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Composite Lichen Thalli of *Sticta* sp. from Brazil, with Morphologically Similar Lobes Containing Either a Chlorobiont or a Cyanobiont Layer

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Abstract

Several specimens of an unidentified species of *Sticta* have been found showing composite thalli ("photosymbiodemes"), with foliose lobes containing a chlorobiont layer that are borne upon a foliose thallus containing a cyanobiont layer. The collections were made in the Serra do Mar, Paraná State, Brazil. No apothecia, soredia, or isidia were observed on either type of lobe. The thallus containing the cyanobiont layer was always the primary component, i.e., the one attached directly to the substrate. The two thallus components were very similar morphologically; the one conspicuous difference was the color of the upper surface imparted by the different photobionts. When viewed from below the two types of lobes were indistinguishable, and no morphological discontinuity between them was evident. The morphological similarity of the lobes appears to contrast with the situation described in other species of Sticta reported to form alternative thalli or lobes in association with chlorobionts versus cyanobionts. Anatomical observations of thallus continuity, algal capture and lobe initiation support the contention that both lobe types are manifestations of a single mycobiont, and that the lobes acquire the alternative photobiont *de novo* from external sources.

Keywords: *Sticta, Nostoc,* cephalodia, photosymbiodeme

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1. Introduction

One of the more fascinating aspects of the lichen symbiosis is the capacity of certain species of lichen fungi, particularly those of the Peltigerineae, to form associations with both chlorophytes (green algae) and cyanobacteria (blue-green algae) as photosynthetic symbionts. In these associations, the cyanobionts may be confined to discrete, determinate structures (cephalodia) upon or within a thallus that contains a chlorobiont layer. In such cases, the cephalodial cyanobiont typically contain a notably high percentage of heterocysts, indicating their primary function as fixers of nitrogen (Hitch and Millbank, 1976). Alternatively, separate lobes or even physically separate thalli may be formed with either a chlorobiontic or a cyanobiontic layer, whose primary function is the production of photosynthate. The two types of lobe or thallus may differ conspicuously in morphology. These dual associations and their significance were first discussed in detail by James and Henssen (1976).

The formation of distinct thallus forms by single fungal species associating with different photobionts poses important questions concerning the morphogenetic and physiological interactions between the symbionts, as well as the ecological significance of the alternative associations. It also represents a major challenge to biosystematists (Jørgensen, 1998), since morphological characteristics of lichen thalli are frequently the basis of species- and genus-level distinctions. If the alternative forms occur independently, they may be easily misinterpreted as distinct species. Thus, it is important that newly discovered composite associations be documented. The present paper describes composite thalli in a species of Sticta from southern Brazil.

2. Materials and Methods

Thalli were collected on tree trunks and branches at the margin of Atlantic forest alongside highway BR-277 (km. 50–51) between Curitiba and Paranaguá in the Serra do Mar, Paraná State, Brazil. At the close of this study, additional collections were also examined from the Serra da Bocaina and Serra da Mantiqueira in Rio de Janeiro State, Brazil. Voucher specimens will be deposited in UFP, the herbarium of the Department of Botany, Universidade Federal de Pernambuco, Recife, Pernambuco State, Brazil.

Anatomical sections approximately 15 μm in thickness were prepared with a cryostat freezing microtome, mounted in water, and observed with the light microscope.

3. Results and Discussion

The gray-colored foliose thallus (cyanomorph) containing a cyanobiont layer was the thallus component which was attached directly to the substrate (Fig. 1). The lobes were irregular in size, ranging from about 2 to 15 mm broad. The upper surface was smooth, slightly undulating to rugose-ridged or impressed in places, particularly near margins of larger lobes. On the lower surface, the ridged areas often showed distinct, raised veins near the margins (Fig. 2). The lower surface was cream to rusty orange in color, with tomental hairs more or less straight, flattened, hyaline, glossy and translucent. The hairs were generally unbranched, although near the central portions of the thallus the tomental hairs often showed a proliferation of very short brush-like furcations near the tips. Some cilia-like hairs appeared along the margin in places, particularly on younger lobes. Cyphellae ranged in diameter from ca. 0.25–1.5 mm, with varying depth (Fig. 3).

Green, foliose, morphologically similar lobes containing a chlorobiont layer were seen emerging from the upper surface (Fig. 4), the margin (Fig. 6), and, most frequently, from the lower surface of the cyanomorph (Fig. 5). The young chloromorph lobes often had some tomental hairs, about 0.1–0.3 mm long, projecting from the margin. The base of the emerging lobes was constricted and often more or less terete at the point of attachment to the parent thallus. Where the green lobes emerged from the upper surface or margins, the basal attachment was often whitish, indicating the discontinuity of the algal layers (Figs. 6, 7). No morphological interruption of the lower surface was apparent where lobes emerged from the margins or lower surface of the parent thallus. The lower surfaces of the two types of lobes were indistinguishable from each other (Fig. 8); it was always necessary to turn the thallus over and view the color of the upper surface in order to distinguish them. No isidia, soredia or apothecia were observed on either thallus component.

The cyanomorph possessed a pseudoparenchymatous cortex, a lax medulla, and a pseudoparenchymatous lower cortex bearing tomental hyphae (Fig. 9). The photobiont appeared to be *Nostoc*; however, no culture studies were carried out. Incorporation of external chlorophyte cells by tomental hyphae was observed (Fig. 10). A comparable role in alternative photobiont capture was previously reported for the upper surface tomentum in *Pseudocyphellaria* (Renner and Galloway, 1982) and for the rhizines in species of *Nephroma* (Jordan and Rickson, 1971). Chlorophyte cells incorporated at the surface of the lower cortex were also observed (Fig. 11). Chlorophytic lobes emerging from the lower surface appeared approximately radial in symmetry, but soon began to express dorsiventrality in the initiation of tomental hairs and in the asymmetric proliferation of the photobiont (Fig. 12). Differential growth reoriented the primordium obliquely toward a more horizontal position; the

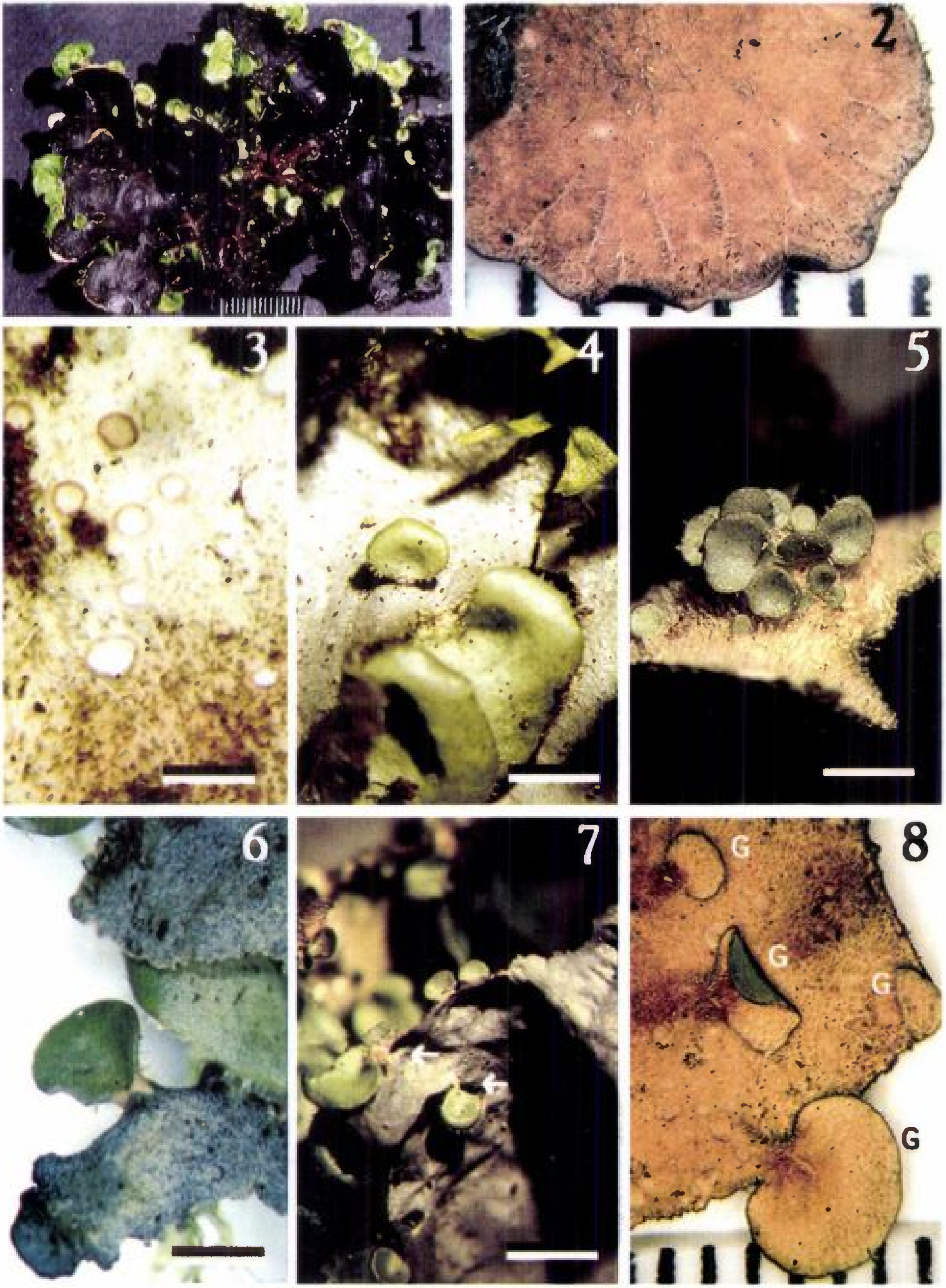

Figure 1. Composite thallus of *Sticta* sp. collected in the Serra do Mar. Thallus has been moistened to highlight color difference between parent thallus (grey-black) containing a cyanobiont layer and emergent lobes (bright green) containing a chlorobiont layer. Scale in mm.

surface with accelerated growth developed into the lower surface of the emergent lobe (Fig. 13). The anatomical continuity of fungal tissues between parent cyanomorph and emerging chloromorph supports the contention that both components are manifestations of the same mycobiont (Figs. 14, 15). The chloromorph showed an anatomy comparable to that of the cyanomorph, although their cortical cells often appeared to have somewhat thicker walls. Internal cephalodia were observed only in the larger green lobes (Fig. 16), suggesting that the chloromorph reacquired cyanobiont cells secondarily from external sources. In one instance, incorporation of cyanobiont cells was observed at the chloromorph lobe margin (Fig. 17).

The identity of the thalli could not be determined with certainty. The cyanomorph fits approximately the vegetative characters of *Sticta ambavillaria* (Bory) Ach. (see Galloway, 1995), recorded from central-southern Brazil by Wainio (1890). However, that species typically possesses abundant apothecia, which were not observed in the material collected in the present study.

The morphological similarity of the two thallus components observed in the present study appears to differ from the situation in other species of *Sticta* previously reported to produce composite thalli. In those *Sticta* composites the chloromorph is foliose while the cyanomorph is usually a branching fruticose structure which in its free-living manifestation had been previously referred to the lichen genus *Dendriscocaulon* (James and Henssen, 1976; Galloway, 1994, 1997, 1998). However, it is certainly possible that yet undiscovered pair relationships exist between the foliose (or stalked-foliose) Stictas currently considered to be distinct species. Joined thalli of two foliose taxa, *Sticta canariensis* (chlorobiont layer) and *Sticta dufourii* (cyanobiont layer) have been documented in the genus (James and Henssen, 1976), suggesting that

Figs. 2–8. See opposite page.

Figure 2. Lower surface of cyanomorph, showing tomentum and vein-like ridges near margin. Scale in mm.

Figure 3. Cyphellae on lower surface of cyanomorph lobes. Bar = 0.6 mm.

Figure 4. Chloromorph emerging from upper surface of cyanomorph. Bar = 2 mm.

Figure 5. Chloromorph emerging from lower surface of cyanomorph. Bar = 1 mm.

Figure 6. Chloromorph emerging from margin of cyanomorph lobe. Note whitish lobe base devoid of algal layer at juncture. Bar = 0.6 mm.

Figure 7. Chloromorph emerging from upper and lower surfaces and margin of cyanomorph lobes. Note whitish stalk-like lobe bases (arrows). Bar = 3 mm.

Figure 8. View of lower surface of parent cyanomorph, showing four emerging chloromorph lobes (G). Note morphological similarity and continuity of lower surface of both types of lobe. Scale in mm.

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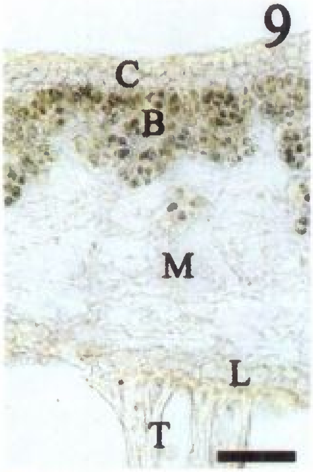

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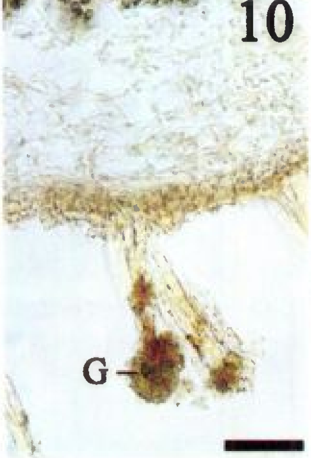

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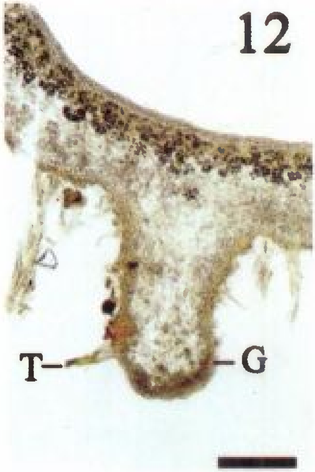

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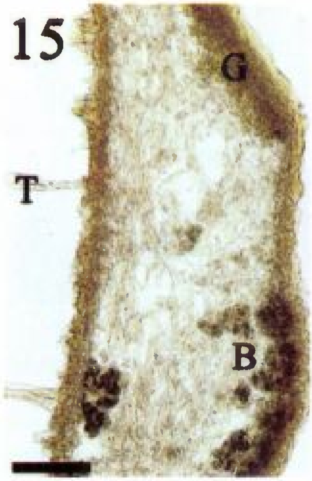

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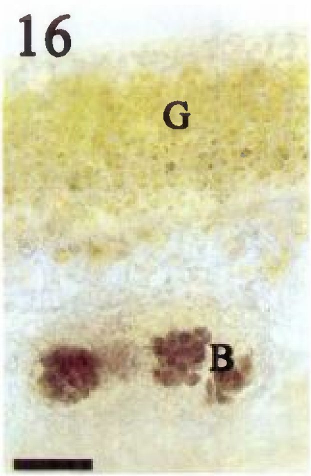

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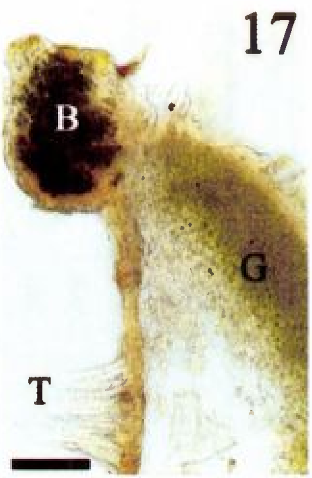

these two lichens are actually formed by the same mycobiont species. While *S. canariensis* and *S. dufourii* thalli are both foliose, they are morphologically quite distinct from each other, differing substantially in lobe shape, branching pattern, and margin morphology (Purvis et al., 1992; Galloway, 1995). The morphological similarity of the two lobe types in the present *Sticta* is perhaps more reminiscent of the patterns reported in *Peltigera aphthosa* (Brodo and Richardson, 1978; Paulsrud et al., 1998) or *Nephroma* (Tønsberg and Holtan-Hartwig, 1983). In the genus *Pseudocyphellaria*, joined thalli of closely similar foliose components differing mainly in the presence of a chlorobiont versus cyanobiont layer are also known (Renner and Galloway, 1982). That genus includes an additional dimension to the phenomenon: in thalli of certain species of *Pseudocyphellaria*, chlorobionts and cyanobionts co-occur in varying intermediate proportions apparently correlated with environmental parameters but seemingly without substantial alteration of thallus morphology (James and Henssen, 1976; Green et al., 1987).

Figs. 9–17. Cryostat sections of thallus, mounted in water. See opposite page.

Figure 9. Cyanomorph, showing cellular cortex (C), cyanobiont layer (B), medulla (M), and lower cortex (L) with tomentum (T). Bar = 43 µm.

Figure 10. Chlorobiont cells (G) captured at tips of tomental hairs on lower surface of cyanomorph. Bar = 43 µm.

Figure 11. Chlorobiont cells (G) incorporated into lower surface of cyanomorph. Bar = 17 µm.

Figs. 12–14. Development of chloromorph primordium from lower surface of cyanomorph. G, chlorobiont forming layer near tip of emerging lobe. T, tomental hairs forming on one side of emergent chloromorph lobe. U, future upper surface of lobe. L, future lower surface of lobe.

Figure 12. Emergence of cylindrical lobe primordium. Bar = 160 µm.

Figure 13. Reorientation of lobe by differential growth on one side of primordium which will define the lower surface of the lobe. Bar = 160 µm.

Figure 14. Detail of Fig. 13, showing continuity of fungal tissues between parent thallus and emerging lobe. Bar = 83 µm.

Figure 15. Longitudinal section through transition zone between parent cyanomorph and green lobe emerging along its margin. Micrograph is oriented with lower surface of thallus at left of figure. T, tomental hairs on lower surface. B, cyanobiont layer of parent thallus. G, chlorobiont layer of emerging lobe. Bar = 83 µm.

Figure 16. Larger (ca. 1.5 cm) green lobe with dense chlorobiont layer (G), and cells of cyanobiont (B) in cephalodial clusters within medulla. Bar = 27 µm.

Figure 17. Longitudinal section at edge of green lobe showing cells of cyanobacteria (B) incorporated into cephalodium at margin. Micrograph is oriented with lower surface of thallus at left of figure. G, chlorobiont layer. T, tomental hairs of lower surface. Bar = 43 µm.

It is not yet known whether the green lobes of the *Sticta* species reported here are capable of existing as independent thalli. The possibility that pair relationships may exist between independent "species" with chlorobiont versus cyanobiont layers will have to be considered in future attempts to understand the biosystematics of the Brazilian Stictas.

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