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Investigations on the Photobiont and Resynthesis of the Tropical Lichen *Coenogonium leprieurii* (Mont) Nvel from the NE Coast of Brazil in Culture

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Abstract

The lichen forming ascomycete *Coenogonium leprieurii* and its trentepohlioid photobiont were isolated and cultured on agarized media. Resynthesis experiments were successful, at least partial ensheathment of the filaments of the green algal photobiont by the fungal partner was observed, typical features of this morphologically very simple microfilamentous lichen. The photobiont was identified as *Printzina lagenifera* (Hildebrand) Thompson and Wujek (*Trentepohlia lagenifera*).

Keywords: Ascolichen, *Coenogonium leprieurii*, culture, resynthesis, *Printzina lagenifera* (*Trentepohlia lagenifera*)

1. Introduction

Coenogonium leprieurii is a yellowish green, filamentous lichen (Coenogoniaceae, Ascolichens) growing on the bark of tropical trees and shrubs in shady locations. The investigated species was described by Xavier-Filho et al. (1983) as one of three species distributed in the NE of Brazil (*Coenogonium leprieurii*, *C. moniliforme* and *C. curvulum*).

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Generally Coenogoniaceae are found growing on the trunks of trees and on soil in tropical and subtropical climates (Meier and Chapman, 1983; Davis, 1994). There are also a few reports on species of the genus *Coenogonium* from temperate zones, but these reports (e.g. Schade, 1932; Uyenco, 1963) need to be verified.

Nakano and Ihda (1996) identified the photobiont of *Pyrenula nitida* as *Trentepohlia lagenifera*. Further results of Nakano and coworkers (Nakano and Handa, 1984) indicated that one *Trentepohlia*-species may be lichenized by different unrelated lichen fungi. Additional examples of *Trentepohlia* ssp. lichenized by different fungal taxa were described by Tschermak-Woess (1988).

On the other hand one *Coenogonium* ascomycete seems to have the ability to envelope different *Trentepohlia*-species, as Uyenco (1965) identified *Trentepohlia umbrina* and *Trentepohlia odorata* as photobionts of *C. leprieurii*.

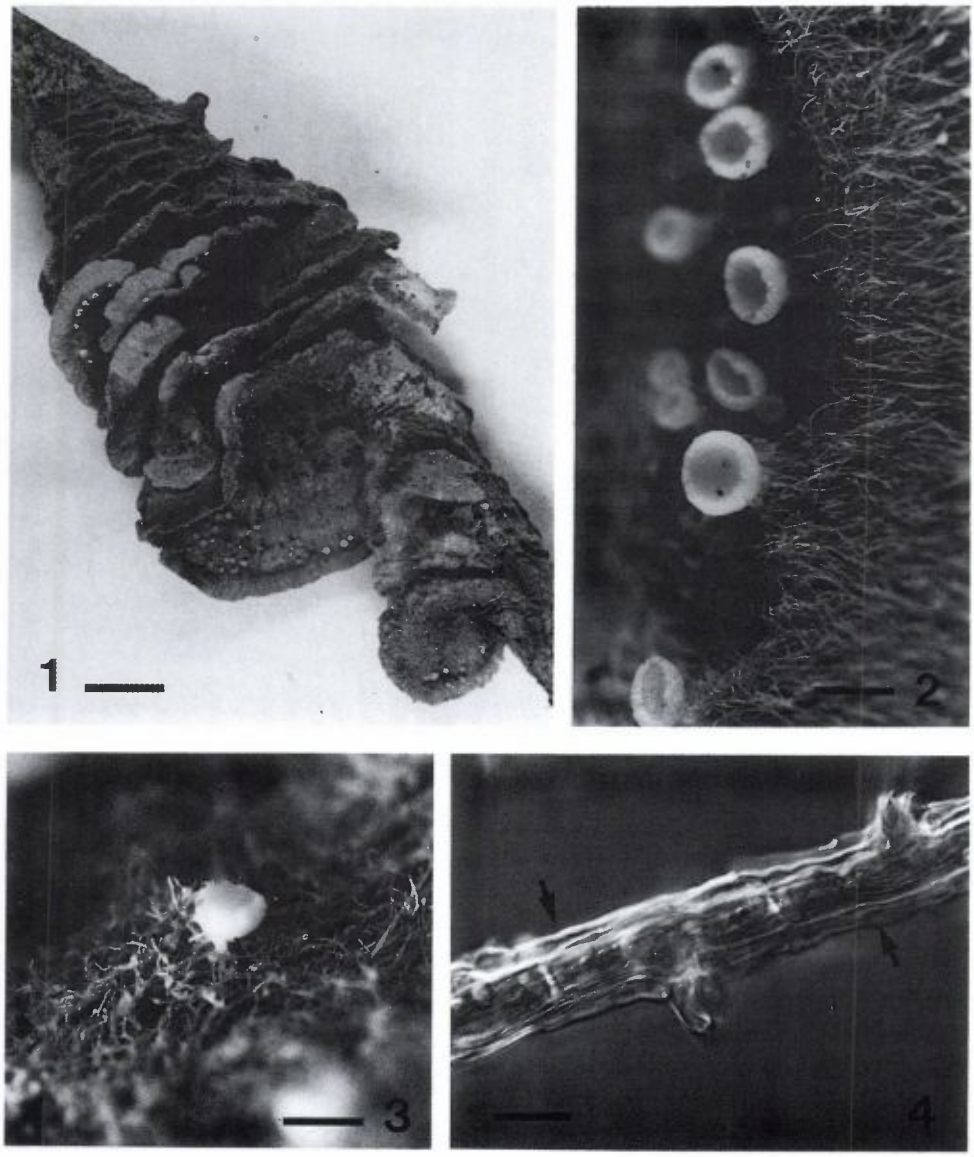
The first objective of this study was to isolate and identify the photobiont of the tropical *Coenogonium leprieurii* from Brazil. An extensive series of tests were initiated to find the optimal culture conditions for the alga. A second aim was to grow the mycobiont from spores and lichenized filaments. Furthermore a partial relichenization is reported in which artificial resynthesis is achieved on agar plates. These investigations have been based on previous culture experiments with tropical lichens (Stocker-Wörgötter et al., 1994).

2. Material and Methods

Thalli of *Coenogonium leprieurii* (Mont) Nvel with rose yellowish apothecia (Figs. 1–3) were collected after the rainy period in August, September, December and January of the years 1992, 1994, 1995 at Alhandra, Paraiba, Brazil. In the very dry season, e.g. January 1996, the lichen was observed to survive as small, lichenized filaments without forming fruiting bodies. Growth and further development of the thalli apparently occurred very quickly during the rainy period.

Photobiont

The photobiont (a species of the genus *Trentepohlia*) was isolated using the method described by Nakano (1988). Small thallus fragments were scratched between glass slides and transferred to sterile agarplates (BBM; Bischoff and Bold, 1963) containing soil/sand extract from the habitat of the lichen prepared using the method described by Esser (1976). Previous investigations had shown that the growth of the isolated *Trentepohlia* was improved by the



Figs. 1–4 *Coenogonium leprieurii.*

Figure 1. Well developed thallus with a great number of fruiting bodies in the natural habitat. Bar = 3.3 mm.

Figure 2. Detail of the thallus showing rose yellowish apothecia. Bar = 130 µm.

Figure 3. One stalked apothecium grown up from lichenized *Trentepohlia*-filaments. Bar = 200 µm.

Figure 4. *Trentepohlia*-filament enveloped by fungal hyphae (arrows). Bar = 10 µm.

inclusion of soil extract. Axenic cultures were obtained by the micropipette method (Ahmadjian, 1973) and after several subcultivations into new media.

The photobiont was cultured on solid and in liquid BBM, incubated at a photon flux intensity of 100 μE m^{-2} s^{-1}, 27–30°C and a light/dark regime of 12:12 h.

Mycobiont

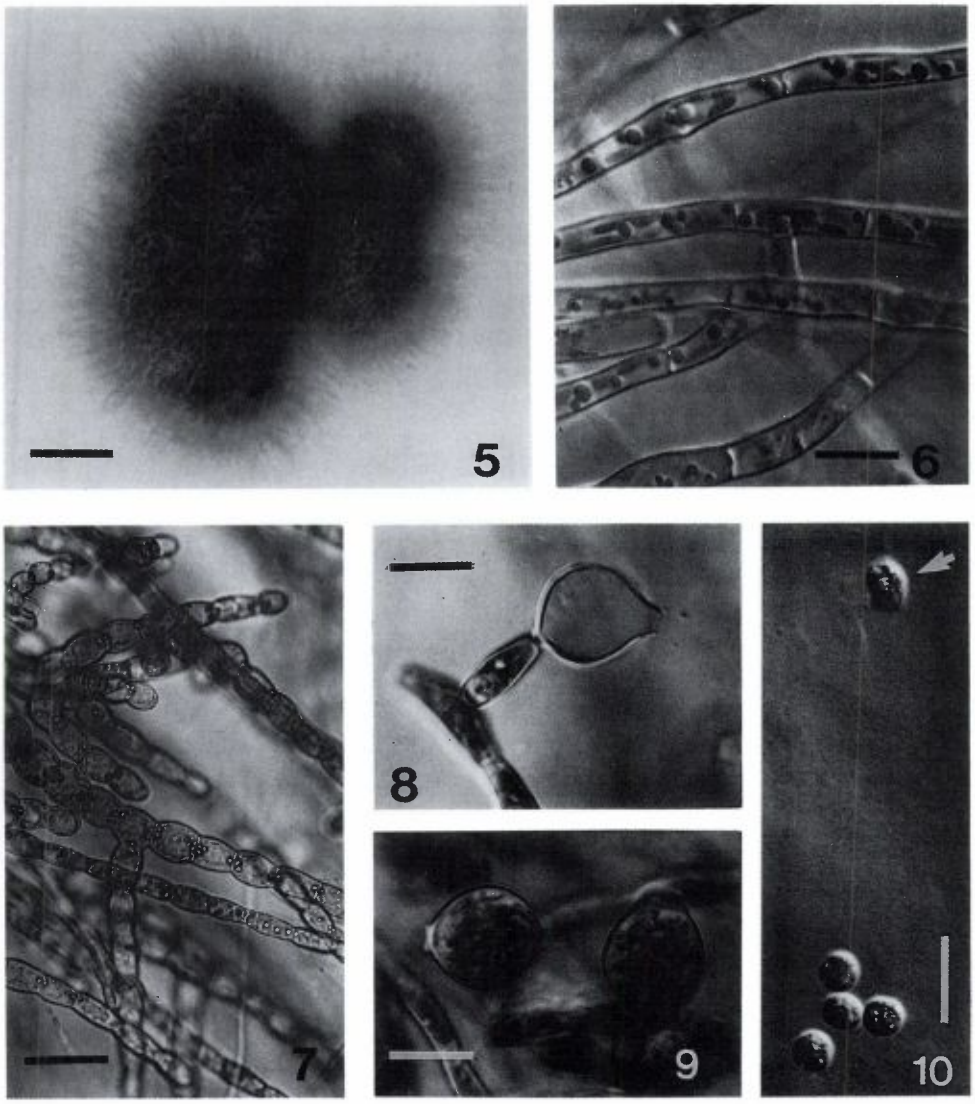
The mycobiont was isolated from spores (Figs. 11 and 12) or lichenized *Trentepohlia*-fragments and after purification grown on LBM-Agar (Lilly and Barnett, 1951) containing 4% erythritol (instead of glucose), the polyol usually transferred from *Trentepohlia* to the lichen fungus.

3. Results and Conclusions

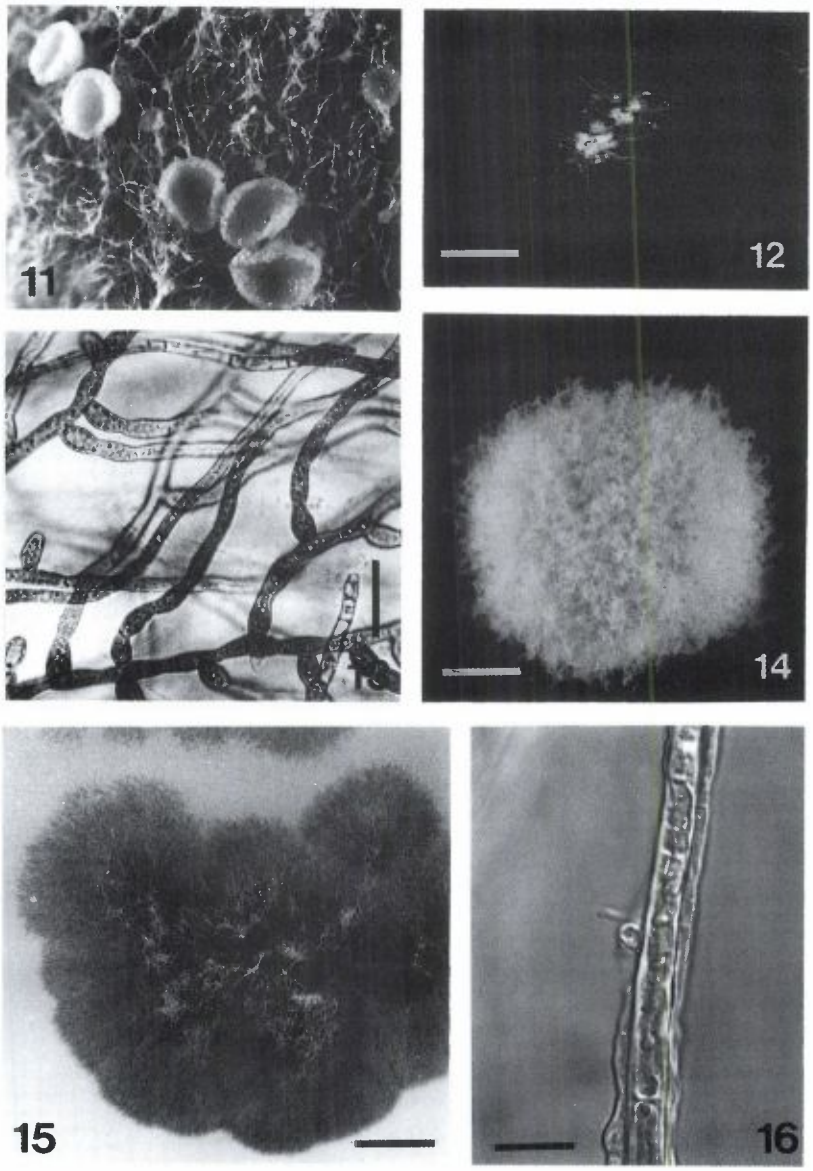
In the lichenized state, the *Trentepohlia*-filaments are tightly enveloped by the fungal hyphae as can be seen in the light micrograph (Fig. 4, arrows). Despite examining a great number of lichenized *Trentepohlia*-filaments, no reproductive organs were found. Small photobiont colonies were formed after 1–2 months on the agarplates. The lower part of the colonies penetrated the agar substrate, whereas in the upper part the algal filaments grew into the air (Fig. 5). Both types of the filaments had parietal, plateshaped chloroplasts and contained hematochromes (droplets of carotenoids). In nearly all examined cultures the filaments on and in the agar substrate and the aerial filaments had cells of different length and shape, the former showing larger cylindrical elongated cells (Fig. 6), the latter smaller more rounded short cells (Fig. 7).

Differences in cell size and shape of cultured *Trentepohlia* have been previously found by Nakano and Handa (1984) in their study of photobionts isolated from species of *Graphis*.

In liquid BBM, the filaments mainly consisted of elongated cylindrical cells (Fig. 13). Growth measurements comparing the rate of increase in dry and fresh weight of the developing colonies (Stocker-Wörgötter, in prep.) revealed a much slower growth rate in the liquid BBM than on the solid medium. The growth measurements are not yet completed (because of the large amount of needed photobiont material), but first available results show that the growth of *Trentepohlia* occurs about three times faster on agarplates than in the liquid medium under comparable conditions. In my cultures a high number of sporangia were formed (Fig. 9). According to the Syllabus of Ettl and Gärtner (1995) the sporangia are interpreted as flask-shaped gametangia housing two flagellate isogametes. The flask-shaped gametangia were found empty (Fig. 8) or filled



Figs. 5–10. *Trentepohlia* lagenifera in culture.
Figure 5. Photobiont culture on agarplate, 3 months old. Bar = 1 mm.
Figure 6. *Trentepohlia*-filament, penetrated into the agar substrate. Bar = 20 µm.
Figure 7. Aerial filaments of the photobiont on agarplate. Bar = 10 µm.
Figs. 8, 9. Flask-shaped gametangia ("sporangia") in empty state and filled with swarmers. Bar = 10 µm.
Figure 10. Above: swarmers just fusing. Below: Settled isogametes, having lost their flagellas. Bar = 15 µm.



Figs. 11–16. Mycobiont of *Coenogonium leprieurii* in culture, resynthesis.
Figure 11. Apothecia used for spore isolation. Bar = 130 µm.
Figure 12. Juvenile mycelia after two months incubation (from spores). Bar = 500 µm.
Figure 13. *Trentepohlia lagenifera*, growing in liquid culture (resynthesis). Bar = 12 µm.
Figure 14. Mycelium after 5 months in culture. Bar = 800 µm.
Figure 15. *Coenogonium* resynthesis. Lichenization occurs in the center on aerial filaments. Bar = 1 mm.
Figure 16. Lichenized *Trentepohlia*-filament from the center of the colony. Bar = 10 µm.

up with gametes (Fig. 9). By adding some water drops, e.g. on a glass slide, the swarmers were released trough the ostiole of the flask, swimming actively and rapidly around. After some minutes they stopped moving, became more rounded and began to settle. During settlement the flagella disappeared (Fig. 10). Sometimes the fusing of two swarmers was observed (Fig. 10, arrow).

The isolated *Trentepohlia* resembled in nearly all characteristics the *Trentepohlia lagenifera* (Hild.) Wille (Nakano and Handa, 1984; Nakano, 1988), except that no four flagellate swarmers were observed. According to Nakano (1988) this alga is found freeliving and lichenized in some species of the *Graphidaceae*. The presented results show that *Trentepohlia lagenifera* is also present in the material of *Coenogonium leprieurii*. According to the Syllabus (Ettl and Gärtner, 1995) the actual systematic name is *Printzina lagenifera* (Hildebrand) Thompson and Wujek (syn. *Trentepohlia lagenifera*).

The mycobiont, isolated by spores (Figs. 12 and 14) grew very well in LBM (Lilly and Barnett, 1951) containing 4% erythritol. After 3–4 months in culture, mycelia with an average diameter of 2–3 mm were obtained (Fig. 14).

For the resynthesis hyphal fragments were mixed with *Trentepohlia*-filaments from liquid BBM (Fig. 13) on agar plate. First lichenization was observed after five months on aerial filaments in the center of one well-developed *Trentepohlia*-mat (Fig. 15). In culture the aerial filaments only were lichenized (Fig. 16) but never the filaments penetrating the agar substrate. Lichenization, in *Coenogonium* did not occur under moist conditions inside the agar substrate, but it needed a very high relative humidity in the air of the culture dish.

In the course of the resynthesis experiment *Trentepohlia*-filaments became tightly enveloped by fungal hyphae. Finally there was no visible difference between fungus-infected filaments isolated from the resynthesis culture (Fig. 16) and the natural thallus (Fig. 4), but the more complicated morphology of the thallus as shown in Fig. 1 was not achieved in laboratory cultures.

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