Proceedings of the Iowa Academy of Science

Volume 76 | Annual Issue Article 55

1969

Fauna of Testate Amoebae from the Rhytidome of Juglans Nigra

Ronald E. Goddard
University of Kansas

Paul A. Meglitsch
Drake University

Fauna of Testate Amoebae from the Rhytidome of *Juglans Nigra*[1]

RONALD E. GODDARD[2]

AND

PAUL A. MEGLITSCH[3]

Abstract. Samples of bark without visible lichen or moss cover were taken from a *Juglans nigra* located in a city lot in Des Moines, Iowa, remote from water. *Euglypha ciliata, E. cuspidata, Microchlamys patella, Centropyxis aerophilus,* and two unidentified species of *Difflugia* were found, ranging from ground level to heights of 35 feet. An estimate of population density, based on a modification of the most probable number count, showed evidence of zonation on the tree surface, with *C. aerophilus* restricted to near ground level, *M. patella* occurring approximately evenly up to 35 feet, and *E. cuspidata* most abundant at moderate heights. Some of the factors related to distribution on the tree surface are discussed.

Recent studies of the microcommunity of tardigrades, nematodes, rotifers, and protozoa associated with tree lichens in Iowa led to the discovery that all but the tardigrades occur also on naked bark. This study was undertaken to describe the fauna of testate amoebae present on the rhytidome of one tree, a *Juglans nigra* located in a city lot in Des Moines, and to describe their distribution on the surface of the tree.

MATERIALS AND METHODS

Pilot studies were carried out on bark samples taken in July and September, 1967. The data presented here are based on samples collected March 24, 1968. Samples were taken at 150 cm (5 feet) intervals, using a scalpel sterilized in 95% alcohol, and were kept in plastic bags until cultured. Qualitative samples were hydrated by swirling in 5 ml of sterile water and observed periodically for 4-5 weeks. Quantitative samples were taken with a sterilized No. 2 cork borer (3); the inner part of the bark was removed, leaving a thin wafer of rhytidome. Five whole and five quarter borings were swirled and hydrated in 5 ml of sterilized water to prepare 10 cultures from each level. The cultures were followed for 4-5 weeks, during which six observations were made for each culture at each level.

An estimate of population density was made, using a modification of McCrady's (7) equation for the calculation of the most probable number. The equation used was:

[1] Contributions from the Biology Department of Drake University, No. 30.

[2] Universiy of Kansas, Lawrence, Kans.

[3] Drake University, Des Moines, Iowa.

$$1-\left(\frac{A-B}{A}\right)^x = \frac{P \text{ or } p}{p+q} \quad 1-\left(\frac{A-B}{A}\right)^x + 1-\left(\frac{A-B}{A}\right)^x = \frac{p}{p+q} + \frac{r}{r+s}$$

where: A = 1000 mm²
B = area of a single whole boring (95mm²)
B' = area of a quarter boring (23¼mm²)
x = the most probable number of organisms per 1000 mm²
p = number of positive cultures (organism present) for whole borings
q = number of negative cultures for whole borings
r = number of positive cultures (organisms present) for quarter borings
s = number of negative cultures for quarter borings

Calculations were made with the aid of a computer.

Temporary mounts were made with sucrose and permanent mounts with polyvinyl alcohol (5), with good results, although the membranes of *Microchlamys patella* seemed slightly distorted by PVA. Squash preparations were used to study shell details (1).

Cultures using rice and barley, with or without an agar base, and inoculated with *Aerobacter aerogenes* were used with indifferent success. Tree bark cultures maintained a reasonable population of Testacea for as long as 5-6 weeks before encystment of the population terminated the cultures.

Measurements were made with an ocular micrometer in phase or bright field illumination, using the 45x objective, except for *Difflugia,* which was measured with a 20x objective.

The Testacean Fauna

The following species were encountered.

Euglypha ciliata (Ehrenberg) Leidy

Dimensions: (N=3) length 64 μ; diameter 50 μ; thickness 47 μ; spine length 7μ.

Distribution: not common; found only at the 10 foot level on north side.

Euglypha cuspidata Bonnet

Dimensions: (N=35) length 33-47 μ (43μ); diameter 12-27 μ.

Distribution: found on north, west and south sides at heights from 5-20 feet.

Microchlamys patella (Cockerell) Claparede and Lachmann

Dimensions: (N=35) diameter 38-52 μ (44 μ); pylome diameter ca 11 μ.

Distribution: the most prevalent species; found on all four sides of tree and from ground level to 35 feet.

Difflugia sp. A.

Dimensions: (N=25) diameter 36 μ; pylome diameter 18 μ (based on 13 measurements).

Distribution: north side; 10 foot level.

Difflugia sp. B.

Distribution: It was found at ground level, 5 and 20 foot levels on the south side.

Remarks: This second species of *Difflugia* was seen only during the quantitative studies. It differed from species A in that it was larger.

Centropyxis aerophilus Deflandre

Dimensions (N=11): length 50-70 μ (61 μ); diameter 40-61 μ (52 μ); pylome length and width ca 19 μ.

Distribution: ground level on south side and 5 foot level on north.

Remarks: the pylome varied from circular to elliptical, and test size was quite variable; pseudopodia reach up to 100 μ.

Population Densities

Sample for quantitative work were taken from the south side of the tree only. Only four species appeared in the samples studied quantitatively. The results, rounded to the nearest whole number, are given in Table 1.

Table 1. Most Probable Numbers per 100 cm^2 of Testate Amoebae on the South Side of *Juglans nigra* at Various Heights.

Species	Height (ft.)						
	0	5	10	15	20	30	35
Centropyxis aerophilus	12	<2	<2	<2	<2	<2	<2
Euglypha cuspidata	<2	<2	<2	12	4	<2	<2
Microchlamys patella	27	27	16	5	21	7	14
Difflugia (sp. B)	2	2	<2	<2	2	<2	<2

The attempt to estimate the population density must be considered a preliminary effort. Several repetitions are needed for statistical reliability but the system used has been tested and found to be usable.

Pseudopodial formation or pulsating contractile vacuoles were observed in every instance for *Microchlamys* and *Euglypha;* population densities are undoubtedly more accurate for these organisms.

Unoccupied tests are found in the greatest number at the lower levels of moss, while active individuals tend to migrate upward (4). For *Centropyxis* and *Difflugia,* therefore, the lower levels may be reported as fallaciously high.

In any case, there is evidence that the testate amoebae may show some stratification, and that different species are distributed differently. *Microchlamys* is found in reasonably high numbers from ground level to 25 feet. *Euglypha cuspidata* appears to be more abundant at moderate heights, and *Centropyxis aerophilus* appears to be abundant near ground level.

Discussion

Testate ameobae have been found in a wide spectrum of habitats, and many investigators have reported them in semi-terrestrial habitats, generally in moist soil or associated with mosses. Deflandre (2) and Schonborn (8a,b,c,9) have demonstrated stratification of species (from aquatic to bog to a semi-terrestrial habitat in moist and often peaty soil). They have been found in mosses on the bark of trees bordering on lakes.

Past investigations appear to have ended with the arboreal mosses. Leidy (6) and Thompson (10) may have observed testate amoebae on tree bark, but their reports do not clearly establish this. It is, therefore, a real surprise to find consistently a testacean population distributed on the surface of a tree placed well away from water, and in areas without visible lichen or moss covering. Unidentified naked amoebae, ciliates, and flagellates have also been seen in samples of bark taken at considerable heights, and without a lichen or moss cover.

The characteristics of the testacea seen tend to confirm the tendencies toward morphological variations associated with zonation in soil and mosses. Upper (and dryer) habitats tend to be associated with hemispherical or subhemispherical tests, shortened in the fundus-pylome axis or flattended laterally; with a cryto- or plagiostomatic pylome, and with a loss or reduction of spines. *Microchlamys patella* has a very short fundus-pylome axis and the species of *Difflugia* observed were relatively flat. The tests of *Euglypha cuspidata* and *E. ciliata* are laterally compressed, and *Centropyxis aerophila* is flattened near the pylome. The spines of *E. ciliata* are reduced, and *Centropyxis aerophila* rarely had spines. Pylomes were approximately circular for *Microchlamys* and the two species of *Euglypha,* as well as in *Difflugia,* and were variable in *Centropyxis.* Testacean species are known to be variable for many of the test traits, and such morphological gradients as have been described make the problem of species diagnosis a difficult one. A critical analysis of morphological traits, and especially those related to the

test, is badly needed.

Quantitative data suggest that some zonation of testacea on tree bark occurs. Zonation of soil and moss forms have been linked with moisture, the quantity and quality of available test material, climatic factors, and pH. While microclimates on the tree surface may vary with northern or southern exposures, and with height, they may also be affected severely by shading in seasons when leaves are present, and runoff patterns resulting from branch and trunk configuration. Moisture-holding capacity of the bark may vary markedly at different heights, and chemical factors may also vary considerably with bark qualities at different heights. The peculiarities of tree surfaces as substrates for animal life have not been characterized, but it seems evident that a new field of habitat analysis is provided by the invertebrate fauna distributed on naked bark.

In any case, the habitat provided at the tree surface is evidently one of considerable stress. The severity of the bark habitat is revealed by population densities. Testacean population densities in sphagnum moss reached 166,300 individuals/cm^3, while the highest density recorded at the surface of *Juglans nigra* was 1 *Microchlamys*/ 3.7 cm^2. The biological stresses of the habitat may provide interesting research potentialities in the future.

Literature Cited

1. Brown, F. A. 1950. Selected Invertebrate Types. New York: John Wiley & Sons Inc., pp. 23-25.
2. Deflandre, Georges. 1928. "Le genre Arcella Ehrenberg." Arch. f. Protistenk 64:152-285.
3. Furniss, M. M. 1962. A circular Punch for Cutting Samples of Bark Infested with Beetles. Canada Entomol. 94-959-64.
4. Heal, O. W. 1962. "The Abundance and Micro-Distribution of Testate Amoebae (Rhizopoda: Testacea) in Sphagnum." Oikos 13: 35-47.
5. Hoffman, Glen L. 1954. "Polyvinyl Alcohol Fixative Adhesive for Small Helminths and Protozoa." Trans. Amer. Microsc. Soc. 73:328.
6. Leidy, Joseph. 1879. Freshwater Rhizopods of North America. Washington, D.C.: Government Printing Office, p. 8.
7. McCrady, M. H. 1915. "The Numerical Interpretation of Fermentation Tube Results." J. Infect. Dis. 17: 183-212.
8. Schonborn, Wilfried. 1962. "Die Okologie der Testaceen im oligotrophen See, dargestellt am Beispiel des Grossen Stechlinsees. Limnologica 1:1101--83.
9. _____. 1962. "Neue Testaceen aus dem Grossen Stechlinsee und dessen Umgebung." Limnologica 1:82-91.
10. _____. 1962. "Zur Okologie der sphagnikolen, bryokolen und terrikolen Testaceen." Limnologica 1:231-253.
11. _____. 1963. "Die Stratigraphie lebender Testaceen im Sphagnetum der Hochmoore." Limnologica 1:315-321.
12. Thompson, Jesse C. 1960. "Ciliated Protozoa from Tree Borne Mosses and Lichens." (Abstract). J. Protozool. 7:17.