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SHORT NOTE

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A dorsiventral mycorrhizal root in the achlorophyllous *Sciaphila polygyna* (Triuridaceae)

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Abstract The star-like root system of the achlorophyllous *Sciaphila polygyna* (Triuridaceae) consists of roots up to 1.4 mm thick and 1 cm long seemingly radiating from a single origin. Internally, the roots show a bilateral symmetry when viewed in cross-section: the third root cell layer contains rather loose coils of the aseptate mycorrhizal fungus from the dorsal to the lateral sides, in contrast to the extremely dense coils of thin hyphae in its ventral part. Additionally, the hyphae develop vesicle-like swellings mainly in the central part of the dorsal side as well as the lateral parts of the third layer. The fourth root layer is anatomically heteromorphic, having exceptionally large cells, reaching up to 320×130 μm in size (giant cells), in the lower lateral parts. The root-colonizing hyphae only degenerate in the fourth layer, most readily in the giant cells, where they may swell to 24 μm in diameter, collapse and end as amorphous clumps. Hyphae in the third layer keep their definite structure. The structures are interpreted to be the result of a dynamic reaction of the root to the actual fungal penetration points in order to maximize the benefit from the subsequent colonization by compartmentation of the root tissue. The function of the third layer is to host the fungus and keep it alive within its cells, while mainly the giant cells serve for its digestion. Many indications suggest an arbuscular mycorrhiza for this association. Similarities and differences to other myco-heterotrophic species are discussed.

Keywords *Sciaphila* · Myco-heterotrophy · Arbuscular mycorrhiza · Root structure · Bilateral symmetry

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Introduction

The Triuridaceae, with six to seven genera and 45–73 species (Rübsamen-Weustenfeld 1991), are invariably characterized by achlorophylly and morphological reductions. Most species are delicate plantlets 5–15 cm in height and only *Sciaphila purpurea* Bentham can reach 1.40 m (Schultes and Cabrera no. 13449 after Maas and Rübsamen 1986; "4 feet and 2 inches" according to R. Spruce, cited after Bentham 1855). Maas-van de Kamer (1995) compiled all information available on the family and stated, that due to such extreme reduction of taxonomic characters its systematic position is hard to ascertain. They were classified in their own order (Cronquist 1988), superorder (Triuridanae, Thorne 1992), or even in their own subclass (Triurididae) by Takhtajan (1997). Embryological features suggest that this group can be placed between the Alismatiflorae and Liliiflorae (Rübsamen-Weustenfeld 1991), and not close to the superficially similar Burmanniaceae, as has often been suspected before. The molecular approach used by the Angiosperm Phylogeny Group (APG) (1998) did not allow the Triuridaceae to be assigned to any order, but in their recent update (APG 2003) the family is placed in the Pandanales.

In 1987 Mori et al. (no. 18631) collected three small achlorophyllous plants from Saül (French Guyana). One specimen was dried and stored in New York, the others were preserved in alcohol and sent to Hiltje and Paul Maas in Utrecht. They cautiously identified it as *Sciaphila* aff. *polygyna* Maas (Triuridaceae): "Assuming the variability of the number of male and female flowers is as large as described above, I think one should identify Mori et al. 18631 as aff. *S. polygyna* till more material of the species has been found. It is without doubt a *Sciaphila* species" (H. Maas, personal communication). In the monograph on the New World Triuridaceae (Maas and Rübsamen 1986) *S. polygyna* is the only species which, due to incomplete material available at that time, is not depicted with its subterranean parts. Reasonably, *S. polygyna* was suspected to be similar in its subterranean

parts to its relatives. But surprisingly, the root system of the specimens of Mori et al. were distinctly different, and not known in the family so far. Because of this deviating root morphology, and since the aberrant mycorrhizas of achlorophyllous plants are a rewarding subject for investigations (e.g. Imhof 1997, 1999a, 2001), one out of the two specimens from the Herbarium in Utrecht was supplied for this study.

Materials and methods

One fruiting specimen of *Sciaphila* aff. *polygyna* Maas in alcohol was supplied by the Herbarium of the University of Utrecht. It was collected by Mori et al. (18631) and has the following label data: The New York Botanical Garden/Plants of French Guiana/ no. 18631/ *Sciaphila* aff. *polygyna* Maas Det. P. Maas, 1993/Saül, 3°37′N, 53°12′W. Circuit Grand Boef Mort. ca. 300 m alt. Non-flooded moist forest. Stem white, pedicels and fruits deep pink. Collection also in ETOH./S. A. Mori, C. A. Gracie, A. E. Hartley & L. Raymond/3 August 1987.

The specimen was investigated for its external morphology, photographed, and then prepared for anatomical observations. After dehydration in an ascending ethanol series, four carefully detached roots were embedded in Unicryl (British Biocell International). Complete serial sections of 3–4 μm were prepared and stained with toluidine blue (1 g toluidine blue O+1 g sodium tetraborate in 100 ml distilled H_2O, after Harris in Krause 1927) and mounted in Corbit balsam. Staining for suberin was done on Unicryl-embedded sections using a saturated solution of Sudan IV in 70% ethanol, heated and differentiated with glycerol (Johansen 1940), and with the technique employing Oil Red O supersaturated isopropanol diluted to 60% with distilled water after Lillie (1944).

For microscopy, a Leitz DMRB microscope equipped with a photographic camera was used.

Results

Roots of the investigated specimen of *Sciaphila* aff. *polygyna* are brittle, up to 1.4 mm thick, and not longer than 1 cm. They form a star-like root system, with the roots seemingly radiating from a single origin. Four independently arising roots radiating into different directions were completely sectioned and the following descriptions hold for all of them, three prepared for cross-sections (Figs. 1, 2, 3, 4, 5, 6) and one for longitudinal sections (Figs. 7, 8, 9).

Beneath the hairless, persistent, but not fortified epidermis cells, measuring about 60 μm in all directions, the hypodermis cells are about 35 μm wide in cross-section but somewhat longitudinally elongated. Sudan IV and Oil Red O detected a faint suberin lamella, which justified calling it an exodermis (after von Guttenberg 1968; Peterson and Perumalla 1990). Short cells could not be distinguished. Hyphal penetrations by the aseptate, exclusively intracellular mycorrhizal fungus only locally pass these layers, and, except for these penetration points, epidermis and exodermis remain fungus free. In contrast, the subsequent third cell layer consisting of larger (80–100 μm each direction) cells become heavily colonized by coiled hyphae. But the first 400 μm following the series of cross-sections from the root tip is free of fungal colonization (Fig. 1). Not until then do the first hyphal coils composed of rather thin hyphae appear, always in the third layer, indicating the side of the root which is referred to as the dorsal side in the following. Shortly after the appearance of the first dorsal coils two other areas show hyphal colonization, located at both sides (lateral sides) in the same layer as the first coil but leaving several cells uncolonized in between (Fig. 2). Simultaneously the lower lateral cells of the fourth layer start to enlarge to enormous sizes (up to 320 μm radially, 130 μm tangentially, here called "giant cells"), whereas the other cells of the fourth layer are smaller than those in the third layer (Figs. 2, 3, 4, 5). Neither an external penetration nor colonization of any other cell layer had occurred so far. The successive penetration points are always (one exception, see Discussion) located at the dorsal side, initiated by a single, sometimes branched external hypha up to 25 μm thick attached to the root (Figs. 3, 4). Following the serial root sections further on in the same direction, the colonization gaps between the initially colonized three parts of the third layer (asterisks in Fig. 2) gradually become colonized as well (Fig. 3). Continuing in the same direction, the central dorsal and the lateral parts develop thicker hyphae and vesicle-like structures (Figs. 4, 7a), whereas the cells in between host coils of thinner hyphae (Fig. 7b), often attaining a peculiar bead-like appearance (Fig. 9a). This distinction is best visible on tangential sections (Fig. 7). Even more pronounced is the difference between the comparatively loosely coiled hyphae in the entire dorsal-lateral sector of the third layer (see Figs. 5, 7), and the much denser coils in cells of its

Figs. 1–6 Successive stages from root tip to root base of *Sciaphila polygyna*, combined from three different series of cross sections

Fig. 1 Root tissues not yet colonized by the fungus; root still showing radial symmetry. *Scale bar* =250 μm

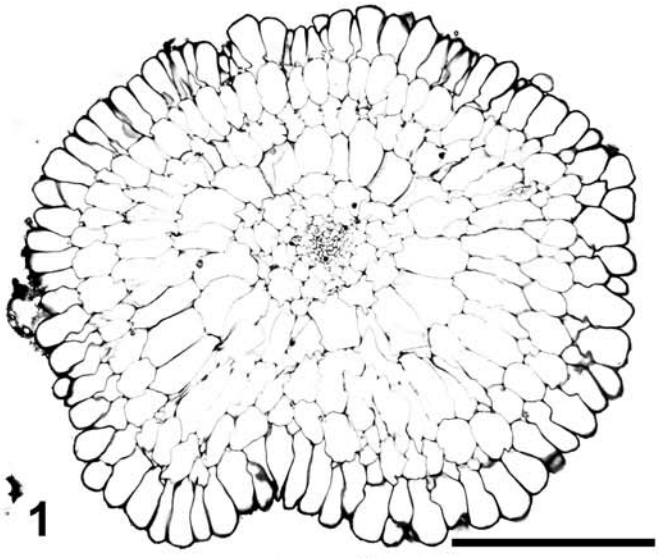
Fig. 2 First hyphae appear in the dorsal and lateral part of the third root layer (➞) with colonization gaps in between (*); heteromorphic development of the fourth layer (*4th*). *Scale bar* =250 μm

Fig. 3 Previously uncolonized gaps between the dorsal and lateral part of the third root layer are colonized. The dorsal cells of the fourth layer already contain hyphae, the giant cells (*g*) are evident, but not yet penetrated. *Scale bar* =250 μm. *ep* Epidermis, *ex* exodermis, *3rd* third layer, *h* external hypha

Fig. 4 Cross-section of the middle part of a root with penetration point (*p*), thick hyphae and vesicle-like structures in the dorsal and lateral parts of the third layer (➞), less pronounced in the cells between. g contain thick hyphae turning into amorphous clumps. The ventral part of the third layer is not yet fully colonized (*). *Scale bar* =250 μm

Fig. 5 Ventral part of the root now fully colonized. Note the structural difference between the hyphal coils of the dorsal and ventral side of the third layer. Due to the heteromorphic fourth layer, the central cylinder is often shifted dorsally. *Scale bar* =250 μm

Fig. 6 Close to the root base the dorsiventral anatomy fades. The ventral colonization fades earlier than the dorsal one, making the distinction between the coarser dorsal and denser ventral colonization pattern of the third layer more obvious through emerging colonization gaps (*). ➞ Show areas with thick hyphae and vesicle-like swellings. *en* Endodermis



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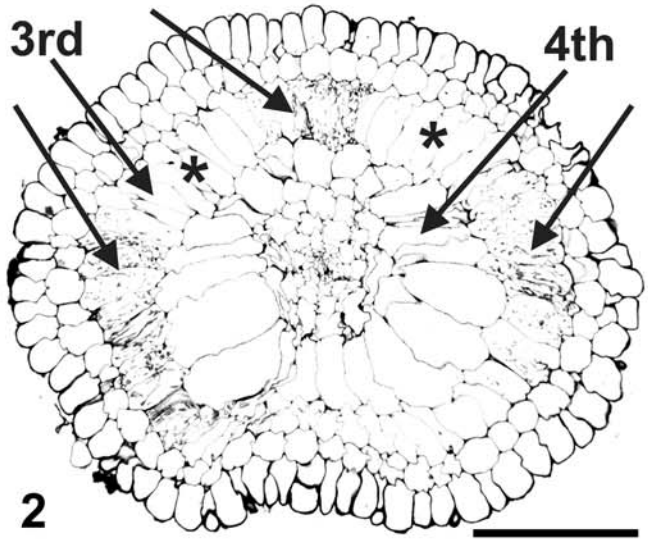

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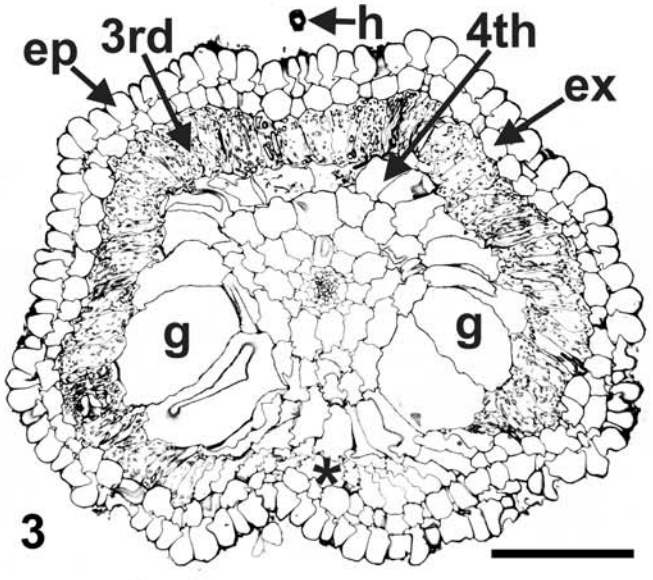

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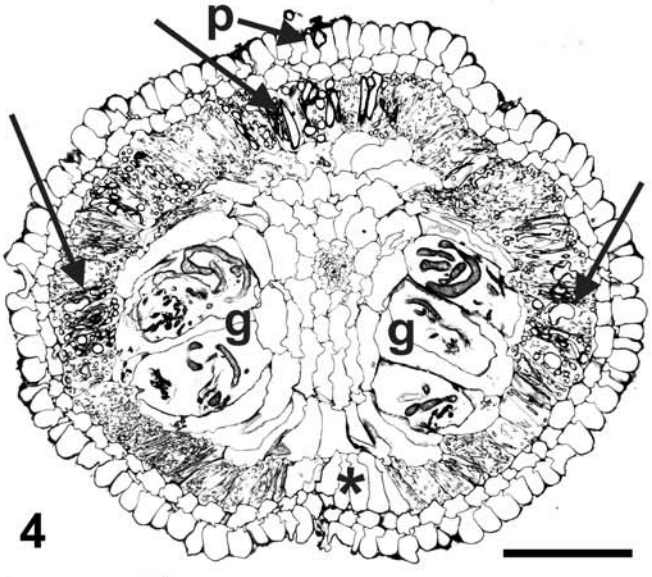

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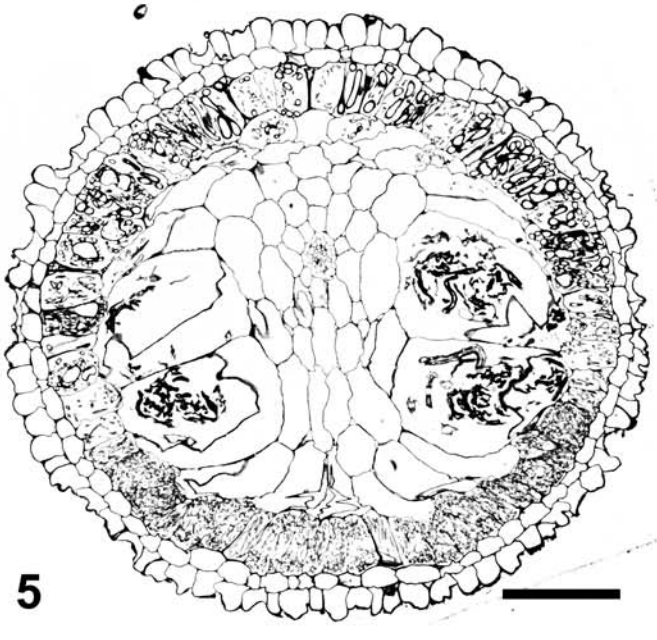

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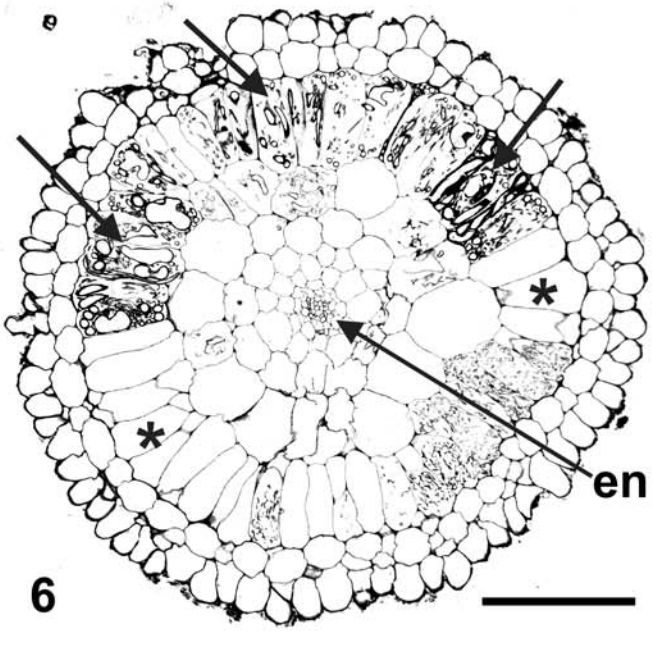

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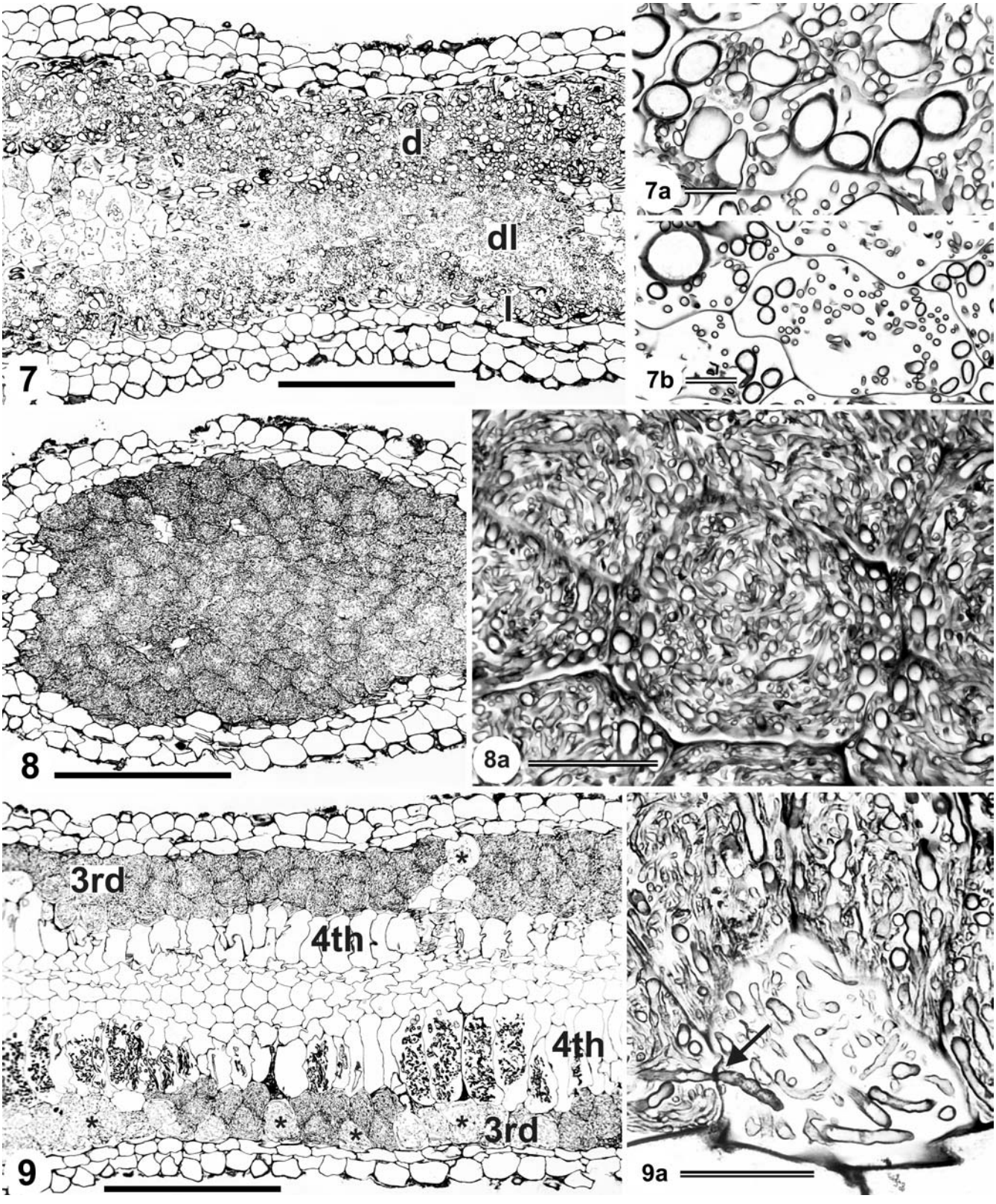

Fig. 7 Tangential section through the dorsal third layer, showing coils with thick hyphae representing the central dorsal part (*d*, detail in **a**, *scale bar* =20 μm) and coils of rather thin hyphae representing the cells between the central dorsal and the lateral parts (*dl*, detail in **b**, *scale bar* =20 μm). The thick hyphae in the lateral parts can just be seen on the lower edge of the depicted tissue (*l*). *Scale bar* =500 μm

ventral side, lacking hyphal swellings (Figs. 5, 8). Moreover, serial sections from the root tip show that the ventral third layer remains uncolonized for still many sections (Figs. 2, 3, 4), and colonization also fades earlier than in the dorsal part when approaching the root base (Fig. 6). Tangential sections through the ventral third of the root reveal the close but distinctly separated two different coil structures (Fig. 9). They are connected by hyphal transitions, albeit rare and inconspicuous ones (Fig. 9a).

The fourth layer at first (seen from the root tip to root base) is penetrated from the central dorsal side, where the cells as well as the hyphae of the fourth layer only slightly differ from those in the third layer (see Fig. 3). Later the giant cells receive hyphae from the lateral parts of the third layer. Within the giant cells the hyphae swell up to 25 μm in diameter, undergoing a deterioration process and ending as amorphous clumps (Figs. 4, 5). The dense coils in cells of the ventral side never project hyphae to the fourth layer; this is best seen on tangential sections through the ventral third of the root (see Fig. 9). Only in the fourth cell layer, mostly in the giant cells, do the hyphae show signs of degeneration; in the third layer the hyphae keep their definite structure (see Figs. 4, 5, 9). The inner cortex comprises some cell layers that are never colonized (Figs. 1, 2, 3, 4, 5, 6). The central cylinder is only about 60 μm wide, surrounded by an inconspicuous endodermis (see Fig. 6) with a very weak suberin lamella.

Due to the size of the giant cells and the non-concentric adjacent cell layers of the inner cortex, the entire root attains a dorsoventral orientation where even the central cylinder can be off its central position (see Fig. 5). The dorsoventral orientation is kept for almost the entire length of the root, most prominently in its middle segment. Close to the root base this orientation becomes less obvious and the heteromorphy of the fourth cell layer fades (Fig. 6). However, the distinction between the coarser coils with swellings on the dorsal and lateral side of the third layer and the dense coils on the ventral side is kept until the fungal colonization ceases. Moreover, the distinction even becomes more obvious due to the colonization gaps developing between the dorsal and the ventral side of the third layer (asterisks in Fig. 6).

Discussion

In order to understand the heterotrophic mode of life of achlorophyllous plants, examination of their subterranean parts is of particular interest. An achlorophyllous plant needs an external carbon source. Two possibilities for this are accepted by science so far: either the plant is parasitic, developing special organs (haustoria) penetrating living tissue of neighbouring plants in order to benefit from them (Weber 1993), or the plant is myco-heterotrophic (Leake 1994), living on carbon and nutrients provided by a mycorrhizal fungus. As *Sciaphila polygyna* has no parasitic organs, and given the highly complicated but consistent colonization pattern in this plant, one can conclude that it is myco-heterotrophic. Considering the hyphal features, which are aseptate, heteromorphic in terms of thickness, producing intracellular coils and vesicle-like structures, the mycorrhiza is strikingly similar to *Paris* -type arbuscular mycorrhizas (AM). This is confirmed by the fact that other specialized forms of this type of AM have been described for the myco-heterotrophic gentians *Voyria truncata* (Standl.) Standl. & Steyerm. (Imhof and Weber 1997), *V. tenella* Hook. (Imhof 1997), *V. obconica* Prog. (Imhof and Weber 2000) and *V. aphylla* (Jacq.) Pers. (Imhof 1999b), supported by molecular work which detected *Glomus* group A (Schüssler et al. 2001) in *Voyria* Aubl. and *Voyriella* Miq. (Miq.) species (Bidartondo et al. 2002). Arbuscules and vesicles were found in the achlorophyllous Burmanniaceae *Burmannia tenella* Benth. (Imhof 1999c) and *Dictyostega orobanchoides* (Hook.) Miers (Imhof 2001), whereas the unique mycorrhizal structure of *Afrothismia winkleri* (Engl.) Schlecht. (Imhof 1999a) was connected to a classic AM in a neighbouring root by direct hyphal bridges. Also, in *Sciaphila tosaensis* Mak., a sister species of the one investigated here, DNA sequence data of the root fungus revealed close proximity to, once again, *Glomus* species (Yamato 2001). Hence, there are good reasons to think that the mycorrhiza in *S. polygyna* is a specialized *Paris* -type AM.

The pattern of mycorrhizal colonization in *S. polygyna* described here is unique. Closest in terms of root anatomy and mycorrhizal structures is *Triuris hyalina* Miers (Triuridaceae), which, however, does not attain the degree of structural complexity found in *S. polygyna*. Whereas the third and fourth root layers in *T. hyalina* are uniform with respect to cell sizes and hyphal coil structure (Imhof 1998), these layers show different types of hyphal coils within a single layer and the fourth layer is anatomically heteromorphic in *S. polygyna*, resulting in dorsiventral anatomy when viewed in cross-section. Also the complex pattern in the longitudinal direction consistently found in all investigated roots is distinct from that of the uniform colonization in *Triuris hyalina*, as there is the tripartite colonization close to the root tip merging later into the also tripartite structure of the hyphal swellings, the precursory colonization of the third compared to the fourth layer, the "shorter" colonization of the ventral side compared to the dorsal side, as well as the limited occurrence of the giant cells along the root. The mycorrhiza of *S. polygyna*, therefore, is another good example showing the tendency of myco-heterotrophic plants towards a complex mycorrhizal pattern, developing struc-

Fig. 8 Tangential section through the ventral part of the third layer. Here only dense coils of thin hyphae occur. *Scale bar* =500 μm. Detail in **a**, *scale bar* =50 μm

Fig. 9 Tangential section through approximately the ventral third of the root. Note the clear distinction between cells with loose coils (belonging to the dorsal/lateral part;*) and the densely coiled hyphae in the cells of the ventral part of the third layer (*3rd*). In the fourth layer (*4th*), only the area close to the cells with loose coils is colonized, since the dense hyphal coils do not project hyphae to the fourth layer. *Scale bar* =500 μm. The detail in **a** shows a transition (→) between both types of colonization. *Scale bar* =50 μm

turally distinct root/rhizome compartments to separate tissues where the fungus is kept alive and where the hyphae are digested, thus achieving a sustained benefit from a few fungal penetrations (see also Imhof 1997, 1998, 1999a, 1999b, 1999c, 2001; Imhof and Weber 1997, 2000). In *S. polygyna* this compartmentation appears to be triggered by the fungal colonization itself. Examination of the complete series of sections indicated that the colonization of all investigated roots was due to one external hypha per root (there was one exception, see below) attached to one side of it, repeatedly penetrating the epidermis and exodermis from there, and thereby defining "dorsal" of the colonization pattern. Without fungal colonization, as seen in the root tip, the root has the usual radial symmetry. Not until the first hyphal coils appear in the cortex the dorsiventral root structure develops. In support of this finding, the only supplementary penetration, found once close to the ventral side near a root base, in fact disturbed the symmetry. The compartmentation in *S. polygyna*, in contrast to other compartmented mycorrhizal roots (Imhof 1997, 1999a, 1999b, 2001; Imhof and Weber 2000), hence, seems to be a dynamic reaction, in response to the actual site of penetration.

Discussing the function or purpose of structures inevitably is speculative. Nevertheless, the hyphal degeneration, especially in the giant cells, suggests that they are the main site of carbon inflow, possibly because the production of digestive enzymes can be concentrated where much fungal material is located. Keeping the fungus alive in the third root layer is beneficial as it achieves a sustained use of the root fungus (see Imhof 1997). Other features, namely the distinction between the dorsal and ventral coil structure and the inability of the latter to pass the fungus to the fourth layer, as well as the tripartite appearance of thick hyphae and vesicle-like structures in the third layer, remain unexplained so far.

Yamato (2001) also reports the presence of bead-like hyphae similar to those described in this paper. Because of its aberrant appearance, Yamato (2001) suspects a second, saprophytic fungus in these structures. However, direct transitions from beaded to non-beaded hyphae in *S. polygyna* proves that the two different types of hyphae belong to the same fungus, and this possibility should be checked in *Sciaphila tosaensis* again.

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