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SELECTIVE PREDATION OF *THECAMOEBA SPHAERONUCLEOLUS* (GREEFF, 1891) ON FILAMENTOUS ALGAE IN NATURAL CONDITIONS

ELOY BECARES* AND SUSANA ROMO[1]

Departamento de Ecologia, Facultad de Biologia, Universidad de León, 24071-León, España
[1]*Area de Ecologia, Departamento de Microbiologia, Facultad de Biologia, Campus de Ciencias de Burjasot, 46100-Burjasot, Valencia, España*

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Predation of algae by amoebae has been commonly reported in laboratory, but scarcely studied in natural ecosystems. Feeding selectivity and behavior of *Thecamoeba sphaeronucleolus* was studied from samples taken from a small eutrophic pond. *Mougeotia* sp. (77%) and *Phormidium autumnale* (12%) were the dominant algal species. *P. autumnale* was the most extensively predated algae by *T. sphaeronucleolus*, although both major algal species were similar in size. Cyanobacteria cell-wall structure and trichome flexibility, together with the capacity for gliding movement seem important to explain this selective predation. The two specific mechanisms for filaments ingestion by this amoeba are described.

In recent years there has been an upsurge interest of limnologist on cyanobacteria, due to their environmental importance in the increasing number of eutrophic freshwaters (*2*). Bacteria, viruses (cyanophages), fungi (e.g. chytrids) and protozoa are the main antagonist of cyanobacteria in high polluted water-bodies (*25*). However, amoebae and myxococci are considered to have the highest potential as control agents of this algal group (*6*). Reports of amoebae predation on cyanobacteria have mainly concerned to laboratory investigations with cultures (*13, 18, 26–29*), while studies based on natural communities are uncommon (*20, 23*). This is specially true for soil habitats and very shallow freshwaters, where however the overall amount of amoebae and cyanobacteria (mainly filamentous species) are usually high (*8*). Although *Thecamoeba sphaeronucleolus* Greeff (Lobosea, Amo-

* Address reprint requests to: Dr. Eloy Becares, Departamento de Ecología, Facultad de Biologia, Universidad de León, 24071-León, España.

ebida) is a species frequently found in soil and in sediment of freshwaters (*14*), it has been scarcely studied (*10,11,14,24*).

The present work deals with the selective predation by natural populations of *T. sphaeronucleolus* on the filamentous cyanobacterium, *Phormidium autumnale* (Agardh) Gomont. This will be also the first report of predation of this amoeba on this species.

MATERIALS AND METHODS

Live water samples were collected from the surface of a small eutrophic pond, located in an agricultural area of the province of Palencia (Spain). The pond is almost circular with a length of 10 m, a surface of 78 m^2 and a mean depth of 0.25 m.

The dominant amoeba was identified as *Thecamoeba sphaeronucleolus* according to Houssay and Prenant (*14*), Grospietsch (*9*) and Page (*19*). About 110 individuals were counted, and the content of the digestive vacuoles was identified and measured, the measuring precision being 1 μm.

The algal composition of the pond was determined according to specific criteria for the different groups (*3,7,15,17*), and following the recent taxonomic classification for cyanobacteria (*1*). Species were counted using Utermöhl's technique (*22*). At least 50 individuals of the most abundant species were measured, the measuring precision being 0.8 μm. The standard deviations of all measurements were used to calculate 95% percentage of significance (*21*).

RESULTS

The main phytoplankton species identified in the study site are shown in Table 1. *Mougeotia* sp. and *Phormidium autumnale* were the predominant algae, amounting to 77% and 12% of the total phytoplankton abundance respectively. Populations of the chlorophyte, *Mougeotia* sp., had longer filaments than those of *P. autumnale* (Table 2), although the diameter of both algae were similar (Fig. 1A).

From 110 individuals of *Thecamoeba sphaeronucleolus* observed, 36% showed

Table 1. Main algal composition of the study eutrophic pond.

Phytoplankton composition	Abundance (%)
Mougeotia sp.	77
Phormidium autumnale (Ag.) Gom.	12
Planktolyngbya sp.	7
Chroococcus sp.	3
Scenedesmus quadricauda (Turp.) Bréb. sensu Chod.	1
Scenedesmus acutus Meyen	0.7
Oedogonium sp.	0.4
Ulothrix sp.	0.1

Table 2. Percentage of predation and morphometric features of the ingested algae by *Thecamoeba sphaeronucleolus* (Greeff).

Phytoplankton composition	Filament length (μm)				Predation (%)	Filament length into the digestive vacuoles (μm)			
	Mean	Min.	Max.	Std.		Mean	Min.	Max.	Std.
Mougeotia sp.	157	60	400	72	25	28	15	38	9
Phormidium autumnale (Ag.) Gom.	88	20	700	63	65	87	30	700	145

algal rest into the digestive vacuoles. Although *Mougeotia* sp. was the most abundant algae, it was the second important prey for *T. sphaeronucleolus*, occurring into 25% of the digestive vacuoles (Table 2). The highest predation took place on *P. autumnale*, which was identified inside 65% of the amoeba individuals. It was observed that *T. sphaeronucleolus* was able to recognize *P. autumnale* between the tangle of algal filaments, where the amoeba was normally moved over (Fig. 1B). Other algae, specially *Oedogonium* sp. and *Chroococcus* sp., were the remaining 10% of food source for this amoeba.

Mechanisms of predation

T. sphaeronucleolus showed two mechanisms of predation depending of the algal prey features.

T. sphaeronucleolus fed only on short filaments of *Mougeotia* sp., the maximum filament size ingested of this chlorophyte was of 38 μm for a mean amoeba size of 105 μm (Table 2). There was not observed a selection a priori of the suitable filaments length of this species. Thus, the amoeba would start the ingestion of a strain too long for its size, which finally must be released due to *Mougeotia* sp. could not be coiled inside the amoeba (Fig. 1C; Table 2). The attack and ingestion of *Mougeotia* sp. filaments always started from one of the apices (Fig. 1C). The amoeba sought and attached to the terminal end and gradually engulfed and ingested the segment (Fig. 1C). Finally, if the length of the filament was appropriated for the amoeba size, the trichome was completely included into a digestive vacuole.

Predation of *P. autumnale* by *T. sphaeronucleolus* was different for short or long filaments. Short filaments were phagocytosed similarly as the aforementioned *Mougeotia* sp. (Fig. 1D). However, feeding on long strains of *P. autumnale* started with the progressive engulfment of the trichome by the extension of a conical pseudopodium which surrounded the filament, so the attack could take place at any part of the trichome (Fig. 1E). Inside the amoeba, the ingested trichome was coiled by the twisting of the pseudopodial channel (PC in Fig. 1E). Plasmodesms between two adjacent pseudopodial coils were joined in order to accommodate the prey into an unique digestive vacuole and occupying the minimum volume (Fig. 1F, G).

The gliding movement of *P. autumnale* helped the ingestion along the complete

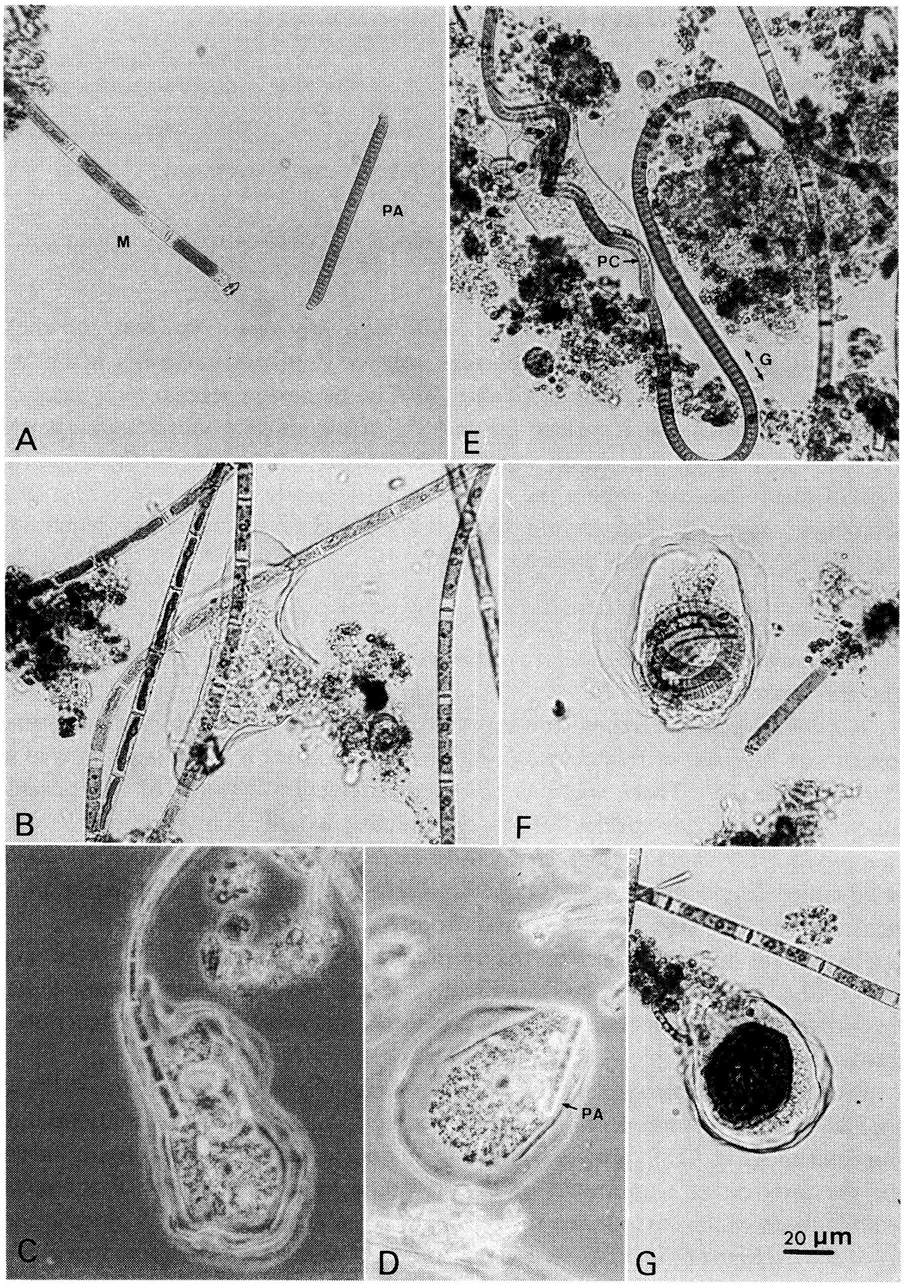

Fig. 1

process. The amoeba took advantage of the backward movement of the algae, by engulfing part of the trichome into the pseudopodial channel when the algae moved toward amoeba direction (G in Fig. 1E). The forward movement of the algae was progressively reduced because the ingested part of the trichome was gradually retained into the vacuole by the effect of the amoebal pseudopodia and the loss of motility at the coiled parts. However, in some few occasions long trichomes of *P. autumnale* could escape to the attack of *T. sphaeronucleolus*, when the engulfment started by the apex and the algae was actively moving.

By the mechanism described, *T. sphaeronucleolus* was able to ingest trichomes of *P. autumnale* approximately seven times its size (up 700 μm, Table 2).

DISCUSSION

In natural conditions, *T. sphaeronucleolus* moved over the tangle of algal filaments and showed to be rather selective for detecting and feeding on filaments of *P. autumnale.* This observation agrees with the general pattern observed in laboratory of marked selectivity of amoebae among the algae (see e.g. *13*), with a special trend to feed on cyanobacteria (*27*). However, biochemical and morphological basis for prey selection remain largely unresolved, although size and shape of the prey appear to be important features for feeding behavior of some amoebae (*12*, *13*). This seems the case of *T. sphaeronucleolus* which predation strategies in the present work seemed conditioned by filament size, motility, cell-wall structure and trichome flexibility of the algae. The amoeba often tried to feed on long filaments of *Mougeotia* sp., but cell-walls inflexibility of this algae probably prevented their predation. In this context, it has been reported the presence in some amoebae species of specific enzymes for the degradation of several cyanobacteria cell-walls (*5*, *16*), but little is known in relation of a similar action on green algae walls. Moreover, predation on *P. autumnale* could be favored by the absence in this algae

Fig. 1. (A) Dominant algae of the study eutrophic pond: M, *Mougeotia* sp. (Chlorophyte); PA, *Phormidium autumnale* (Agardh) Gomont (Cyanobacteria).

(B) Movement of *Thecamoeba sphaeronucleolus* Greeff over a filaments tangle of *Mougeotia* sp. The amoeba only ingest short filaments of this algae.

(C) Attack of *T. sphaeronucleolus* by the apex, on a long filament of *Mougeotia* sp. Due to the algae length, the amoeba finally desisted.

(D) Amoeba with a digestive vacuole containing a short phagocytosed filament (46 μm) of *P. autumnale.*

(E) Starting of the ingestion of a long trichome of *P. autumnale* (700 μm) by the extension of an conical pseudopodium (PC). The gliding movement (G) of the cyanobacterium helps to engulf the trichome (for details see text).

(F) A recent ingested trichome of *P. autumnale* (280 μm length) completely included in a digestive vacuole.

(G) Amoeba containing a trichome of *P. autumnale* in an advanced stage of digestion.

Bar scale is for all the photographs.

of thick sheaths, which can cause in *T. sphaeronucleolus* difficulties for trichomes ingestion (*10*). This could also explain the greater selectivity and intensity of predation observed by *T. sphaeronucleolus* on *P. autumnale* than in other studied cyanobacteria, such as *Oscillatoria* sp. (*10, 11*).

The two mechanisms of predation of *T. sphaeronucleolus* observed in the study under natural conditions, are similar in general to those described for cultures of this species (*10, 11*), and those found in other related species, such as *Thecamoeba verrucosa* (Ehr.) *sensu* Gläser (*4, 9*). However and contrary to Haberey's interpretation about this amoeba predation on *Oscillatoria* sp. (*10*), our results showed that the gliding movement of *P. autumnale* favored the ingestion by the amoeba. The causes for losing of motility at the ingested parts of the filament were probably due to degradation of the algal cell-wall and the effect of amoebal micropseudopodia inside the pseudopodial channel (*10, 11*).

The direct evidence of selective predation of *T. sphaeronucleolus* on *P. autumnale* suggests that maybe this amoeba could be one of the factors involved in the regulation of this algae populations in the study eutrophic pond.

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