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Original Article

Protists and other organisms on a minute snail periostracum

A. López de la Fuente[a*], *R. J. Urcuyo*[a] *and G. H. Vega*[b]

[a]Centro de Malacología, Universidad Centroamericana – UCA, Rotonda Rubén Darío 150mt Oeste, Managua, Nicaragua

[b]Estación Biológica Juan Roberto Zarruk, Universidad Centroamericana – UCA, Rotonda Rubén Darío 150 m Oeste, Managua, Nicaragua

*e-mail: alosi@uca.edu.ni

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(With 11 figures)

Abstract

Since the foundation of the Malacological Center in 1980, Universidad Centro Americana (UCA), Managua-Nicaragua, has been monitoring and collecting the marine, terrestrial, fluvial and lake mollusk population of the country. Many specimens have been photographed by Scanning Electronic Microscope (SEM), and in one of these, observation of the hairy periostracum reveals a seemingly thriving population of minute protists in possible symbiosis with their host. Adequate magnification and comparison with previous studies allowed the determination of these hosts as diatoms, testaceous amoebae, yeast, phacus, spores and other undetermined organisms which occur in tropical forests on rocks, trees and leaves. Here illustrated are diatoms and other organisms detected for the first time on the periostracum of a tropical rainforest mollusk.

Keywords: Nicaragua rainforest, minute snail, SEM electrograms, diatoms, protists.

Protistas e outros organismos em um pequeno periostracum de caracol

Resumo

Desde a fundação do Centro Malacológico em 1980, a Universidad Central Americana (UCA), Manágua-Nicarágua, vem acompanhando e coletando a população de moluscos marinhos, terrestres, fluviais e lagoas do país. Muitos espécimes foram fotografados pelo microscópio eletrônico de varredura (SEM) e, em um deles, a observação do periostracum peludo revela uma população aparentemente próspera de protistas de minuto em possível simbiose com o hospedeiro. A ampliação adequada e a comparação com estudos anteriores permitem a determinação dessas diatomáceas, amebas testaceas, leveduras, phacus, esporos e outros organismos indeterminados que ocorrem em florestas tropicais em rochas, árvores e folhas. Aqui estão as diatomeas e outros organismos pela primeira vez detectados no periostracum de um molusco tropical.

Palavras-chave: floresta tropical da Nicarágua, pequeno caracol, eletrogramas SEM, diatomáceas, protistas.

1. Introduction

The UCA University Malacological Center (UCACM) has been monitoring Nicaraguan malacology since 1981 with greater emphasis on the Matagalpa and Jinotega rainforest especially at Juan R. Zarruk Biological Station, in Santa Maura Hacienda managed by the university, which has shown exceptionally high diversity and abundance of species, including some of undetermined genus of which some are Protists.

Protists are among the most common components of plankton in water, benthos in aquatic habitats and edaphos on land, associated with illuminated and moisture milieus (Freckman et al., 1997; Palmer, 1997; Alekperov and Snegovaya, 1999; Audesirk et al., 2012); they participate in food webs (Bere and Tundisi, 2011;) and are used as indicators of climatic changes, eutrophication and acidification (Nascimento et al., 2010). They have been reported on rocks, trees, leaves, turtle carapace and inside the thallus of algal lichen (Lakatos et al., 2004; Wu and Bergey, 2017).

Diatoms and testaceous amoebae are free-living, calcareous protists 2 to 2 000 µm in size (Licea et al., 1996; Moreno et al., 1997). Phacus, 2 to 20 µm, are very thin, leaf-shaped euglenids (Lüftenegger and Foissner, 1991; Gallegos-Neyra et al., 2014).

Also extant, are other organisms like yeasts classified as ascomycetes (Kreger, 1984), and algae capable of an oscillatory motility (Trainor, 1978) which mesh together to form a blueish layer on the surface of the water (Catalán, 1984).

2. Material and Methods

2.1. Study area

From numerous sites in the Nicaraguan countryside many minute specimens were collected of an undetermined snail genus (cf. *Radiodiscus* sp.) with a hirsute periostracum. The specimen discussed here (approximately 2 mm. in

diameter) was taken from Rincón del Diablo rainforest, Ciudad Darío municipality of Matagalpa, Nicaragua (Figure 1), which is centrally located in the national territory, with 8.523 km^2 in extension and a varied climate and topography (Incer, 1998).

2.2. Methods

The specimen was collected with a small trowel from a pile of gravel and debris in the Matagalpa rainforest (Figure 2). Freshly collected specimens are often covered with dirt to be cleansed in an ultrasonic cleaner previously to study (Pérez and López, 2002). Since this process can also eliminate attached organisms which might be part of the snail's environment contributing to its life cycle and development, the single specimen under study was photographed in raw state before cleaning, with a HITACHI SEM S-3000N instrument at Los Angeles UCLA Microscopy Center at 2K to 12K magnification, revealing numerous microscopic organisms attached to the periostracum. Some of these were determined as diatoms with the aid of the

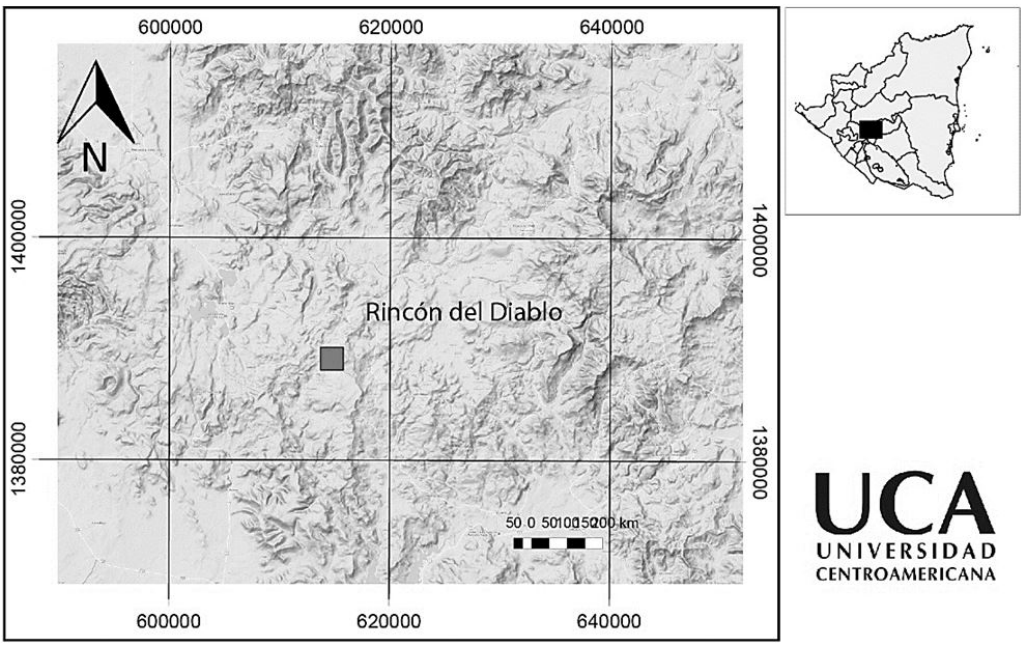

Figure 1. Rincón del Diablo, Matagalpa rainforest of northern Nicaragua.

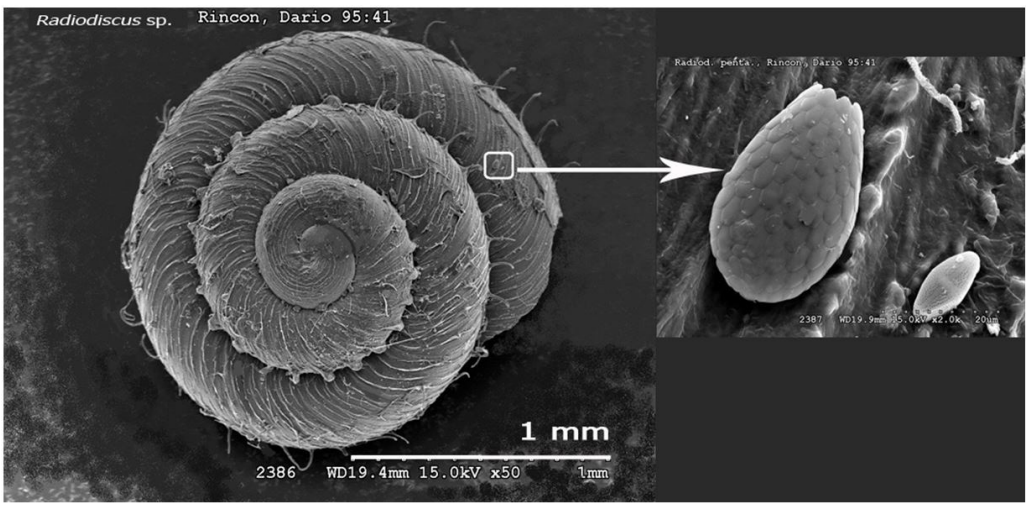

Figure 2. Left Undetermined genus terrestrial snail (cfr. Radiodiscus gen.) from the cloud forest of Jinotega, NICARAGUA. Right, closeup (15K x) shows amoeba Difflugia and diatom Luticola settled on the hairy periostracum.

personnel at the Centro de Investigación de los Recursos Acuáticos de Nicaragua (CIRA/UNAN). Others were determined by appropriate literature as quoted in the text. Yet others remain unidentified.

3. Results and Discussion

This study, the result of monitoring by the UCACM in 1995, allowed determination of protists and other organisms found on a rainforest minute gastropod specimen of Radiodiscus sp. for the first time, by SEM prints at 100X magnification which revealed microscopic accompanying organisms inserted in the hirsute periostracum, possibly in symbiosis with the host (Figure 2).

An unrelated study, UCA-CIDEA (Hernández, 2002), reported diatoms, amoebae and other organisms in Nicaraguan local estuary systems and fish rearing ponds with prevalence of Bacillariophyceae, Chlorophyceae, Cyanophyceae and Dinoflagellidae species.

Aquatic environments are ideal for protists which have cilia and flagella that allow them to move through the water. As single-celled organisms, it is difficult for protists to move on land, but some make their homes in moist terrestrial areas as in the soil and beneath fallen leaves, so it is not surprising that yeast, ostracods and other organisms can live in humid moss or periostracum. Increasing magnification to 10K and using available literature as specified in the text enabled genera determination of most of these as diatoms, testaceous amoebae, phacus and other undetermined organisms such as yeasts and spores, some to generic or specific level while others remain undetermined (Figures 1-11).

3.1. Diatoms

Three genera of diatoms were determined, two of which, the genus *Luticola* (Figure 3) and the genus *Pinnularia* (Figure 4, center) are associated with polluted sites (Bere and Tundisi, 2011). Another *Luticola* sp. (Figure 3 center), clearly shows occlusions protruding from the lateral wall, which have been associated with diatom displacement (Jackson, 1905). Figure 3 right, shows the internal stauross, or "cross", a structure that gives rigidness to diatoms. A third species, *Humidophila* sp. (Figure 4 left) illustrates three individuals, apparently in reproduction.

3.2. Testaceous Amoebae

They are represented by two genera: Difflugia (Figure 1 right) and Trinema (Figure 4 right and Figure 9). Both are associated with polluted water (Bere and Tundisi, 2011). *Trinema* genus test is composed of large circular plates and smaller circular or broad elliptical plates, subterminal aperture with rows of toothed apertural plates and a rim with small oval scales. They are very common in water, between mosses and in soil.

3.3. Phacus

Several specimens of the family Euglenidae genus *Phacus* were observed (Figure 5). Their somewhat contorted appearance and both front and rear views illustrate the movement they can perform (Gallo and Shrével, 1982; Suzaki and Williamson, 1985).

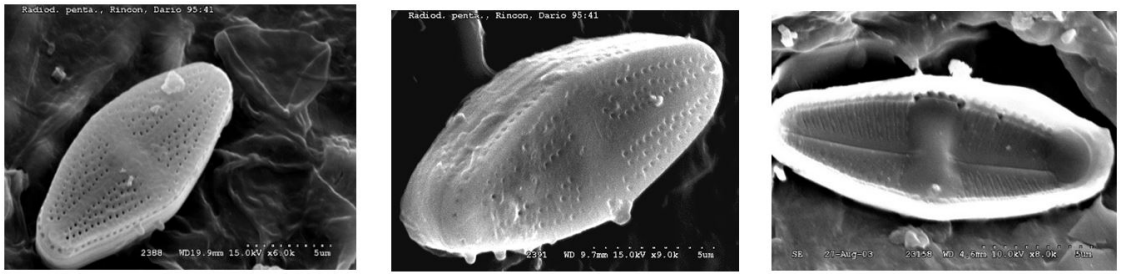

Figure 3. Diatoms on the Matagalpa specimens. Left and center shows Luticola sp. with lateral extrusions associated with displacement. Right shows internal stauross structure.

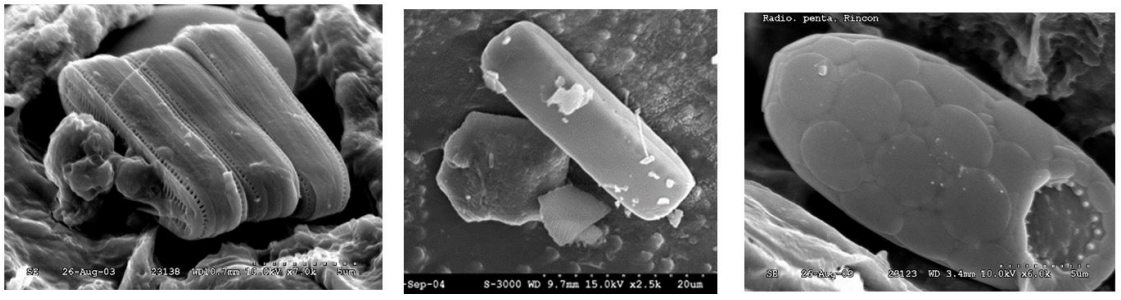

Figure 4. Left, Humidophila sp., apparently in reproduction; center, diatom cfr. Pinnularia. Right, amoeba Trinema (see also Figure 8).

3.4. Other unicellular organisms, some of undetermined genera

Phacus-like organisms. Similar in form, structure and size (Figure 6)

Oscillatory algae (Figure 7, left).

Algae-like organism, similar in appearance to ***Myrionecta rubra*** "Red tide" algae (Figure 7, right).

Yeast (1.5 to 4.5 µm in diameter grains) were some of the more abundant organisms observed on the host specimen and distributed at random in groups containing one to 25 grains (Figures 7-8).

Ascus, a bag-like structure that usually contains 8 ascospores during reproduction in ascomycetous fungi such as yeasts and mildews (Dawes, 1991) (Figure 9).

"Chain" on yeast (left) **and spore** (right) including undetermined organisms (Figure 10).

Flagellate protozoa and minute organism encircled. Nodules with the appearance of deflated globules (Figure 11).

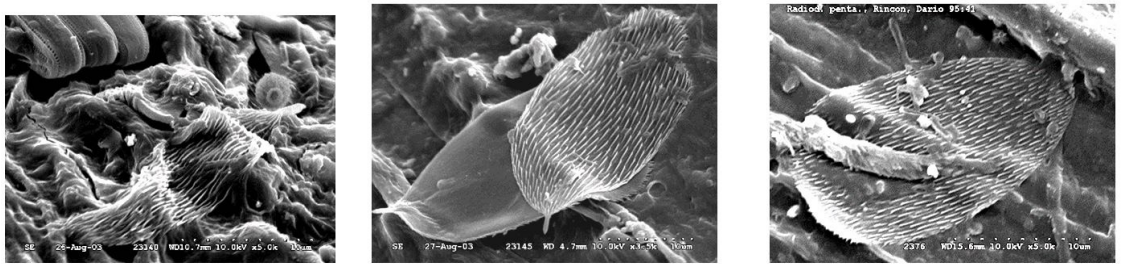

Figure 5. Leaf-like, Phacus protists observed on cf. Radiodiscus gastropod specimens from Rincón del Diablo, Ciudad Darío-Matagalpa.

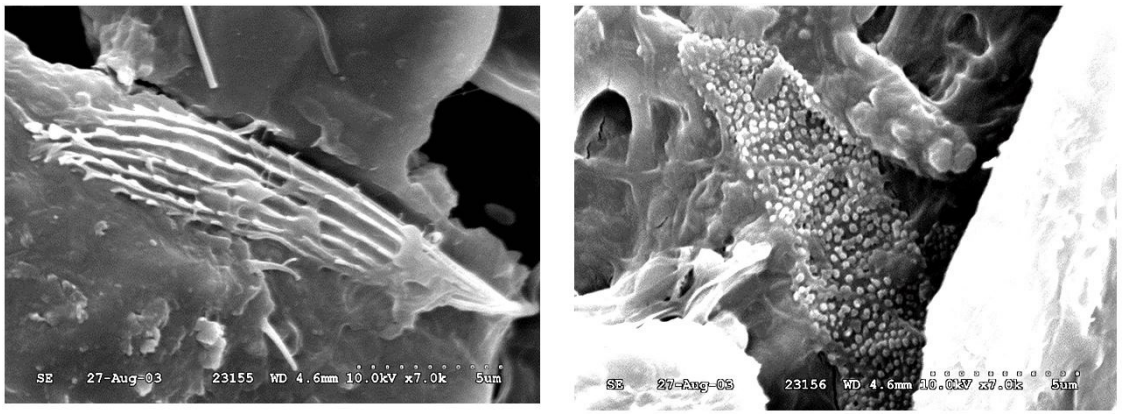

Figure 6. Additional Phacus on same specimen. Figure 5 left shows two additional Phacus sp, the one on the left similar to Gyrodinium spirale, from the Gulf of California (S. Licea et ali, 1995).

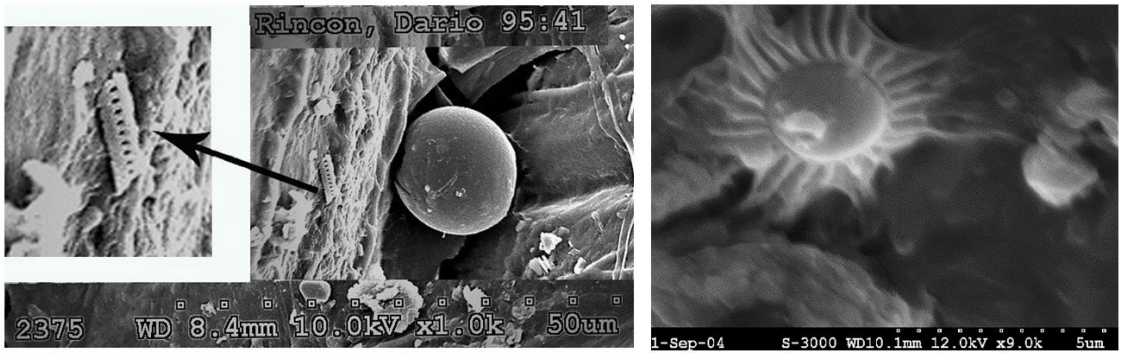

Figure 7. Yeast on Radiodiscus sp., Matagalpa. Arrow indicates oscillatory algae filament. Right, undetermined organism, similar to Maryonecta rubra, the "red tide" marine algae

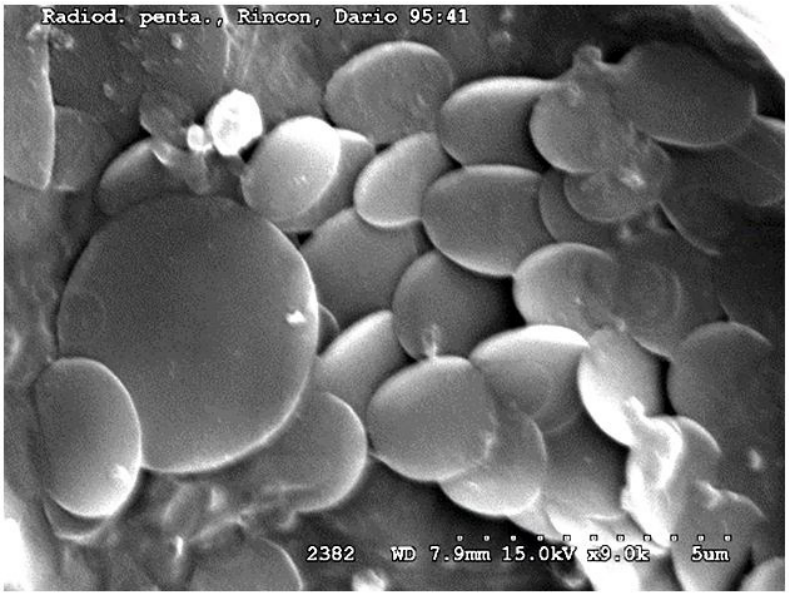

Figure 8. Yeast on Radiodiscus sp., Matagalpa.

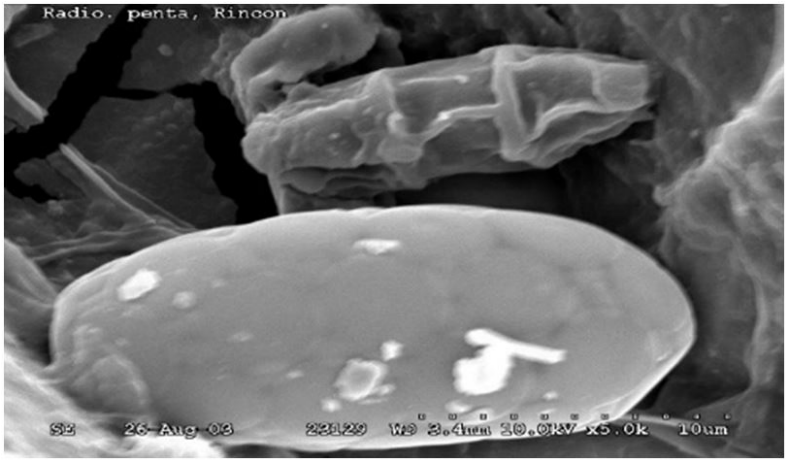

Figure 9. Spore bearing Ascus (above) and testaceous amoeba Trinema (below).

Figure 10. Undetermined organisms, including "chain" on yeast (left) and spore (right).

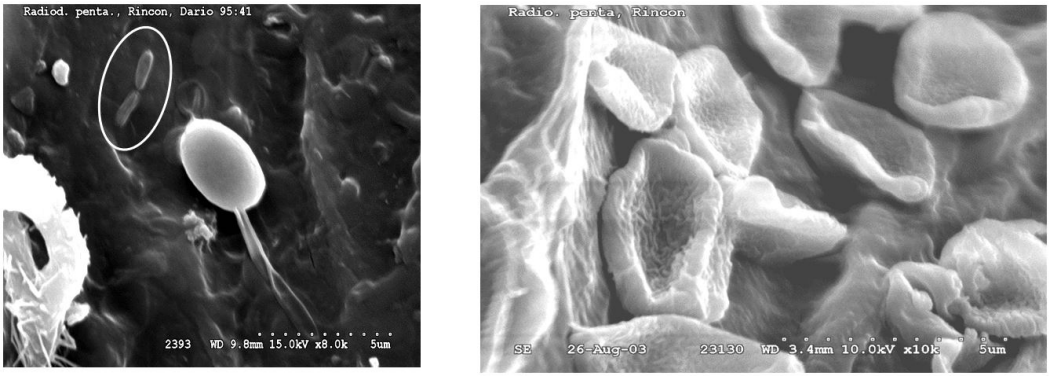

Figure 11. Flagellated protozoa and another "chain" encircled. Right, undetermined objects on Radiodiscus sp.

Concluding, a similar search on other rainforest snails will surely reveal more microorganisms living in a humid periostracum.

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