticus*.

The word tardigrade means "slow walker" which describes their rather sluggish, clumsy movement. They use their short legs and claws to cling to a substrate and waddle along like "Water Bears" or "Moss Piglets" (**Kinchin 1994**). Tardigrades are very common and have been found on every continent (**McInnes 1994**). They have been recorded in every biotope: both salt and fresh water; the humidity of rain forests, the altitude of mountains, the dryness of deserts, and the isolation of remote islands and Antarctic nunatacks.

All tardigrades are aquatic. They need to be in water to live, to find food, to breathe, to reproduce, and to move. There are marine, freshwater, and limno-terrestrial species. The latter are the subject of this booklet and live in the water droplets trapped in the space between the leaves of moss cushions, the thalli of lichens, and leaf litter. Here they share a micro-world with other organisms (collembola, mites, rotifers, and nematodes) and endure extreme environmental cycles from flood to drought.

The reason tardigrades are “interesting” is that they have developed ways to survive these environmental swings. They survive times of flood or lack of oxygen by swelling up like a balloon (anoxybiosis) and floating around for a few days. They survive until the environment dries, then return to the active state for feeding, growing, and reproducing (Figure 3).

When the environment dehydrates in dry weather, tardigrades desiccate into a reversible state of metabolic suspension called cryptobiosis. They shrivel to about one-third their former size into a wrinkled “tun” (Figure 3). Individuals have been observed to come and go from the cryptobiotic state repeatedly and tardigrades have been reported to survive more than 100 years (Kinchin 1994).

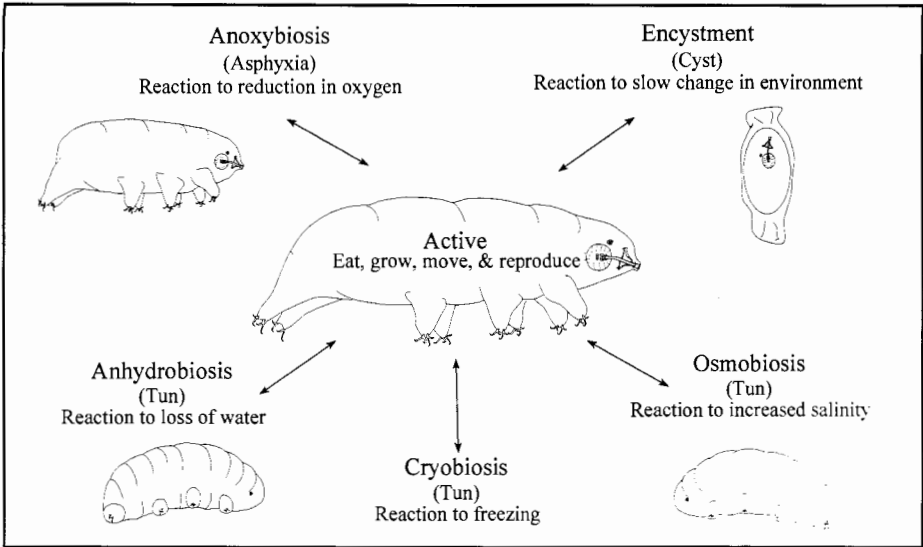
Cryptobiosis is of great interest in the study of cryogenics and tardigrades have been subjected to laboratory experiments which verified their ability to survive. Tardigrades have tolerated temperatures below freezing at 0.05K (-272.95°C) for 20 hours and -200°C for 20 months. They

have survived 120°C, pressures of 1000 atmospheres, and high vacuums. In the cryptobiotic state, tardigrades have shown resistance to hydrogen sulfide, carbon dioxide, ultraviolet light, and X-rays (Kinchin 1994). We could speculate that tardigrades could be transported through outer space in their existing form.

Despite these “capabilities,” tardigrades are still little understood. In the 200 years since the waterbear was first described, we have not identified any specific medical, commercial, or environmental effect of tardigrades. We have identified three Classes, five Orders, 15 Families, 94 genera and more than 750 species (Table 1).

**COLLECTING TARDIGRADES**

Tardigrades are easy to collect. No special equipment is needed; most is available at the grocery store. Limno-terrestrial tardigrades can be found in moss cushions growing on rocks, soil, or the side of houses. Lichens that grow on the trunks of trees and rocks support a good mix of tardigrade species.



**Figure 3.** Life stages of limno-terrestrial tardigrades.

**Table 1. Classification of the Phylum Tardigrada**

Class	Order	Family	Number of genera	
Heterotardigrada	Arthrotardigrada	Stygarctidae*	7	
		Halechiniscidae*	28	
		Renaudarctidae*	1	
		Coronarctidae*	1	
		Batillipedidae*	1	
	Echiniscoide	Echiniscoididae*	2	
		Oreellidae	2	
		Echiniscidae	12	
	Mesotardigrada	Thermozodia	Thermozodidae	1
	Eutardigrada	Parachela	Macrobiotidae	9
Eohypsibidae			2	
Calohypsibidae			6	
Necopinatidae			1	
Hypsibidae			19	
Apochela		Minesiidae	2	

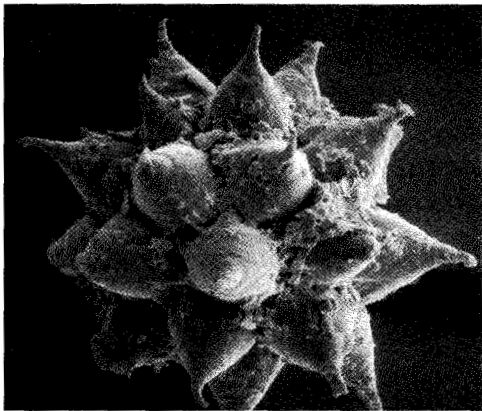
Modified from Kinchin 1994, \* Marine tardigrades, others are primarily limno-terrestrial species.

To collect moss, simply pull off a clump of the cushion with your fingers and place it in a small paper bag. School lunch bags work best. Do not use plastic bags since the moist moss will not dry and allow the tardigrades to become cryptobiotic. A pocket knife can be used to scrape lichens into a bag. For precise, comparative samples, use a core sampler, to collect equal areas. A cubic sample of moss can be analyzed for an internal habitat (Miller, Miller & Heatwole 1994).

Collect only a sufficient quantity for plant and animal identifications. Do as little environmental damage as possible. Write your collection code on the paper bag and on a map to mark the location. Enter the collection code in your field notebook along with the description of the location, plant type, substrate, conditions, and other relative data such as exposure, sea spray, or road dust.

**WORKING WITH TARDIGRADES**

Place part of the moss or lichen sample in bowls or cups and add 100 ml of distilled water. Plastic picnic cups work well. However, tap water containing chlorine may prevent your tardigrades from emerging from cryptobiosis. Keep the remainder of the sample for plant identification.



**Figure 4.** Egg of *Macrobiotus* sp.

To observe active tardigrades after only an hour or two of soaking, pipette a small quantity of the debris from the bottom of the bowl into a petri dish. Place the dish under a dissecting microscope at 40X. Normal transmitted light will work but reflected light directed from the side of the dish at a 45° angle produces a clearer image. Use a black background under the dish.

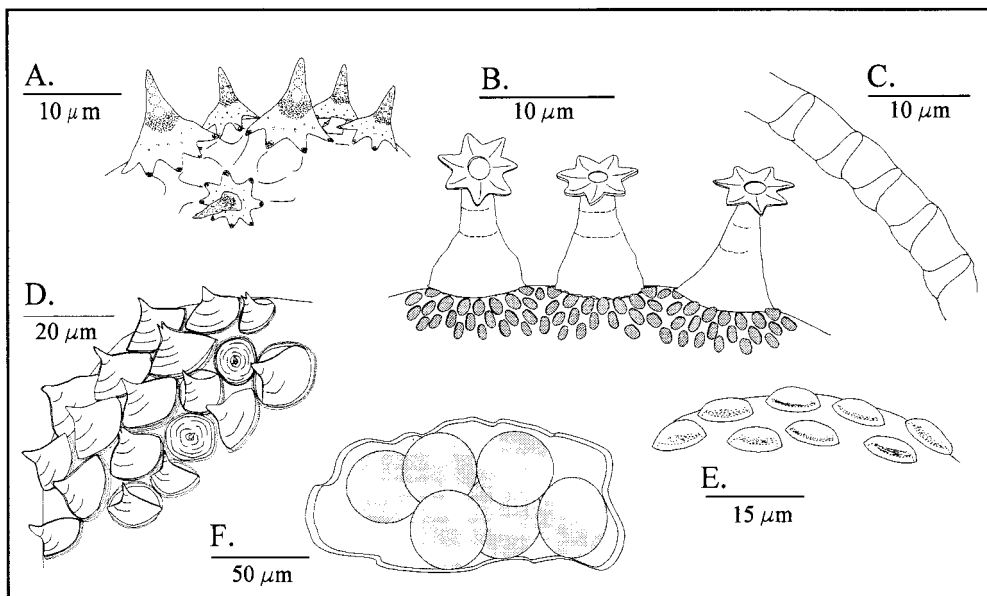
Tease the debris apart with a fine needle or insect pin taped to a pencil. Tardigrades will be seen wiggling on the bottom of the dish or crawling along a piece of debris. The first one is the hardest to see; the rest will be easy. For the best view, put a drop of water on a microscope slide, insert a tardigrade and drop on a coverslip. The action is spectacular, both the internal and external features can be seen, you will fall in love with tardigrades at this point.

Eggs are very important for identification of some species, so spending the time to look for them is

necessary. They will appear as small white or bright sparkles, little Christmas tree ornaments or sea mines (Figures 4 & 5). Search under 40-80 power. This may be hard on the eyes but possible with practice.

To accumulate specimens for study, let the sample stand in the water for 24 to 36 hours. Then squeeze the plant material with your hand or fingers to dislodge any remaining animals. The asphyxiated (anoxymbiotic) specimens can be separated and moved with a micro-pipette or a very fine inoculation loop (Irwin loop). Specimens should be preserved in small vials of Ethel alcohol at a 70% concentration or buffered formalin at a 5% solution. Insert a label with the collection code into the vile and mark the outside with a permanent marker.

To make slides, tardigrades are generally mounted in Hoyer's medium (Table 2) which acts both as a mounting medium and a clearing agent. Preserved or asphyxiated tardigrades may be mounted.



**Figure 5.** Tardigrade egg ornamentation.

**Table 2. Hoyer's Medium formula**

Ingredient	Quantity
Water	50 gm
Gum arabic	30 gm
Chloral hydrate	200 gm
Glycerine	20 cc

Mix at room temperature, in above sequence.

Center a drop of Hoyer's on glass slide and transfer specimens to the medium. Gently place a glass coverslip onto the drop. Close attention should be paid to the final location of the specimen as the cover slip will cause smaller specimens to flow with the medium as it evens out. To reduce the searching time later, use a fine pointed felt tip pen and place a dot on the coverslip to mark the location of the animal. Eggs are mounted in Hoyer's medium the same as the animals but because of their small size (0.050-0.100 mm) are more challenging to handle.

Slides should be immediately labeled with the collection code and put aside to dry for a couple of weeks. After drying, seal the coverslip by applying a coat of epoxy paint to the edge with a fine brush. Slides must be stored flat because the Hoyers never completely dries and the specimens will move if stored on their edge. Slides may be examined under medium power in a day or two. Slides should be dry and sealed before observing under oil emersion high power magnifications.

Most tardigrades can be identified to genus at the medium powers (200-400X). High power (1000X) is generally required for determining species. Tardigrades are generally drawn with a camera lucida attachment. Photography can be accomplished with camera attachments. A video image may be viewed on a television. Video may also be digitized on a personal computer, printed on a laser printer, and stored on a disk. Most of

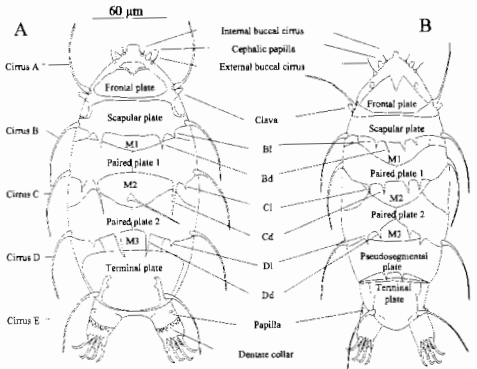
the drawings in this booklet are made from digitized video images, a technique that preserves dimensions and relationships of structures.

**STUDYING TARDIGRADES**

In your collection of moss or lichens, two basic types of limno-terrestrial tardigrades will be found. Armored specimens are in the Class Heterotardigrade, Order Echiniscoidae (Figure 1; Table 1). Naked specimens are in the Class Eutardigrada, Order Parachela or Apochela (Figure 2; Table 1).

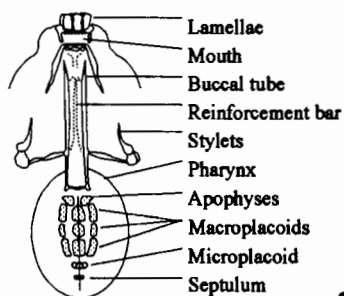
The armored Heterotardigrades will have spotted, pitted, or sculptured dorsal and lateral plates of varying number, size, and shape (Figure 6). Many are red in color. They may have spines of varying length and thickness projecting from the plates (Figure 6). Heterotardigrades have four separate claws on each foot (Figure 8B). The mouthparts (Figure 7B) are often obscured by the cuticular plates. Variations of these features determine genus and species.

The naked Eutardigrades do not have the dorsal plates but a few may exhibit some reticulations, sculpturing, bumps, or even spines (Figures 10, 11). They have a pair of branched claws that vary in size and shape on each leg (Figure 8C-I). Detail of the buccal apparatus is generally visible

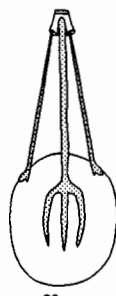


**Figure 6. External structures.**

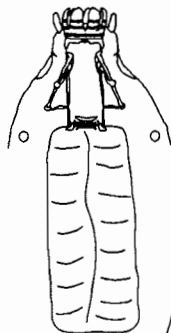




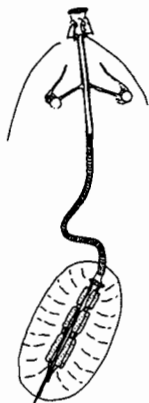
A. Diagrammatic



B. *Echiniscus*  
& *Pseudechiniscus*



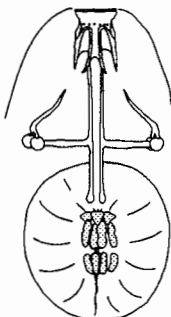
C. *Milnesium*



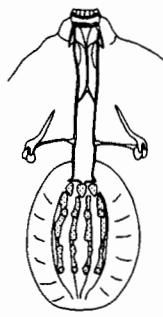
D. *Diphascon*



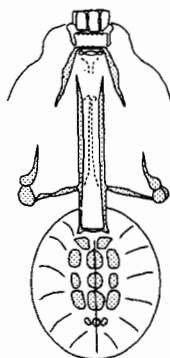
E. *Ramazzottius*



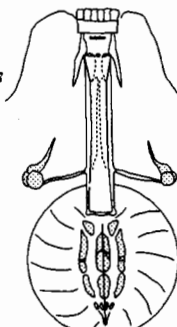
F. *Hypsibius*



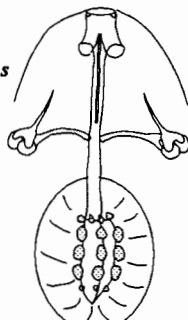
G. *Dactylobiotus*



H. *Macrobiotus* cf. *harmsworthi*



I. *Macrobiotus* cf. *hufelandi*



J. *Minibiotus*

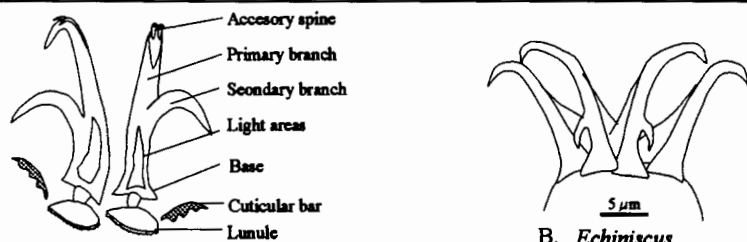
**Figure 7.** Buccal apparatus of various genera of tardigrades.

(Figures 7C-I). Different genera and species may have placoids, teeth, and lamella of varying number and size and exhibit variation in other structures that can be used for identification.

### WHAT YOU ARE LIKELY TO FIND

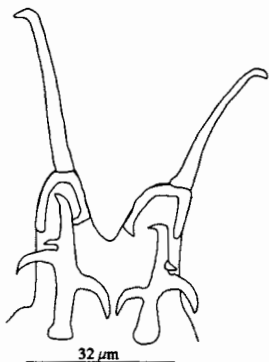
Members of the genus *Echiniscus* are cosmopolitan, armored Herteotardigrades of moderate size (0.10-0.35 mm in length). They are identified by the number, shape, and



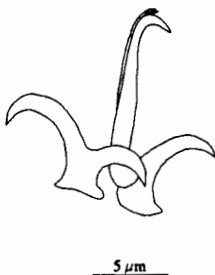


A. Diagrammatic

B. *Echiniscus*  
& *Pseudechiniscus*



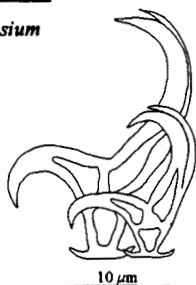
C. *Milnesium*



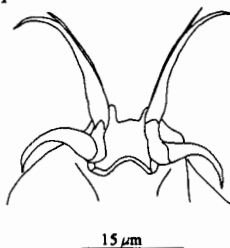
D. *Diphascion*



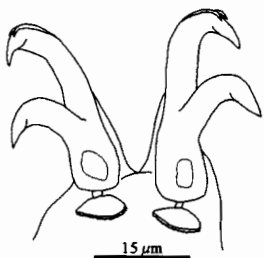
E. *Ramazzottius*



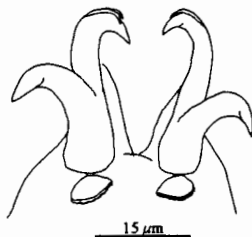
F. *Hypsibius*



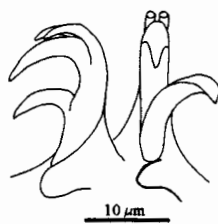
G. *Dactylobiotus*



H. *Macrobiotus* cf. *harmsworthi*



I. *Macrobiotus* cf. *hufelandi*



J. *Minibiotus*

Figure 8. Claws of various genera of tardigrades.

placement of their dorsal armored plates and spines or cirrus (Figure 6). A dentate collar on the fourth pair of legs has teeth and the internal claws may have spurs, the external claws are smooth (Figure 8B). The eggs are oval and deposited in the exuvium (Figure 5).

Two general types of *Echiniscus* are recognized, the first has only one spine, the most anterior cirri "A." The granulation on the plates is dense and uniform. The internal claws have large curved spurs and the dentate collar on fourth pair of legs has many sharp teeth

(Figures 6, 8B). The other type is distinguished by having several spines present on the edges of the dorsal plates (Figures 6, 14). They generally have red eyes, a double granulation composed of a very fine, uniform sculpture, and a courser set of depressions or pores of irregular size and arrangement. The dentate collar has small irregular teeth.

**Pseudechiniscus** are small (0.10-0.20 mm) red, armored Heterotardigrades with a pseudo-segmental plate inserted between median plate three and the terminal plate (Figure 6). They have large, black eyes and very fine granulation. The only appendages are a cirrus and clavae arising between the head and first plate, other appendages are absent (Figures 1, 6). Medial plates I and II are divided; median plate III is single. The internal claws on all legs have a spur just above base. A dentate collar is not present on the fourth pair of legs.

**Milnesium** is a genus of large (up to 1.0 mm in length), very common, cosmopolitan tardigrades. *Milnesium* is distinguished by its anteriorly sloping body, the very wide buccal tube, the muscular pharynx without placoids. Six oral papillae and triangular lamellae surround the mouth opening (Figure 7C). The claws are distinctive with a long, thin primary branch and a short, multi hooked secondary branch (Figure 8C). It is a carnivorous species and is occasionally observed eating nematodes. The mouth parts of rotifers and the buccal apparatus of other tardigrades may occasionally be observed in its gut

**Diphascon** is a genus of medium-sized (0.20-0.40 mm), smooth, eyed tardigrades with long, thin bodies. The buccal tube is also very thin. The esophageal tube is very long, thin and leads to an elongated pharynx. Long, thin macroplacoids are

present. A microplacoid and a septulum may be present (Figure 7D). Macroplacoids usually increase in size from the first to the last. Cuticular bars are often present at the base of hypsibius-type claws.

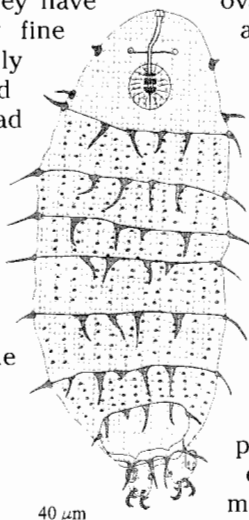
The **Ramazzottius** genus contains common cosmopolitan mid-sized (0.20-0.35 mm in length) tardigrades with a granular cuticle that has a pigmented (red or brown) pattern often appearing in longitudinal rows and transverse bands. The buccal tube is narrow, the pharynx oval to round (Figure 7E). An apophysis is present as are two roundish, smooth macroplacoids, nearly equal in size. The claws are oberhaeuseri-type (Figure 8E) with the primary branch of the external claws very thin, long, and whip-like.

**Hypsibius** is a common genus of smooth tardigrade with or without eyes and up to 0.35 mm in length. They have a short, broad buccal tube and a round pharynx. In the pharynx is a large apophysis, two elongated, globular macroplacoids, and no microplacoid (Figure 7F). The claws are typical hypsibius-type in the 1-2-1-2 pattern (Figure 8F). The

eggs are generally smooth and left in the cuticle, but some species do leave free eggs with small projections (Figure 6C).

**Dactylobiotus** is a large (0.30-0.50 mm), smooth, aquatic genus of tardigrade. A broad buccal tube leads to an oval pharynx with two slender macroplacoids, the first twice the length of the second (Figure 7G). No microplacoids are present. The large, thin claws are connected at their base with a chitinous bar (Figure 8G). The eggs have small, cone shaped projections and are laid free (Figure 6D).

The **Macrobiotus** cf. **harmsworthi** group contains medium to large (0.20-0.50 mm), cosmopolitan tardigrades with a smooth cuticle and eye spots. The



**Figure 9.**  
*Calohypsibius ornatus.*

supported buccal tube is wide. The oval pharynx has three macroplacoids and a large microplacoid (Figure 7H). The claws are Y shaped with small lunules. Eggs have prominent projections that



**Figure 10.** *Isohypsibius cf. sattleri*

look like cones rising from the surface (Figure 6A). Eggs are required for identification.

Members of the **Macrobiotus** cf. **hufelandi** group are large (0.30-0.50 mm), white tardigrades with a smooth cuticle and eye spots. The supported buccal tube is moderate in width. Two macroplacoids and a microplacoid are found in the elongated pharyngeal bulb. The first macroplacoid is longer than the second and often constricted to appear as two (Figure 7I). They have Y-shaped claws with prominent lunules and accessory points (Figure 8I). The eggs are deposited free, with ornamentation that resembles inverted egg-cups or goblets (Figure 6B). Many species in this group are very difficult to separate even for experts. Eggs are required.

The **Minibiotus** genus is composed of small (0.10-0.25 mm), smooth tardigrades with large eye spots. They have three round equal sized macroplacoids in a round pharyngeal bulb and a tiny microplacoid (Figure 7J). The stylet supports are joined to the buccal tube at the midpoint of its length. The claws are small of the hufelandi type with large accessory spines and large lunules (Figure 8J). The eggs have smooth dome-like projections (Figure 6E).

## TARDIGRADE LIFE

Naked Eutardigrades hatch as small versions of the adults and grow by

molting through instars that are similar but larger. The armored Heterotardigrades change from one molt to the next, achieving their distinctive morphology as adults. Changes include the number and size of dorsal spines, texture of plates, and the appearance of the gonopore.

The active life of a tardigrade may last only a few months, although it may be spread over several years if interrupted by cryptobiotic periods. Growth and molting occur throughout the active life and from six to 12 instars are common (Higgins 1959). Molting begins with the 'simplex' stage where the feeding apparatus is lost. The old cuticle is shed and left behind. Then a new feeding apparatus is regenerated. Tardigrades do not feed during molting which may take hours to a few days.

Tardigrades exhibit both sexual and asexual reproduction. Parthenogenetic strains are known where females produce females, without fertilization.

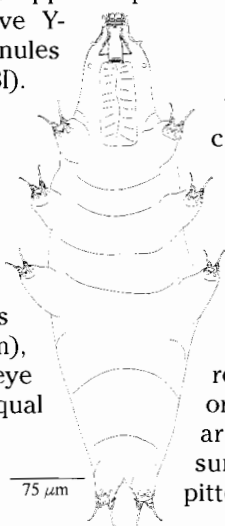
For bisexual limno-terrestrial tardigrades, periods of reproduction occur quickly because of the erratic nature of cryptobiosis. Many tardigrades leave their eggs in their discarded skin when they molt. Others release their eggs free into the environment. Egg producing activity generally declines in the winter.

The egg may be smooth, have rounded projections, thin spines, or robust cones. Some projections are split or branched and the surface of the egg may be smooth, pitted, or patterned (Figures 4, 5).

The size, shape, and pattern of the projections and the surface of the egg are the only known

differences between some species of tardigrade, thus eggs are needed for identification.

The buccal apparatus of tardigrades show great variation in detail but great similarity in structure and function. Generally the mouth and buccal tube are flanked by two piercing stylets.



**Figure 11.**  
*Milnesium tardigradum.*

A pharyngeal tube leads to the bulbus, sucking pharynx that opens into the alimentary system (Figures 7). Mouthparts are used in identification. The claws also show great diversity from simple, smooth sword-like to multi branched tree-like structures (Figures 8). They are heavily used in taxonomic determination.

## **TARDIGRADE LITERATURE**

As with any biological project one must first find the general, basic knowledge of the subject upon which to build observations and develop questions. Three major references for tardigrades are available today. The first, *Fresh-Water Invertebrates of the United States* (Pennak 1978) provides a good general overview and simple key but was published 20 years ago and is not taxonomically current. The second, *Il Philum Tardigrada* (Ramazzotti & Maucci 1983) is the last monograph that attempted to list and describe all known species. It is in Italian but an English translation is available. Finally the third, *The Biology of Tardigrades* (Kinchin 1994) provides an updated summary of the state of knowledge and theory of tardigrades but does not describe species. All three have good bibliographies.

Most libraries will have journals such as *Invertebrate Biology* that have published papers about tardigrades. In the last twenty years, six international symposia devoted to tardigrades have been held. The third (Nelson 1982), the fourth (Bertolani 1987), and the sixth (McInnes & Norman 1996) have produced excellent volumes from the papers presented. Copies of published articles are generally available from living tardigradologists for the cost of a polite letter.

## **WHAT WE DO NOT KNOW ABOUT TARDIGRADES**

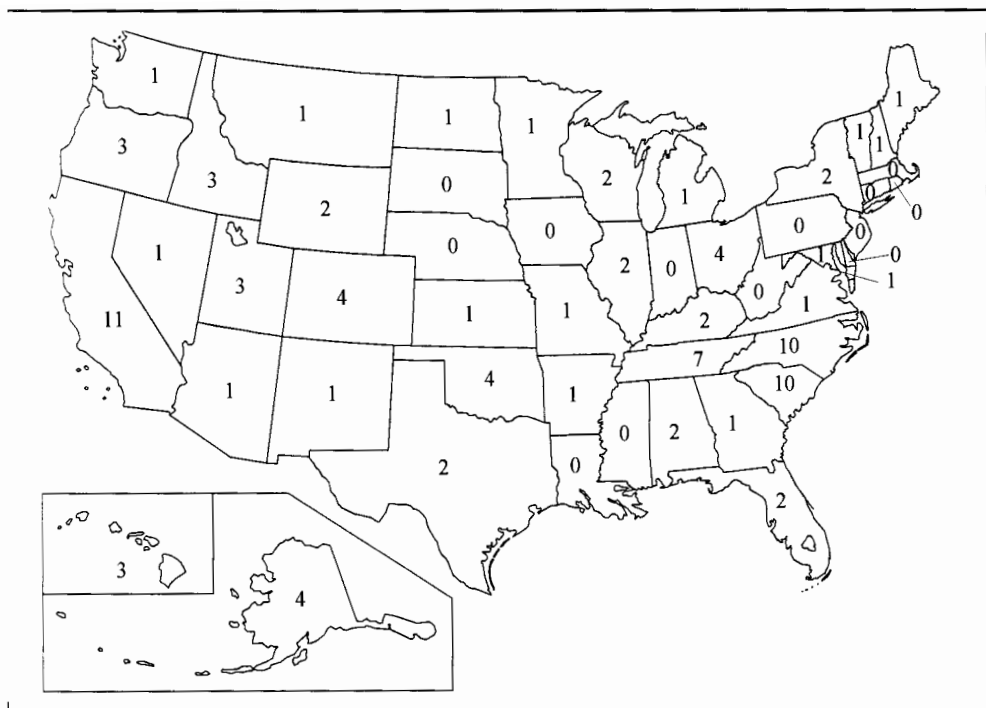
It is generally believed that tardigrades in the cryptobiotic state can

be carried on the winds. This would account for their worldwide distribution. Tardigrades have been found on remote volcanic islands where dispersion could only have been by wind or birds. The debate is supported by circumstantial evidence and awaits direct proof. What is not understood is why some apparently suitable microhabitats are not inhabited and why tardigrades may be more common in temperate and polar regions than in the tropics.

Do animals that undergo cryptobiosis have fewer generations? Does cryptobiosis allow tardigrades to inhabit microenvironments that most other animal groups would find too harsh? Are the most advanced tardigrades the ones that have been in the most stable environment for the longest time? From what did tardigrades evolve?

Eighty percent of the tardigrades described are limno-terrestrial Eutardigrades. Is this the actual makeup of the phylum or result of the collections of the last 200 years being concentrated in terrestrial mosses and lichens? Are there more species to be found? Will there be more new marine tardigrades because the salt water environment is less studied and harder to sample?

We know little of how tardigrades go about living normally. What do tardigrades do when active in the moss? Tardigrades have been observed eating nematodes, rotifers, and each other, but how often and how many? How do they find food or each other? Are they drawn to or away from light, cold, heat, oxygen or CO<sub>2</sub>? Are tardigrades positively or negatively associated with their microscopic neighbors: nematodes, rotifers, and springtails? How do they fit into the micro ecosystems? What are the effects of pollutants on tardigrades? Comparisons of tardigrades from moss and lichen samples from pristine localities and those exposed to air, chemical, or thermal pollution has not been done.



**Figure 12.** Number of reports of tardigrades by state in the United States, per McInnes 1994.

Our records of world distribution are based on only a few hundred observations (McInnes 1994). Most of those collections are from Europe. The other continents have very few records. The reports of tardigrades in the United States are scarce and scattered (Figure 12). Patterns of distribution within localities and affinities for or against particular environments or elements of the habitat are only now beginning to emerge. Almost any verified collection will add to our knowledge of distribution.

### SOLVING PUZZLES

The tardigrade lives a paradox. How does it maintain life while passing through the freezing or boiling points were the water molecule needed for metabolism expands as it changes states? How does it prevent the cells from rupturing? Answers to these puzzles are beginning to emerge with research. For example, in the anhydrobiosis type of cryptobiosis a tun is formed as the tissue dries out and the

water is replaced by the disaccharide sugar *trehalose*. Metabolism is stopped and the trehalose forms membranes that inhibit the expansion of the remaining fluids (Roser and Colaço 1993).

Many puzzles about tardigrades and life await to be solved. For example, only ten years ago Kristensen (1987) stated that the 130 plus species of the genus *Echiniscus* were completely parthenogenic, that is composed of only females. He proposed that over time males became smaller and less frequent as a percentage of the population. Thus the presence of large and abundant males is thought to be a statement of an evolutionarily older and more primitive condition. Pilato (1979) speculated that parthenogenesis was an adaption for successful passive distribution of cryptobiotic tardigrades because a single animal reaching an isolated location has better chance of surviving and procreating than if two individuals of different sexes are required.

About the same time, Dastych (1987) identified males in the genus *Echiniscus* by describing differences in the gonopores of specimens collected in the Himalayas and Antarctica. A few years later Claxton (1991) reported *Echiniscus* with large and numerous males in the mountains of Southern Australia. Then Miller and Heatwole (1994, 1996) found males on the Mawson Coast and in the Prince Charles Mountains of East Antarctica. Most recently, Claxton (1996) has described several new species of *Echiniscus* from Australia that exhibit sexually dimorphic characteristics in addition to the gonopore. Because of this recent evidence, we must rethink the basic assumptions of the role of parthenogenesis and cryptobiosis as adaptations for survival in harsh, isolated situations.

Like a puzzle or a mystery, the pieces of evidence unfold, one report at a time. You work with your data, you read what others find, and you look for patterns of sameness or contradiction. You challenge how each fact fits into the puzzle. This is the adventure of science. There are many biological puzzles waiting to be answered by the observant student. Most tardigrade work does not require expensive equipment, only the tools found in a biology

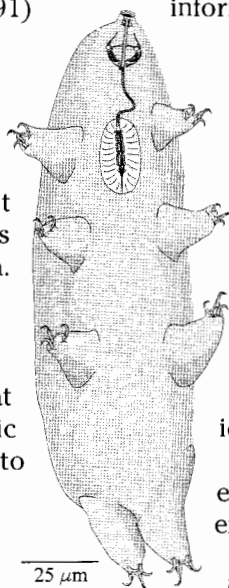
classroom. It just takes a little interest and patience.

## WORKING WITH EXPERTS

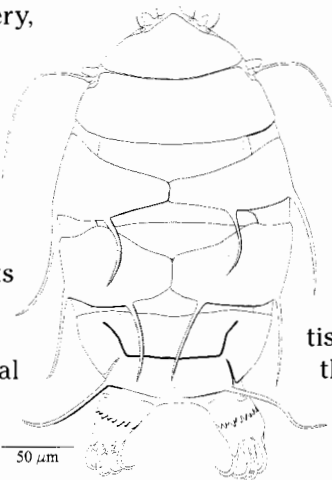
Experts can be a great source of information. To contact an expert write and explain who you are, what you are doing, and ask for copies of papers that are relevant to your work. If you seek additional help, first ask permission to submit questions and/or specimens. An expert will not mount your specimens nor identify your whole collection. Most will be glad to assist by confirming examples of your identifications.

When sending specimens to an expert, send only a few good examples of each type of animal you have found. If they are *Macrobiotus*, you must have eggs. Each slide must be clearly labeled and sealed with the epoxy paint. Include a data sheet that identifies each slide, your identification, and questions; leave space for the specialist to write a response.

For shipment use a small sturdy slide box, many science supply houses have a good one that holds 25 slides. Use tissue to pack the slides so they do not rattle. Tape the box tight. Pack the slide box in the center of a shipping container surrounded by plastic "peanuts."



**Figure 13.**  
*Diphyscon pingue*.



**Figure 14.**  
*Echiniscus springer*.

When shipping specimens internationally, use the following statement on the green customs slip to clearly describe what you are sending and avoid explanations of what tardigrades are: "Preserved, dead insects on microscope slides for scientific research." Expect the package to be opened in the other country, so include a page that gives your return address, phone numbers, fax numbers, etc. Tardigrades are not endangered species. Do not send moss or lichen with soil attached; most countries do not allow soils to be imported. If you are requested to send unmounted specimens, send them in small vials of formalin, not alcohol.

If you find something important enough to warrant publication, like a new species, ask your specialist to help you with the paper. Remember an author on a scientific paper should contribute to the science, not just pick up the moss.

## TEACHERS

Pick up some moss or scrape a lichen from a tree. Soak it in a dish. Look at it under a scope. A whole micro-ecosystem is there. Try it and you'll be ready when a student asks for an idea for a unique science project or wants to learn about something different. Add tardigrades to your classes by developing a simple project that can be repeated annually.

Tardigrades offer low cost, visible results, and great biology questions. In addition, you teach sampling, field work, lab preparation, observation, measurement, identification, literature, and writing. And maybe the adventure of finding something new, the reward for wrestling with Tardigrades: Bears of the Moss.

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