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# Contribution to the study of *Normandina pulchella*: a cytological approach

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**Key words:** *Normandina pulchella*, ultrastructure, mycobiont-photobiont interface.

**Abstract.** A cytological study was undertaken on *Normandina pulchella* (Borr.) Nyl. in order to contribute to a better knowledge of the structure of the lichen and to acquire useful information on its taxonomic arrangement. Micro- and submicroscopic observations revealed that the tallus is heteromerous, with a medullary «tissue» composed of a network of hyaline fungal threads surrounding small clumps of algal cells. These were thick-walled and exhibited a single lobate chloroplast in which a conspicuous metameric pyrenoid, small starch granules and lipophilic plastoglobuli were frequently present. The mycobiont had hyphae with simple perforated septa with associated Woronin bodies. The relationship between the two partners was merely of very close proximity of their cell walls and involved no fungal penetration into algal cells. The presence of Woronin bodies, exclusive of the *Ascomycetes* and of plastoglobuli, that were described only in the *Chlorococcales* phycobionts in ascolichens, indicates that in all likelyhood *Normandina* is a member of the ascolichens and not of basidiolichens, as previously reported by some workers.

**Resumen.** *Contribución al estudio de* Normandina pulchella: *aproximación citológica.* Para contribuir a un mejor conocimiento de la estructura de *Normandina pulchella* (Borr.) Nyl. y para obtener informaciones útiles para su correcta colocación taxonómica, se ha emprendido un estudio citológico sobre el liquen. Observaciones al microscopio óptico y electrónico han revelado que el tallo del liquen es heterómero, con un tejido medular compuesto por un entrelazamiento de hifas hialinas de hongos, que rodean pequeños grupos de células algales. Éstas presentaban una pared gruesa y un cloroplasto lobulado simple, en el cual frecuentemente estaban presentes un voluminoso pirenoide metamérico, pequeños granos de almidón y plastoglóbulos lipófilos. El micobionte tenía hifas con septos simples y corpúsculos de Woronin asociados. La relación entre los dos componentes consistia en una simple y densa aproximación de sus paredes, sin una penetración del hongo en las células algales. La presencia de los corpúsculos de Woronin, exclusivos de los Ascomicetes y de los plastoglóbulos, que han sido descritos sólo en los clorococales ficobiontes de los ascolíquenes indica que, con toda probabilidad, *Normandina* es un miembro de los ascolíquenes y no de los basidiolíquenes, como había sido previamente afirmado por algunos autores.

## Introduction

*Normandina pulchella* (Borr.) Nyl. is an ombrophytic and hygrophytic small lichen, which is widely distributed in the Northern and Southern hemispheres, in localities with suboceanic conditions. This species is one of the most striking

examples of «hyperepiphytism», growing both on rocks or bark and on the thallus of other epiphytes, chiefly liverworts, mosses and even other lichens (Tretiach and Nimis 1988 and references therein). The thallus, whose colour may vary from glaucous to pale grey or greenish-grey, consists of squamules (1-2 mm) that are more or less rounded or kidney-shaped. The phycobiont is *Nannochloris normandinae* Tsch.-W. (Tschermak-Woess 1981). Fruiting structures do not seem to be produced by this lichen. In fact, the perithecia observed in the thallus by some workers (Ozenda and Clauzade 1970, Poelt 1974) are ascribable to the invading parasitic fungus *Sphaerulina chlorococca* (Leight.) R. Sant. Therefore, the identity of the mycobiont caused considerable taxonomic problems. In particular, whether *N. pulchella* is an ascolichen or a basidiolichen has remained an open problem in published accounts (Ozenda and Clauzade 1970, Poelt 1974, Wirth 1987). In fact, *N. pulchella* was considered for a long time as a member of basidiolichens (Henssen and Jahns 1973) because of the close morphological similarity with *Coriscium viride*, a lichen currently regarded as the imperfect stage of the basidiomycete *Omphalina hudsoniana* (Oberwinkler 1970). On the other hand, the subsequent finding of lack of dolipore septa in *Normandina*, which are typical of *Basidiomycetes*, made its attribution to basidiolichens unlikely (Henssen 1976). Thus, the systematic position of *Normandina* is still to be clearly defined.

In this context, a cytological investigation was undertaken on this lichen, focusing the attention on the organization of the thallus, the structural characteristics of both myco-and photobiont and the nature of the fungal-algal contact.

The purpose of this study was to contribute to a better knowledge of the structure of the lichen, and to acquire useful information for its taxonomic arrangement.

## Materials and methods

*Normandina pulchella* (Borr.) Nyl., grown on *Frullania dilatata* (L.) Dum., was collected on *Quercus pubescens* L. at Opicina (250 m) in the Karst Region (Trieste, Italy). Micro- and submicroscopic observations were carried out on thallus pieces soaked for 12 h in tap-water in a thermostatically controlled incubator at 25 ± 1°C under continuous light (50 $\mu E \cdot m^{-2} \cdot S^{-1}$ at the surface).

Microscopic examinations were performed with a Zeiss Axiophot photomicroscope equipped with conventional or fluorescent attachments (BP 436, FT 460, LP 470 filter set). For transmission electron microscopy (TEM), the specimens were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 6.8, at 4°C for 3 hours, rinsed for several hours in buffer and postfixed with 1% $Os0_4$ in the same buffer. After dehydration in a graded ethanol series, they were embedded in Epon-Araldite resin, cut with a LKB Ultratome III and stained with uranyl acetate and lead citrate. Observations were made with a Zeiss EM 109 electron microscope at 80 kV. Scanning electron microscopy (SEM) was carried out on glutaraldehy-

de fixed thallus pieces that were briefly postfixed with 1% $Os0_4$, rapidly dehydrated in acetone, critical point dried and gold coated, as previously described (Mares and Fasulo 1990). Observations were made with a scanning electron microscope Cambridge Stereoscan 360 at an accelerating voltage of 20 kV.

## Results and discussion

As observed by the light microscope, the thallus of *N. pulchella* has the same morphological characteristics previously described by Henssen (1976). In particular, it is heteromerous with an upper pseudoparenchymatous or evanescent cortex, whereas a distinct lower cortex is absent. The medullary «tissue» of the lichen is composed of a network of hyaline fungal threads, which surround small clumps of algal cells (Fig. 1.l). These latter have no well defined distribution pattern in the thallus and are present in two different forms, that probably correspond to two different stages of their life cycle. More precisely, the photobiont population consists of both large and thick- walled mature cells, and smaller young mitospores, which can be free in the thallus or still ensheated by the mother cell wall.

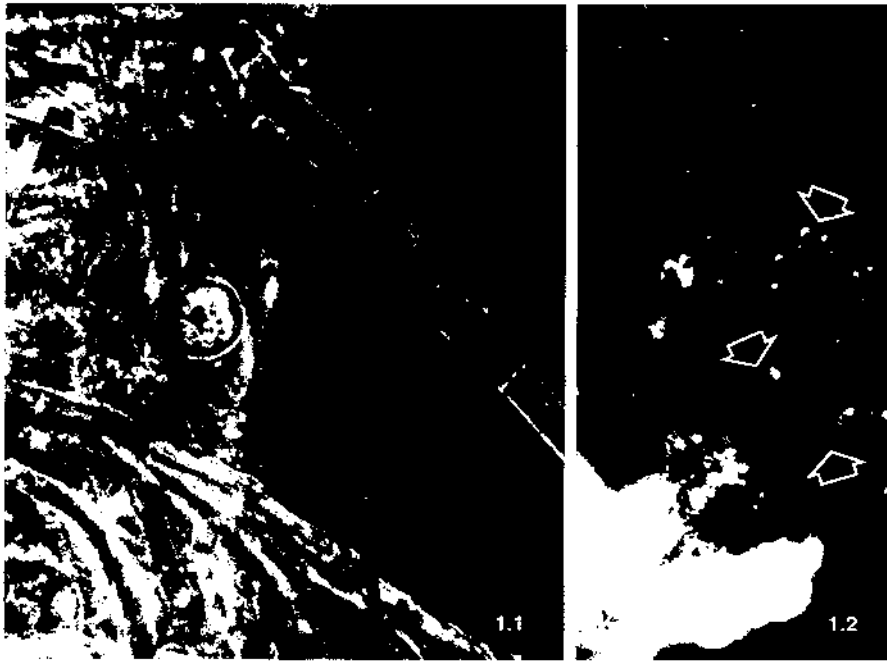


Figura 1.1 Light micrograph of the thallus of *N. pulchella* showing a network of hyaline mycobiont cells enveloping small groups of algal cells. (x 400). 1.2 Fluorescence microscope aspect of the lichen showing the photobiont population with a well defined red fluorescence which is more intense in the smaller mitospores (arrows). (x 250).


Figura 2. Fine structure of *N. pulchella* association showing numerous mycobiont hyphae (my) adjacent to a phycobiont cell (ph) in which a single lobate chloroplast with a central metameric pyrenoid may be observed. The physical contact between the symbionts consists of a simple relationship of wall-to-wall proximity. Bar = 1 μm.

When observed under UV light, all the algae exhibit a well defined red fluorescence which is more intense in the younger ones. (Fig. 1.2).

At TEM, the cells of the phycobiont have a rather darkly stained wall with a variable thickness according to the stage in life cycle. However, the algal wall is always thicker than that of the fungal partner. The centre of the cell is occupied by a single lobate chloroplast with a conspicuous pyrenoid having a metameric structure (Fig. 2). Small starch granules and numerous plastoglobuli are present close to the thylakoid membranes (Fig. 3).

The mycobiont shows hyphae with multilayered walls, sometimes infiltrated by electron-dense granular precipitates uncertain in nature. The septa are always simple structures, typically perforated, hardly ever linear, but often warped, and frequently associated with microbodies and Woronin bodies. The latter organules are spherical (about 0.2 μm in diameter), bounded by a single unit membrane and contain a homogeneous electron-opaque matrix. Woronin bodies appear singly in

Figura 3. In the phycobiont, the chloroplast matrix presents some small starch granules (arrowheads) close to the thylacoid membranes and many plastoglobuli (pg) peripherally distributed. Bar = 1 μm.

Figura 4. Hyphal septum with a single central pore having Woronin bodies (arrows) associated. Bar = 1 μm.

the pore or as a pair at both sides of the septa (Fig. 4), Dolipore septa or clamp connections, typical of the *Basidiomycetes*, were never observed.

As it normally happens in the squamulose forms of lichens (Honegger 1985), each phycobiont appears to be encircled by various mycobiont hyphae (Figs. 2,5). The physical connection between the two symbionts is merely the close proximity of their cell wall without the formation of fungal haustoria. This was established by observations on 30 serial lichen sections, from three resin blocks, and by SEM specimens.

In our opinion, the structural aspects observed in *N. pulchella* can furnish some structural details useful for a better knowledge of this interesting species of lichen. As viewed, in fact, the physical contact between the symbionts, consists of a simple relationship of wall-to-wall proximity with no fungal penetration into the algal cells. According to Galun et al. (1970), the prevention of the hyphae from entering into the phycobiont could be related to the presence in the alga of a very thick wall.

Another interesting finding in our samples is the constant presence in the mycobiont of simple septa. This aspect was already described by Henssen (1976)

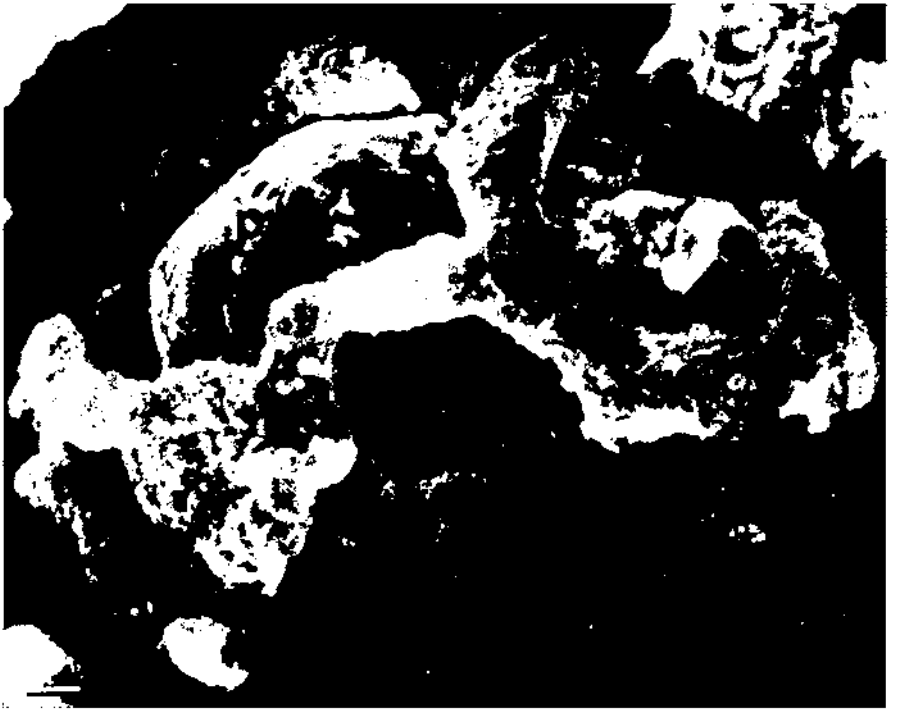

Figura 5. SEM micrograph of an algal cell contacted by numerous adhering mycobiont hyphae. Bar = 1 μm.

which, just on this basis, ascribed *N. pulchella* to ascolichens. Our finding of Woronin bodies associated with the simple pores substantiates this affiliation, because these structures are considered to be exclusive of the *Ascomycetes* (Moore 1965, Beckett et al. 1974, Vannini and Mares 1975, Withrow and Ahmadjian 1983). Thus, we think that the mycobiont of *N. pulchella* is the sterile stage of an *Ascomycete*. A support to this hypothesis seems to be offered by the presence of plastoglobuli in the phycobiont. In fact, in other lichens having *Chlorococcales* as photobionts, these plastidial inclusions were found only when the algae are in symbiosis with *Ascomycetes*, whereas the same phycobionts do not show plastoglobuli when the symbiosis is with *Basidiomycetes* (Peveling and Galun 1976).

Therefore, on the basis of our results, we are led to believe that *N. pulchella* is a member of ascolichens.

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