NOTES ON THE LABYRINTHULAN PARASITE OF THE EEL-GRASS *ZOSTERA MARINA*

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The present series of investigations was carried on at Salsbury Cove, Maine, on the grass of that region and at Harvard on grass from Plymouth Harbor, Massachusetts. The objects were first to determine the specific rank of the Labyrinthulan parasite of the eel-grass, *Zostera marina,* and second to study more fully certain of its features. During the past seven years the wasting disease of the eel-grass has caused much interest on both sides of the Atlantic, but it was not until 1934 that Dr. Renn established one of the *Labyrinthulae* as the causative agent. This little known group of organisms is of doubtful phylogenetic relationship, showing affinities both for the *Proteomyxa* and for the *Mycetozoa.*

The method of study has been largely microscopic examination of the parasite in hanging drop cultures after it had migrated out of the infected host tissue. This was reinforced by staining, microchemical tests, and micromanipulation, and in addition a series of tests to determine host range was carried out.

In a hanging drop culture the organization of the parasite is seen to be that of a large number of filaments formed into an intricate lacey network on which the cell bodies in the shape of fusiform spindles migrate, either as separate individuals or as individuals massed in thick rope-like pseudoplasmodia. This complex of tracks and cell bodies grows radially from the point of emergence from the host. At the periphery or advance fringe of the track network the spindles, as the cell bodies are called, are massed in clumps prior to the formation of more filaments for subsequent advance. No matter how densely massed the spindles may be at any time, however, they never lose their discrete individuality

The spindles vary in shape from long fusiform to short ovoid and range in size from 8.5 to 30 microns in length by 1.5 to 7 microns in diameter with an average of about 4 by 18 microns. They may be actively motile or quiescent. In the active state the anterior tip is slightly differentiated, being more tenuous and less easily distinguishable from the track than is the well-rounded distinct posterior tip. In the quiescent cell both poles are clearly marked. The spindles are very plastic, being curved or constricted to conform to bends in the track or to external impingements. It is difficult to detect any more definite cell membrane than that possessed by an amoeba. The color varies from gray to green and the whole cell has a foamy appearance due to minute granulation. Often a dozen or so prominent larger greenish granules are seen. These seem to be associated with nutrition. Vacuoles of a contractile nature are occasionally observed and also seem to be associated with nutrition. There is always a central circular nucleus with a darker nucleolus, but both are of varying distinctness.

As yet little is known about the production and structure of the tracks. They are obviously an excretion product of the spindle, but the mechanism and the locus in the cell of production is unknown. At the advance fringe of a culture a spindle is often seen to shoot out a section of track as much as six to eight times its own length. This section appears to originate at the forward tip and is seen to gyrate. When two such sections meet they fuse and become apparently quite stable allowing subsequent advance of the spindles. The track complex has been regarded as an inert rigid structure, but by micromanipulation, I have shown it to be quite elastic and somewhat glutinous. There is some evidence that the track may be finely fibrillar. It does not dissolve in alcohol, nor test for fats or starch and is not stained with neutral red, methyl blue, carmine, thiorin, or tannic acid.

Volkanov believed that the track was an infinitely elastic hollow tube in which the spindles migrated, but this has proved erroneous. The spindles are on the outside of the track, held to it by some kind of secreted adhesive. Sometimes this adhesive weakens allowing the spindle to swing free of the track at one tip and sway around with the other tip as a point of attachment. The motion of the spindle is usually a graceful effortless gliding like a drop of glycerine flowing down a taut silk thread, but occasionally advance may be made by irregular short jerks. The mechanism of motion appears to be like that of a snail gliding on its secreted slime. The rate of motion varies from an almost imperceptible advance up to some 150 microns a minute. The rate of the different spindles varies so that it is not uncommon for one to overtake another and pass it without either appearing to leave the track. There is on the whole a peripheral migration, but a spindle may start out peripherally, switch to a side track and return to the starting point several times before reaching its goal.

The life cycle is still incompletely worked out. In the previously described vegetative stage there is a rapid increase in numbers by fission. This is of two kinds. In the simpler type a fragmentation of the cell by partitions perpendicular to the long axis occur. This is a more or less degenerative fission. The more complex type occurs in the active culture and consists of a partition at first median and perpendicular to the long axis of the spindle and later shifting till it is almost parallel to the long axis at which time the two daughter cells may glide apart. This process consumes about two hours, during the earlier part of which the nuclear changes are obscured.

At the close of the vegetative phase one of three things occurs. The culture may disintegrate and die; the spindles may all mass together in one huge glutinous pseudoplasmodial clump; or the spindles may round up either individually or in small groups and encyst. The pseudoplasmodial clumps, in which the cells are smaller but still discrete individuals migrate around apparently carrying on phagocytosis for twenty-four to seventy-two hours before dying wholly or in part leaving a few cysts. The regeneration of a new vegetative phase has not yet been observed.

I have carried on two experiments with this parasite, one to study its host range and the other to study the effect of saline concentration upon it.

In the former, twenty marine algae collected at random were tested with the results noted in the chart. I want to acknowledge here the kind assistance of Miss Margery Poole of Radcliffe in identifying these algae. All the algae were subjected to the same experimental conditions.

HOST RANGE OF THE LABYRINTHULA PARASITIC ON ZOSTERA MARINA

Class	*Successful Inoculations*		*Unsuccessful Inoculations*	
	Genus	species	Genus	species
Chlorophyceae	Cladophora	hirta	Cladophora	gracilis
	Chaetomorpha	linum	Chaetomorpha	cannabina
	Chaetomorpha	melagonium		
			Enteromorpha	intestudinalis
			Enteromorpha	compressa
			Monostroma	undatum var. Farlowii
			Ulva	latissima
Phaeophyceae	Fucus	furcatus	Fucus	vesiculosus
	Ectocarpus	confervoides		
			Ascophyllum	nodosum
			Chorda	filum
			Alaria	filum
			Laminaria	longicrurus
Rhodophyceae	Cystoclonium	purpurescens	Chondrus	crispus
			Rhodymenia	palmata
			Polysiphonia	fastigiata

In addition I ran across two new hosts in nature: *Ruppia maritima* var. *rostrata*, and *Zannichellia palustris* var. *major*—the former from Mount Desert Island, Maine and the latter from Plymouth Harbor, Massachusetts. These two grasses belong in the family *Naidaceae* along with *Zostera*. It should be emphasized here that the parasites from both natural and induced hosts appear in all respects quite similar to those normally found on *Zostera*.

In the second experiment I was able to grow the parasite in viable condition not only in normal sea water but in all concentrations from pure distilled water to salt water of double the normal salinity. In 25% and 200% sea water, however, the tracks were but variably present, while in pure distilled water the cell bodies alone appeared without any track system at all.

Thus, while the specific rank of the present *Labyrinthula* is not definitely established, several points of contention concerning this genus have been cleared up; and from all indications, the final analysis of the situation will lead us to lump most at least of the seven recorded species of *Labyrinthula* under the original name of Cienkowski—*L. macrocystis*.