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Life History of Macrobiotus islandicus Richters With Notes on Other Tardigrades from Colorado

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LIFE HISTORY OF MACROBIOTUS ISLANDICUS RICHTERS WITH
NOTES ON OTHER TARDIGRADES FROM COLORADO

by

Robert Price Higgins

B. A., University of Colorado, 1956

A thesis submitted to the Faculty of the Graduate
School of the University of Colorado in partial
fulfilment of the requirements for the Degree

Master of Arts

Department of Biology

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This Thesis for the M.A. degree by
Robert Price Higgins
has been approved for the
Department of
Biology
by

[Signature]

Sam Shushan

Date 14 May 1958
The primary purpose of this investigation was to establish the probable number of ecdyses in the life history of *Macrobiotus islandicus* Richters under natural conditions.

An isolated patch of moss taken from the Colorado montane zone yielded 800 individuals of *Macrobiotus islandicus* which were induced to undergo asphyxy by allowing them to remain in water for at least 24 hours. In this condition the tardigrades became completely extended and could be accurately measured after mounting in glycerin.

Body length and buccal apparatus length were measured and length-frequency curves constructed. Each curve showed a series of high and low points; each peak represented an instar and each low point corresponded to an ecdysis. Six such ecdyses were suggested.

The correlation coefficient between length of buccal apparatus and body length was shown to be very high. Eleven individuals in the population were positively shown to be in the process of molting at the moment they were killed; their lengths correlated precisely with the suggested points of ecdysis in the curves.

Colorado tardigrades found in this investigation included: *Macrobiotus islandicus* Richters, *Hypsibius granulifer* (Thulin), and *Echiniscus granulatus* (Doyère)
(all new to North America); Macrobiotus harmsworthi J. Murray, Hypsibius scoticus (J. Murray), and H. tuberculatus (Plate) (new to the United States); Macrobiotus hufelandi S. Schultze and Milnesium tardigradum Doyère (previously reported from this country).

This abstract of about 250 words is approved as to form and content. I recommend its publication.

Signed
Instructor in charge of dissertation

Robert W. Pennak
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I'TTR ODUGTIOIT

Tardigrades or "water-bears" are distributed throughout the world wherever suitable habitats exist, yet this phylum has been almost completely neglected in the scientific literature of the United States. These hygrophilous invertebrates are found on both fresh-water and marine substrates and especially on mosses and other cryptogams. Tardigrades puncture the cell wall of such plants and suck out the contents. They are also found in ponds and lakes, and even in the psammolittoral where they live between the grains of sand and feed upon algal cells. Only one species, Milnesium tardigradum Doyère, is considered to be carnivorous.

Although tardigrades are undoubtedly encountered incidentally in many studies, they have been reported from only a few localities in the United States. Such published records are as follows: California (Mathews, 1938); Oregon (Fairbanks, 1958); Washington (Mathews, l.c.), (Whittaker and Fairbanks, 1958); Wisconsin (Mathews, l.c.), (Pennak, 1940); Illinois (Newhaus cited by Mathews, l.c.); Michigan (Mathews, l.c.); New York (Marcus, 1929); Vermont (Mathews, l.c.); Maine (Packard, 1873), (Beal, 1880); Massachusetts (Marcus, 1946); District of Columbia (Marcus, 1928), (Mathews, l.c.), (Curtin, 1948); North Carolina (Hay, 1917);
and Texas (Mathews, l.c.), (Chitwood, 1951).

Almost all of the literature on tardigrades has stemmed from European investigations and dates back to 1773 when Goeze first mentioned the "little water bear." Although there is a reasonable amount of information available on the biology of the tardigrades, most of which is summarized by Marcus (1929, 1936), the life history and number of molts have apparently never been determined for any species. It is generally believed that four to six molts occur under natural conditions (Pennak, 1953), but such information appears to be largely speculative. Mathews (1938) mentions some tardigrades kept for seven months in a laboratory and reports that during this time they shed their exoskeleton almost every four weeks. He further mentions that ecdysis can be induced by chemical injury.

The primary purpose of the present investigation was to establish the probable number of ecdyses in the life history of *Macrobiotus islandicus* Richters under natural conditions. The procedures used were suggested by the very familiar technique of determining the composition and annual growth for a population of a fresh-water species of fish by means of a length-frequency curve. Since a tardigrade sheds its exoskeleton in a typical arthropod fashion, a length-frequency curve should not be a smooth, bell-shaped curve but should rather exhibit a series of high and low points. Each peak should presumably correspond to a particular instar (or size class) and each low point should correspond
to an ecdysis (or the end of one instar and the beginning of the next). Since the buccal apparatus is also shed during ecdysis, the length of the buccal apparatus plotted against frequency should result in a similar curve.

A second purpose of this investigation was to add to our information on the occurrence of tardigrades in the United States.

The author wishes to gratefully acknowledge the generous help of Dr. Robert W. Pennak who suggested this problem and directed this thesis. Acknowledgements are also made to Dr. W. T. Edmondson who kindly made the tardigrade section of the forthcoming revision of *Fresh-water Biology* available to me, and to Dr. B. G. Chitwood who aided in the identification of some of the specimens.
MATERIALS AND METHODS

All tardigrades used in this section of the investigation were taken from a moss, Tortula ruralis (Hedw.) Smith, collected from a north-facing slope of Boulder Canyon near "Castle Rock" about 17 miles west of Boulder, Colorado, at an approximate elevation of 7,800 feet. This spot is on the south bank of Middle Boulder Creek and is part of the lower montane zone of the Central Rocky Mountain Province.

Fifteen grams (dry weight) of Tortula ruralis, found as an isolated microstand (in contact with no other vegetation) yielded 1,131 tardigrades. One hundred seventy of these were identified as Echiniscus granulatus Doyère. The remaining 961 tardigrades were probably all Macrobiotus islandicus Richters. Eight hundred individuals of the latter species were mounted and measured.

The moss was collected on 3 June 1957 and allowed to dry for a four-month period. On 5 October the moss was immersed in a 500 ml. beaker filled with tap water. At the end of 20 minutes a preliminary examination of the washings revealed the emergence of the tardigrades from anabiosis. All of the Macrobiotus islandicus were yellow, and the Echiniscus granulatus were bright orange. The tardigrades were removed from the water with an Irwin loop and transferred to a watch glass containing tap water.
The tardigrades were kept in the tap water for at least 24 hours in order to induce them to undergo asphyxy, a phase in their life cycle which occurs when the hypo-dermal cells apparently lose their ability to control water content (Mathews, 1936). This is an important feature in examining tardigrades since it induces turgidity and causes the animals to extend fully. Marcus (1929) remarks that many investigators have made measurements of tardigrades while the animals were not fully extended or while stretched due to the pressure of a coverslip. For this reason, he is skeptical about many published tardigrade dimensions. Furthermore, investigators have not used a standard length; some include the posterior legs and claws, while others measure only the longitudinal axis of the trunk.

An effort was therefore made in the present investigation to use only fully extended tardigrades. Such specimens were taken from the tap water after they were completely extended and placed in 95 per cent ethyl alcohol. When all individuals were in alcohol, glycerin was added in small amounts and the alcohol allowed to evaporate until a 100 per cent glycerin medium resulted. The tardigrades were then transferred to a large drop of glycerin within the concavity of a shallow depression slide and arranged in a suitable manner. A coverslip was placed over the glycerin and pressed down firmly. The exuded glycerin was carefully removed with alcohol and the coverslip sealed.
with Murrayite cement. This method of mounting tardigrades seemed to be the most satisfactory. It permitted the use of the dry high power objective of a microscope and afforded reasonably clear, well-defined specimens.

A filar micrometer was used to obtain measurements to the nearest micron, and it was found that the accuracy of such measurements was \( \pm 3 \) microns.

The body length of each tardigrade was considered to be the length of an imaginary line running parallel with the ventral surface of the body from the anterior margin of the head to the most posterior junction of the fourth pair of legs with the trunk. The buccal apparatus was measured in a similar plane and was considered as that length from the anterior margin of the buccal apparatus to the posterior margin of the muscular pharynx.

Series of tabulations were made on each specimen. These included the length of the body, the length of the buccal apparatus, the number of developing eggs, and their diameter and length of processes. Eggs found free in the moss washings were mounted along with the tardigrades, and similar measurements were taken on these eggs. In some cases the buccal apparatus was not clearly discernible and the measurements were omitted.
RESULTS AND DISCUSSION

Growth and Ecdysis in Tardigrada

Hatching in the tardigrades is accomplished by splitting the egg shell with the stylets. After the young have emerged, growth is accomplished by the enlargement of the cells already present. There is a constancy of cells in all tardigrades; Mathews (1938) gives 25 epithelial cells and 39 muscle cells for Eutardigrada. Preceding an ecdysis, the tardigrade shrinks within its cuticular exoskeleton which then ruptures at the antero-ventral end. Hypodermal cells then begin to secrete a new cuticle and the tardigrade crawls out of its old exoskeleton. Claws remain attached to the old cuticle and are replaced in the newly molted tardigrade by secretions from special glands at the tip of each leg. A few days before a tardigrade molts, the hardened portions of the buccal apparatus are ejected. These hardened portions are restored after the molt by secretions from the salivary glands, and their size is increased in proportion to the new body length. The restoration of the hard parts may or may not be apparent by the time the tardigrade has completed a full ecdysis.
Body Length-Frequency

The curve resulting from plotting body length against the 800 individuals (Fig. 1) indicates six probable points where ecdyses occurred in the total population. The first point is between 321-360 microns, and the great majority of individuals in the first instar thus ranged between 211-360 microns in length and consisted of individuals which had not yet shed their exoskeleton. This size class is substantiated by one individual (Fig. 4D) which was just hatching from the egg at the time of fixation; the body length of this tardigrade was 237 microns. It is probable that the irregularities of the curve at the left section of this graph are partially due to the fact that the smaller individuals were obscured by debris and overlooked.

A second ecdysis is indicated between 401-440 microns, and most of the tardigrades in the second size class would therefore be between 321-440 microns long.

The third ecdysis is indicated between 471-520 microns, with most of the third instar tardigrades ranging between 401-520 microns in length.

The fourth area of probable ecdysis is indicated between 531-570 microns; most of the tardigrades of the fourth size class are between 471-570 microns in length.

The fifth and sixth ecdysis points are not as obvious as are the preceding points. The peaks and depressions at this end of the curve are significant only when the number
Figure 1. Body length plotted against number of individuals. Measurements are arranged in groups of ten (the first point on the abscissa includes tardigrades between 201-210 microns in length). The solid line indicates the actual curve obtained from the measurement of 800 Macrobiotus islandicus Richters; the dotted lines suggest idealized overlapping curves for each of the instars implied by the solid line; instars are indicated by Roman numerals (I to VII); areas of probable ecdyses are indicated by vertical parallel lines; points of known ecdyses in 11 tardigrades are indicated by heavy dots.
of individuals within these last two size classes are considered. Because of the large size of these individuals, it is very probable that all tardigrades within these size groups were collected from the moss washings, and consequently the resultant curves at the right end of the graph are more accurate than those at the left. The fifth ecdysis point is between 621-640 microns, and the great majority of individuals in instar V are between 531-650 microns in length.

The sixth ecdysis is indicated between 651-670 microns, with most of the sixth instar tardigrades ranging between 621-670 microns in length.

The last or seventh size class ranges between 651-710 microns in length.

To be sure, these size ranges and ranges of ecdyses are somewhat arbitrary. The author would readily agree that the figures for any one tardigrade could vary as much as ±20 microns or more. It is also probable that environmental conditions would have a great deal of effect on the points at which ecdyses would occur.

The suggested positions of ecdyses in Figure 1 are substantiated by inserting the points at which individuals in the population were positively shown to be in the process of molting when killed. This is evidenced by the remnant of the old exoskeleton, which in eleven cases was still in contact with the freshly molted individual. One of these individuals had a body length of 360 microns, and this
figure aligns significantly with the suggested ecdysis range for this size class as shown in the graph. A second tardigrade in this same condition measured 430 microns, which matches the second suggested ecdysis point. Five other molting tardigrades measured 472, 489, 510, 519, and 520 microns; this group of individuals matches the third ecdysis range within reasonable limits. The fourth ecdysis range is substantiated by two molting individuals measuring 553 and 561 microns in length. The fifth ecdysis range is substantiated by one individual whose length at emergence from the old exoskeleton was 622 microns. The last ecdysis point is substantiated by a similar individual measuring 653 microns in length.

A progressive increase in the number of individuals making up each of the size classes is shown in Figure 1. It is suggested that this is merely a function of duration spent by the tardigrades in each size class; the first instar is of rather short duration while the second through the fifth instars show progressively longer durations. The sixth instar as well as the seventh is indicated by very few individuals; possibly this is due to an increase in mortality after the fifth instar or possibly is some other expression of the population structure at the time of collection.

**Buccal Apparatus Length-Frequency**

The curve resulting from plotting length of buccal apparatus against number of individuals (Fig. 2) similarly
Figure 2. Buccal apparatus length plotted against number of individuals of Macrobiotus islandicus Richters. See legend of Fig. 1 for additional data.
shows six probable ecdysis points. Six hundred eighteen individuals were used in making this graph; 182 of the original 800 had poorly defined buccal apparatus and could not be considered.

The first probable ecdysis point is indicated between 58-63 microns, the second between 74-85 microns, the third between 90-101 microns, the fourth between 104-111 microns, the fifth between 112-119 microns, and the sixth between 120-125 microns. Seven out of the 11 tardigrades positively shown to be in ecdysis at the moment of their death possessed buccal apparatus which could be accurately measured. The measurements of these buccal apparatus correlated significantly with the suggested ecdyses ranges of the curves and thereby substantiate these suggested positions.

**Correlation Between Buccal Apparatus Length and Body Length**

Assuming that the buccal apparatus length increases proportionately with the increase in body length after an ecdysis, a positive correlation between these two measurements should exist. This can be shown graphically by plotting body length against length of buccal apparatus as in Figure 3; the diagonal pattern ascending upward to the right side of this graph indicates a very high coefficient of correlation between the two lengths. Accordingly, it is suggested that either buccal apparatus length or body length may be used as an indicator of ecdysis when plotted against the total number of individuals.
Figure 3. Body length plotted against buccal apparatus length to illustrate the high correlation between these two measurements. This graph contains data for 618 *Macrobiotus islandicus* Richters.
Ideas concerning tardigrade classification have vacillated considerably during the last century, and there is little agreement among present investigators. The present author considers them as a phylum which probably arose as an isolated group from annelid stock which also gave rise to the primitive arthropod groups. Many authors consider the tardigrades as a class of the Arthropoda (Marcus, 1936), although the tardigrades have an enterocoel while the arthropods possess a schizocoel. Others have included the tardigrades with the arachnid groups (Borradaile et al., 1958). Occasionally one finds the tardigrades placed in the Onychophora (Barros, 1942). The tardigrades have been compared with almost every group of invertebrates from the nematodes through the arthropods, and, to be sure, the tardigrades have certain characteristics in common with each of such groups, but the more recent views concerning their affinities place them in a separate phylum (Pearse, 1949).

On the basis of the present investigation, it is suggested that the tardigrades should not be included with the arachnids. One reason is the fact that there are typically four ecdyses in the latter group. Furthermore, egg production in Macrobiotus islandicus was found to commence with the third instar (the smallest individual showing egg development was 419 microns in length), whereas eggs are developed only during the final instar of the arachnids.
NOTES ON OTHER COLORADO SPECIES

Mosses with a rather dense growth form yielded the greatest number of tardigrades in the Boulder Canyon area. These mosses were collected by removing the entire plants from their substrate, for during dry conditions the tardigrades may often be concentrated in the more basal regions of the plants where there is higher humidity.

The tardigrades are easily removed from moss merely by immersing the moss in a beaker of water and agitating it intermittently until samples of the washings taken from the bottom of the beaker show the active or perhaps asphyxiated tardigrades. Eggs will similarly show up in the washings and commonly appear as tiny pearl-like spheres among the debris. The eggs may occur singly or in groups of from two to five; sometimes they are found in the transparent discarded exoskeleton of the tardigrade which produced them.

The author has considered it important to include figures of the tardigrade species encountered in the present study because of the general unavailability of such figures in American literature. Pertinent notes are included in the brief discussion of each species found in Colorado.
Macrobiotus islandicus Richters (Fig. 4).

Nine hundred sixty-one specimens were collected from Tortula ruralis (Hedw.) Smith. This is the first record of this species for North America. All specimens were yellow in color and varied from 221 to 710 microns in length. Sixty-two eggs (Fig. 4c) were found free in the moss washings. These eggs had a diameter of 75 to 100 microns; most of the hatched eggs were between 90 to 95 microns in diameter. The processes measured between 9 to 11 microns in length. Indications of developing eggs were found in individuals whose body length was from 419 to 710 microns.

The identification of this species is primarily based upon the claws of the Macrobilotus hufelandi-type, the slightly punctate cuticle, the two macroplacoids, and the processes of the eggs.

Macrobiotus hufelandi S. Schultze (Fig. 5).

This very common species has been previously reported from both the United States and Canada. The author collected 93 specimens from Dicranum sp. All specimens appeared to be banded with white pigmented areas. Fourteen eggs (Fig. 5c) were found free in the debris. All eggs from this particular population match that of type 1 (Cuénot, 1932) in that the number of projections around the periphery varied from 21 to 26. Other samples taken recently from Bluebell Canyon, southwest of Boulder, Colorado, showed 38 to 48 processes around the periphery (ten eggs); the former samples were taken on 18 March 1957 (same locality.
Figure 4. *Macrobiotus islandicus* Richters. A, lateral view of adult; B, ventral view of adult; C, egg; D, first instar emerging from egg.
Figure 2. Ventral view of anterior arm of ascidians. A. C. orei; B. ventral view of posterior arm of ascidians. A. B. and C. ventral view (of same species).
Figure 5. *Macrobiotus hufelandi* S. Schultze. A, lateral view of adult; B, ventral view of adult; C, egg; D, ventral view of buccal apparatus. (A, B, and C to same scale)
as *M. islandicus*), while the latter samples were collected on 20 February 1958. This difference in egg type has been also noted recently by Petersen (1951). The specimens collected in Boulder Canyon varied from 227 to 588 microns in body length.

**Macrobiotus harmsworthi** J. Murray (Fig. 6).

In North America, this species has been previously reported only from British Columbia. It is quite similar to *Macrobiotus hufelandii*, and other than minor differences in the macroplacoids, it must be identified on the basis of the projections of the egg (Fig. 6C). Twenty-six specimens were collected along with seven specimens of *Milnesium tardigradum* Doyère and 92 specimens of *Echiniscus granulatus* Doyère. These were all found in five grams (dry weight) of *Grimea calyptrata* Hook collected 21 March 1957, north ridge of Boulder Canyon, 7 miles west of Boulder, Colorado. Five eggs were found in this sample. The length range for the individuals found was 250 to 700 microns.

**Hypsibius tuberculatus** (Plate) (Fig. 7).

In North America, this species has been previously reported only from British Columbia. It is a very distinctive tardigrade having ten transverse rows of bosses, the first and tenth with an odd number (5) of them. Five specimens were collected in Boulder Canyon (same locality as *Macrobiotus islandicus*) by the present author.
Figure 6. *Macrobiotus harmsworthi* J. Murray. A, lateral view of adult; B, ventral view of adult; C, egg; D, ventral view of buccal apparatus. (A, B, and C to same scale.)
Figure 7. *Hypsibius tuberculatus* (Plate). A, lateral view of adult; B, dorsal view of adult.
In North America, this species has been previously reported only from Canada, but here specimens were collected in the present study (same locality as in Euplatus setigerus). The south american representatives appear to be distinct as well.

This species and others were collected from elephant dung (same locality as in Euplatus setigerus). Indistinctly separated from the cardinal, these specimens are distinguished by the presence of six additional papillae that are pear-shaped and secondarily branched. Joker's work to isolate and study these specimens was invaluable to this study.
Hypsibius scoticus (J. Murray) (Fig. 8).

In North America, this species has been previously reported only from Canada, but four specimens were collected in the present study (same locality as M. islandicus). Colorado specimens had a dull brownish-gray color. The mouth appears subterminal; three macroplacoids were barely distinguishable.

Hypsibius granulifer (Thulin) (Fig. 9).

This species is new to North America. Six individuals were collected from four grams (dry weight) of Grimea calypttrata along with four specimens of Milnesium tardigradum (same locality as M. harmsworthi). In this species the secondary branch of the outer (posterior) claw (Fig. 8C) forms a right angle with the common base of the claw. The back of the animal shows minute wart-like papillae. Three macroplacoids are present although the first two may be indistinctly separated.

Milnesium tardigradum Doyère (Fig. 10).

This species, probably the most distinctive of all tardigrades in its appearance, has been previously reported from both the United States and Canada. It is easily distinguished by its "shrew-like" appearance and the presence of six sensory papillae around the mouth with two additional papillae slightly posterior. The sucking pharynx is pear-shaped and contains no placoids. Twenty-nine specimens were collected, and all contained a light
Figure 8. *Hypsibius scoticus* (J. Murray). A, lateral view of adult; B, ventral view of adult; C, ventral view of claw I; D, ventral view of claw IV. (A and B to same scale, C and D to same scale.)
Figure 9. *Hypsiobius granulifer* (Thulin). A, lateral view of adult; B, ventral view of adult; C, lateral view of claw IV; D, lateral view of buccal apparatus.
Figure 10. Milnesium tardigradum Doyère. A, lateral view of adult; B, ventral view of adult.
reddish-brown pigment. They were reasonably common but never very abundant in a given moss sample.

**Echiniscus granulatus** (Doyère) (Fig. 11).

This species has never before been reported from North America. In Boulder Canyon it was very common, and at least a few individuals were found in almost every moss sample. It is a bright orange tardigrade, and commonly the gut is a bright green. The orange color seems to be a result of a red pigment located in the central portion of each polygonal cuticular emargination and orange colored droplets which seem to be an oily substance within the body cavity. The present author found that the most satisfactory method of mounting this tardigrade is in Hoyer's mounting medium which has a greater clearing action than glycerin. An important feature in the identification of this species is the presence of one to three thorns on the base of the outer hooks of the third and fourth pairs of legs.
Figure 11. *Echiniscus granulatus* (Doyère). A, lateral view of adult; B, dorsal view of adult.
The following list includes and five species of Brandt's marine species and the three marine species of Bacillus. These species include Bacillus species (A).
TARDIGRADES REPORTED FROM THE UNITED STATES

The following list is compiled from the papers of Marcus (1936, 1946), Mathews (1938), Curtin (1948), Chitwood (1951), and Whittaker and Fairbanks (1958). It includes three marine species, eleven freshwater or "terrestrial" species, and two species which are somewhat questionable (Species Incertae Sedis).

- *Batillipes mirus* Richters
- *Batillipes pennakii* Marcus
- *Bathyechiniscus tetronyx* Steiner
- *Echiniscus arctomys* Ehrenberg
- *Echiniscus blumi* Richters
- *Pseudoechiniscus suicillus* (Ehrenberg)
- *Macrobiotus hufelandi* S. Schultze
- *Hypsibius canadensis* (J. Murray)
- *Hypsibius convergens* Urbanwicz
- *Hypsibius dujardini* Doyère
- *Hypsibius oberhaeuseri* (Doyère)
- *Hypsibius prosostomus* (Thulin)
- *Hypsibius shaudinni* (Richters)
- *Milnesium tardigradum* Doyère

Species Incertae Sedis:
- *Hypsibius americanus* (Packard)
- *Hypsibius augusti* (J. Murray)
The following species, reported in this paper, are additions to the tardigrade fauna of the United States:

**Echiniscus granulatus** (Doyère) (new to North America)

**Macrobiotus harmsworthi** J. Murray

**Macrobiotus islandicus** Richters (new to North America)

**Hypsibius granulifer** (Thulin) (new to North America)

**Hypsibius scoticus** (J. Murray)

**Hypsibius tuberculatus** (Plate)
SUMMARY

Fifteen grams (dry weight) of Tortula ruralis (Hedw.) Smith were collected from a north-facing slope in Boulder Canyon 17 miles west of Boulder, Colorado. Washings from this moss yielded 1,131 tardigrades; of these, 800 Macrobiotus islandicus Richters were suitably mounted in an extended position (asphyxy).

Measurements of body lengths in microns were plotted against the number of individuals. This length-frequency curve showed a series of high and low points. Since tardigrades shed their exoskeleton periodically, in a typical arthropod fashion, each peak corresponds to an instar and each depression to an ecdysis. Six probable ecdyses and seven instars were indicated.

A second length-frequency curve was similarly constructed using the buccal apparatus lengths. Six probable ecdyses were likewise indicated by this curve.

Eleven individuals were in the process of molting at the moment they were killed; their lengths correlated precisely with the suggested points of ecdyses in the curve.

A high correlation coefficient exists between body length and buccal apparatus length; either of these two measurements can therefore be used in determining the number of ecdyses by a length-frequency curve.
Eggs are developed beginning with the third instar and are found thereafter in some individuals of subsequent instars.

The phylogenetic affinities of the tardigrades are discussed. Based on the present study, it is suggested that the tardigrades constitute a distinct phylum (Tardigrada) and should not be considered as part of the arachnid group.

The eight species of tardigrades found in the vicinity of Boulder, Colorado, include: Macrobiotus islandicus Richters (new to North America), M. hufelandi S. Schultze, M. hamsworthi J. Murray (new to the United States), Hypsibius tuberculatus (Plate) (new to the United States), H. scoticus (J. Murray) (new to the United States), H. granulifer (Thulin) (new to North America), Milnesium tardigradum Doyère, and Echiniscus granulatus (Doyere) (new to North America).


Marcus, R. 1929. Tardigraden i Öroningen des Floraenska Museet Lund. 1-60.


LITERATURE CITED


