Morphological Evidence for the Symbiotic Character of *Turgidosculum complicatulum* Kohlm. & Kohlm. (= *Mastodia tesselata* Hook.f. & Harvey)

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Abstract

The symbiotic nature of the association between mycobiont and photobiont in the Antarctic lichen *Turgidosculum complicatulum* has been questioned. Some authors consider the lichen *T. complicatulum* to be an alga parasitized by the fungus. The photobiont *Prasiola crispa* ssp. *antarctica* (Kützing) Knebel also occurs free-living in adjacent microhabitats. The initial developmental stages of this Antarctic lichen or lichen-like association are described here for the first time. The aim of this study was to examine the early developmental stages of *T. complicatulum* in order to characterize the nature of the association between photobiont and mycobiont. SEM and light microscopy revealed a decisive role of unicellular aplanospores and other few-celled stages of the alga in the process of lichenization. There is a slight morphogenetic influence of the mycobiont on the photobiont, but no cortex differentiated. The outer layer of the thallus does not contain phenolic substances

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other than melanin. On the basis of morphological investigations and some physiological and chemical properties *T. complicatulum* can be described as a lichen with simple organisational level, although certain features challenge usual concepts of lichen symbiosis.

1. Introduction

Lichen symbiosis has been characterized as controlled parasitism, the ability of the photobiont to survive fungal envelopment and formation of haustoria is seen as result of successful co-evolution of the bionts (Ahmadjian and Jacobs, 1981). The multilinear evolution of lichens is thought to be the cause for both the existence of highly differentiated lichen species with a long history of co-evolution and lichen species with a low degree of differentiation and possibly a short history of co-evolution (e.g. Ahmadjian, 1967). A possible example for an association of fungus and alga with a low degree of differentiation may be *Turgidosculum complicatulum* Kohlmeyer & Kohlmeyer (= *Mastodia tesselata* Hooker f. & Harvey). This association has been described as a lichen, an alga and a fungus (Lamb, 1948; Brodo, 1976).

The nomenclature of *T. complicatulum* is as insecure as its symbiotic state. Some authors place *T. complicatulum* among the pyrenocarp Antarctic lichens under the name *Mastodia tesselata* (Lamb, 1948; Dodge, 1973; Lindsay, 1974). The mutualism of the association between mycobiont and photobiont has been questioned, partly due to a lack of morphogenetic influence of the fungus on the alga (Ahmadjian, 1967) and partly because the alga has been considered to be parasitized by the fungus (Kohlmeyer, 1974). The association has been named *Turgidosculum complicatulum* (Kohlmeyer, 1974; Kohlmeyer and Kohlmeyer, 1979) while other authors refer to it as *Turgidiusculum complicatulum* (Kappen, 1993). Meyers (1957) as well as Johnson and Sparrow (1961) describe the association as being between the alga *Prasiola* and a parasitic fungus *Guignardia* (= the mycobiont of *T. complicatulum*) as parasitic rather than symbiotic. More recently, the association has been described as a transitional stage between a parasitized alga and a true lichen symbiosis (Gärtner, 1992; VanDonk and Bruning, 1992). The odd nomenclature of *T. complicatulum* and the lack of a detailed description indicate the need for study.

The alga *Prasiola crispa* ssp. *antarctica* (= *P. crispa* in the following) occurs in the free-living state in overlapping microhabitats with *T. complicatulum* and is, according to Lamb (1948), the photobiont of the lichen. This nitrophilous alga is widespread in Antarctica (Davey, 1989; Wiencke and

Dieck, 1990). *P. crispa* prefers slightly wetter habitats in or near meltwater streams, while *T. complicatulum* is found on exposed coastal rocks (Gremmen et al., 1995). Colonisation of the latter habitat seems to start in rock crevices. *T. complicatulum* has a bipolar distribution (Lamb, 1948; Brodo, 1976) and is common in the maritime Antarctic. As the symbiotic nature of *T. complicatulum* is under discussion, the question arises how the process of association or lichenization is initialized. The aim of this study was to investigate the early developmental stages of *T. complicatulum* in order to characterize the nature of the association.

2. Material and Methods

Samples of *Turgidosculum complicatulum* and *Prasiola crispa* ssp. *antarctica* were collected from supralittoral coastal rocks on Adelaide Island (67°34'S, 66°8'W) and Léonie Island (67°35'S, 68°20'W), Antarctica.

Specimens on stones were examined morphologically by stereomicroscope and by SEM. Semi-thin sections stained with basic fuchsin methyleneblue were examined using a light microscope.

The absence of secondary substances was confirmed by TLC (Culberson and Ammann, 1979) analysing acetone extracts of up to 100 mg/ml dry lichen material. The presence of melanin was based on the chemical properties of the black pigment (Ravishankar et al., 1995; Butler and Day, 1998). The organic solvents used were hexane, acetone and ethanol.

3. Results

In the field, *P. crispa* can be observed in different growth forms. Unicellular forms can develop into filaments and subsequently into sheet-like thalli. The thalline form (status prasiolae) is the most obvious growth form. Unicellular aplanospores of *P. crispa* were abundant along water marks on rocks and pebbles from the supralittoral, near meltwater streams and in crevices, the natural habitat of both lichen and alga (Figs. 1 and 2). Some of the specimens collected were coated with 'green dust' consisting mainly of unicellular forms and tetrads of *P. crispa*. The alga was identified on the basis of morphological and anatomical characteristics. Other species of algae or cyanobacteria were seldom observed. The unicellular forms of *P. crispa* (Fig. 3) occasionally develop into thread-like forms (status hormidii and status schizogonii) (Fig. 3). Underneath thalli of *T. complicatulum*, gelatinized fungal hyphae cover the substrate together with numerous unicellular and thread-like forms of *P. crispa* (Fig. 3).
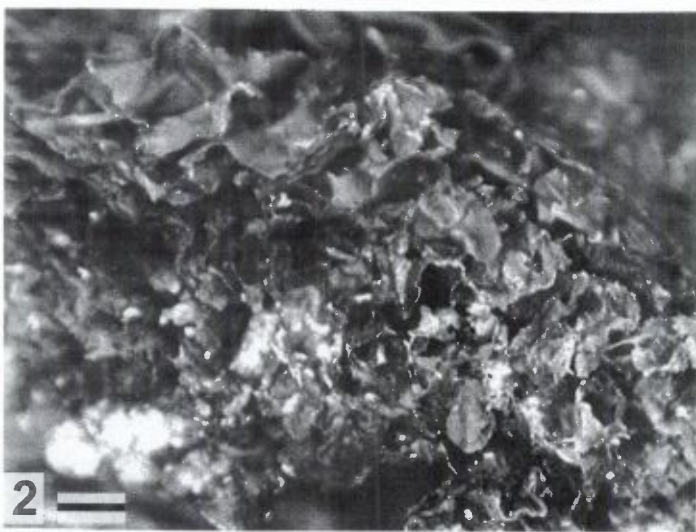
Figure 1. A thallus of *T. complicatulum* on supralittoral rocks. Bar = 0.2 cm.
Figure 2. Thalli of *P. crispa* (status prasiolae) collected from a melt water stream. Bar = 0.1 cm.

Evidence was found for a decisive role of unicellular aplanospores and other few-celled stages of the alga in the process of lichenization (Figs. 3 and 4). Accumulations of tetrads of algal cells were found to form packages interwoven with fungal hyphae. Fungal hyphae attach the packages to the substrate. Some of the packages have a slightly gelatinized outer surface (Fig. 4).
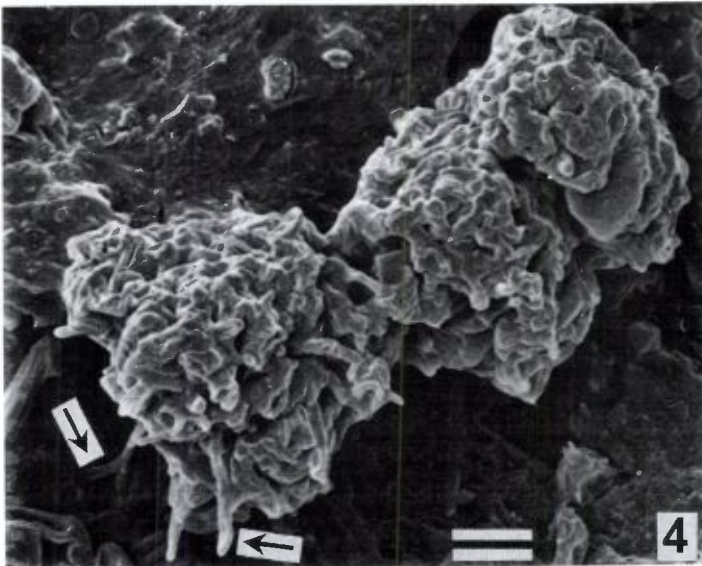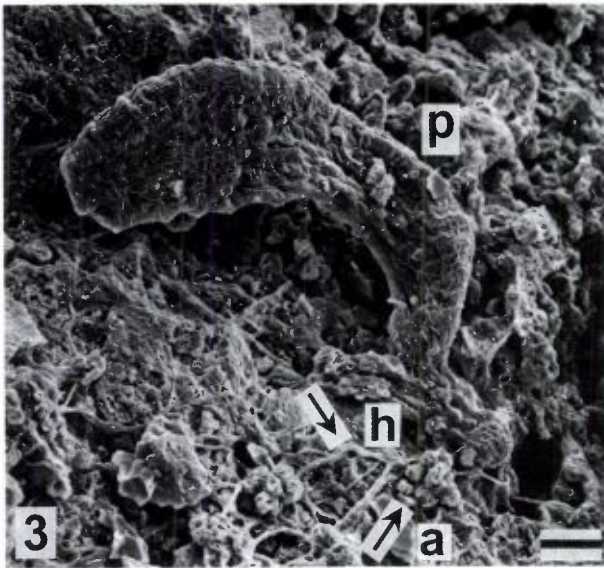
Figure 3. A thread-like thallus (status hormidii or schizogonii, p) of *P. crispa* among unicellular aplanospores of the alga (a) and gelatinized fungal hyphae (h) on the rock surface. Bar = 25 μm.

Figure 4. An aggregate of algal cells interwoven with fungal hyphae (arrows), the hyphae starting to attach the aggregate to the substrate. The accumulations are connected via mucilage. Bar = 10 μm.
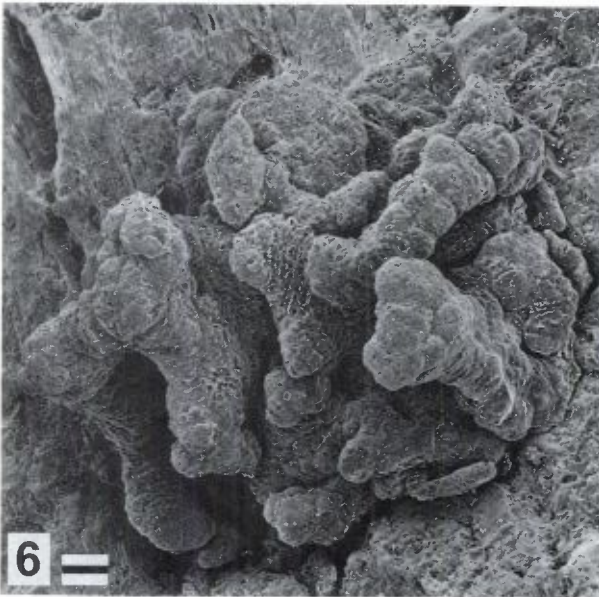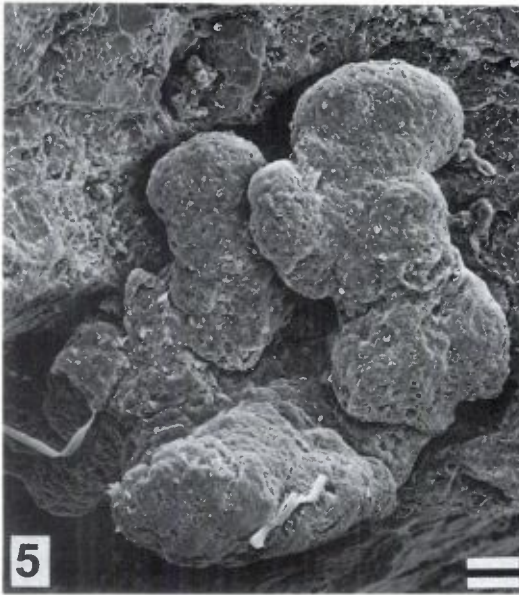
Figure 5. Early stage of a lichen thallus: single aggregations growing in a crevice differentiate into lobes of *T. complicatulum*. The surface is completely gelatinized. Bar = 40 µm.

Figure 6. A small thallus of *T. complicatulum* with gelatinized fungal hyphae forming the outer layer of the lobes. Bar = 100 µm.
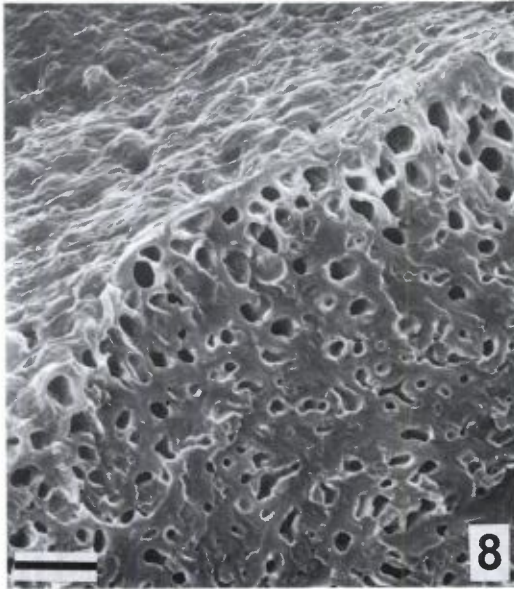
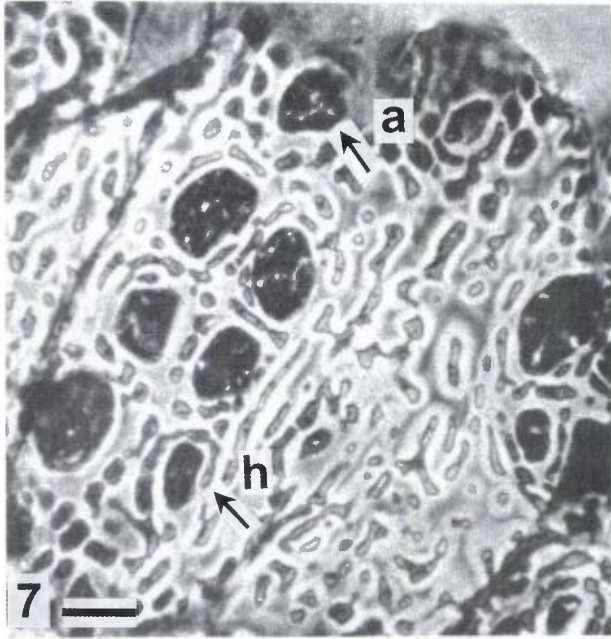Figure 7.   Morphology of a lobe of *T. complicatulum*: *P. crispa* grows in groups of cells within the thallus of *T. complicatulum*, the algal cells (a) are closely surrounded by and interwoven with fungal hyphae (h). Bar = 10 µm.

Figure 8.   There is no differentiated cortex, the thallus is enclosed by a compact layer of mucilage. The morphology of the thallus is unusual for a green-algal lichen. Bar = 10 µm.

As the first tiny lobes differentiate (Fig. 5), the accumulation of hyphae and algal cells is enclosed by a compact layer of mucilage (Figs. 5 and 6). Finally fungal hyphae form an outer layer arround the algal cells within the thallus. The tendency of the fungus to envelop algal cells requires some degree of coordinated growth processes of both bionts. The development of these lobes seems to occur preferably in tiny hollows or crevices (Figs. 5 and 6). Several lobes can form a thallus of *T. complicatulum* (Fig. 6). Lobes of this size already exhibit an inner organisation comparable to lobes of adult thalli. Perithecia are observed in larger thalli only.

Thallus lobes of *T. complicatulum* are relatively compact (Figs. 7 and 8). Gelatinized hyphae separate tetrads or pairs of algal cells and closely surround each algal cell (Fig. 7). Between the fungal hyphae, groups of algal cells within the adult thallus are arranged in more or less regular bands parallel to the surface of the lobe, the lichen thallus shows slight morphogenetic influence of the mycobiont on the photobiont.

There is no differentiated cortex (Fig. 8) but the lobes are dorsiventral to some extent. The outer boundaries of the thallus surface appear blackened underneath the light microscope, which often is more pronounced on the light exposed upper boundary of the lobe. The lichen did not contain phenolic substances other than melanin. The pigment was identified on the basis of the following physical and chemical characteristics: this pigment is black, insoluble in water and organic solvents, but soluble in hot alkali and precipitable in acid solutions. Furthermore it is decolored by H_2O_2 and it is able to decolorize solutions of Azure A.

4. Discussion

The question whether *T. complicatulum* can be interpreted as a lichen remains difficult to answer. The formation of aggregations of fungal hyphae and algal cells alone is no indication of specific interactions between the bionts. Such soredia-like, undifferentiated aggregations of algal cells and hyphae do not necessarily develop further into lichen thalli (Ott, 1987; Galun, 1988). The formation and differentiation of the lichen thallus as a functional unit requires compatibility of the bionts (Galun, 1988). The key observation of our investigations is the development of thalli of *T. complicatulum* in well defined stages.

Gelatinization has already been described as an important step during the initial stages of lichenization (Ott, 1987). As the early developmental stages of *T. complicatulum* already show some degree of differentiation (an outer surface consisting of mucilage), the groups of algal cells appear as symbiotic partners rather than being attacked or infected by the fungus as proposed by

Huiskes et al. (1997). The foliose thalli of *T. complicatulum* could be interpreted as result of a morphogenetic influence of the fungus on the photobiont, where the fungal hyphae thoroughly enclose each algal cell within the thallus and provide the outer layer of the thallus (Brodo, 1976). As in some other Antarctic pyrenocarp lichens such as species of the genus *Verrucaria* (Feige and Jensen, 1992), adult thalli of *T. complicatulum* do not contain phenolic substances in the surface layer. The outer layer of the thallus, especially the upper surface, is melanized. Thus the association can be described as a lichen with simple organisational level.

Further indication for a lichenized organism can be derived from ecological observations. For some authors, the success of *T. complicatulum* in colonizing its habitats accounts for the composite being a lichen (Brodo, 1976). Both bionts of *T. complicatulum* partly share habitats and this opens the opportunity to study environmental factors influencing the symbiotic state.

Some thalli of *T. complicatulum* in the field appear bright green others dark olive-green. The existence of green or black thalli may be related to environmental conditions during growth or represent stages in the process of lichen formation. One of the important questions is whether green and black forms represent ecotypes, developmental stages or thalli differing in balance between the bionts. Water is a key factor in Antarctica, and there is some evidence that differences in water relations determine growth forms of the lichen (Huiskes et al., 1997). Differences in salt contents of the thalli may also be important as *T. complicatulum* can cope with or even benefit from high salt concentrations (Huiskes and Moerdijk-Poortvliet, 2000). Further research is clearly needed to discover how *T. complicatulum* responds to changing environmental factors. An interpretation of the association as an alga parasitized by a fungus is improbable as we could not find adult thalli of *Prasiola* infected by the fungus on investigating 40 adult thalli of *P. crispa* from a melt water pool on Léonie Island. A parasite should be able to infect the thalli but the close contact cannot be established from hyphae and leaf-like algae.

Inside the lichen the hyphae envelope algal cells or more often groups of algae. This form of contact is probably supported by the fact that *Prasiola* always consists of packages of cells inside a musculagineous matrix. This structure may help in lichenization. It is also of interest that a systematic proximity between Prasiolales and other green alga which include the genus *Trebouxia*, the most common lichen photobiont, is discussed (Vanden Hoek et al., 1995; Sherwood et al., 2000).

We will not try to solve the taxonomic muddle described in the introduction. The question whether we are dealing with a lichen or a parasitized alga should not be of relevance for the name of the fungus. The name of the fungus has to be determined by its taxonomic relation to other fungi. If we accept the

association as a lichen, then this name is also the name of the lichen.

The results presented here indicate that juvenile thalli of *T. complicatulum* are symbiotic in nature. Our findings also confirm a decisive role of unicellular or few-celled stages in the lichenization process which had previously been hypothesized (Huiskes et al., 1997). The unicellular and oligocelled forms obviously are of special importance in the initial phase of the lichenization process because our observations showed that leaf-like structures of *Prasiola* are not lichenized by the fungal hyphae. Finally it can be stated that *T. complicatulum* may best be interpreted as a lichen with a very low level of differentiation and special adaptation of the bionts. The organism can therefore serve as a model for the way on which the lichenization process may have occurred in the past. More detailed investigations and experiments of the cellular contact between fungus and alga to clarify the symbiotic state is needed and in progress.

Acknowledgements

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