

## Ectomycorrhizae of *Phellodon niger* on Norway spruce and their chlamydospores\*

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**Abstract.** The ectomycorrhizae of *Phellodon niger* are comprehensively described and compared to other ectomycorrhizae of Thelephoraceae species. *P. niger* ectomycorrhizae are distinguished from all previously described ectomycorrhizae by the unique shape of their chlamydospores.

**Key words:** Ectomycorrhizae – Characterization – Identification – Chlamydospores – *Phellodon niger*

### Introduction

The only three ectomycorrhizae of the family Thelephoraceae which have been comprehensively described are those of *Hydnellum peckii* (Agerer 1992), *Sarcodon imbricatus* (Agerer 1991a) and *Thelephora terrestris* (see Agerer and Weiss 1989; Schramm 1966). Shorter descriptions exist for a few other species (cf. Agerer 1991a).

Methods to characterize ectomycorrhizae have been previously explained by Agerer (1986, 1987–1991, 1991b); a glossary of terms has already been published (Agerer 1987–1991).

### *Phellodon niger* (Fr.: Fr.) Karst

Reference specimen: Germany, Bavaria, Riedberg bei Mittenwald, ca. 1500 m NN, under *Picea abies*, leg. R. Treu, 25 August 1987, fruit body and ectomycorrhizae in Herb. RA 11270 (in M).

### Morphological characters (Fig. 1a)

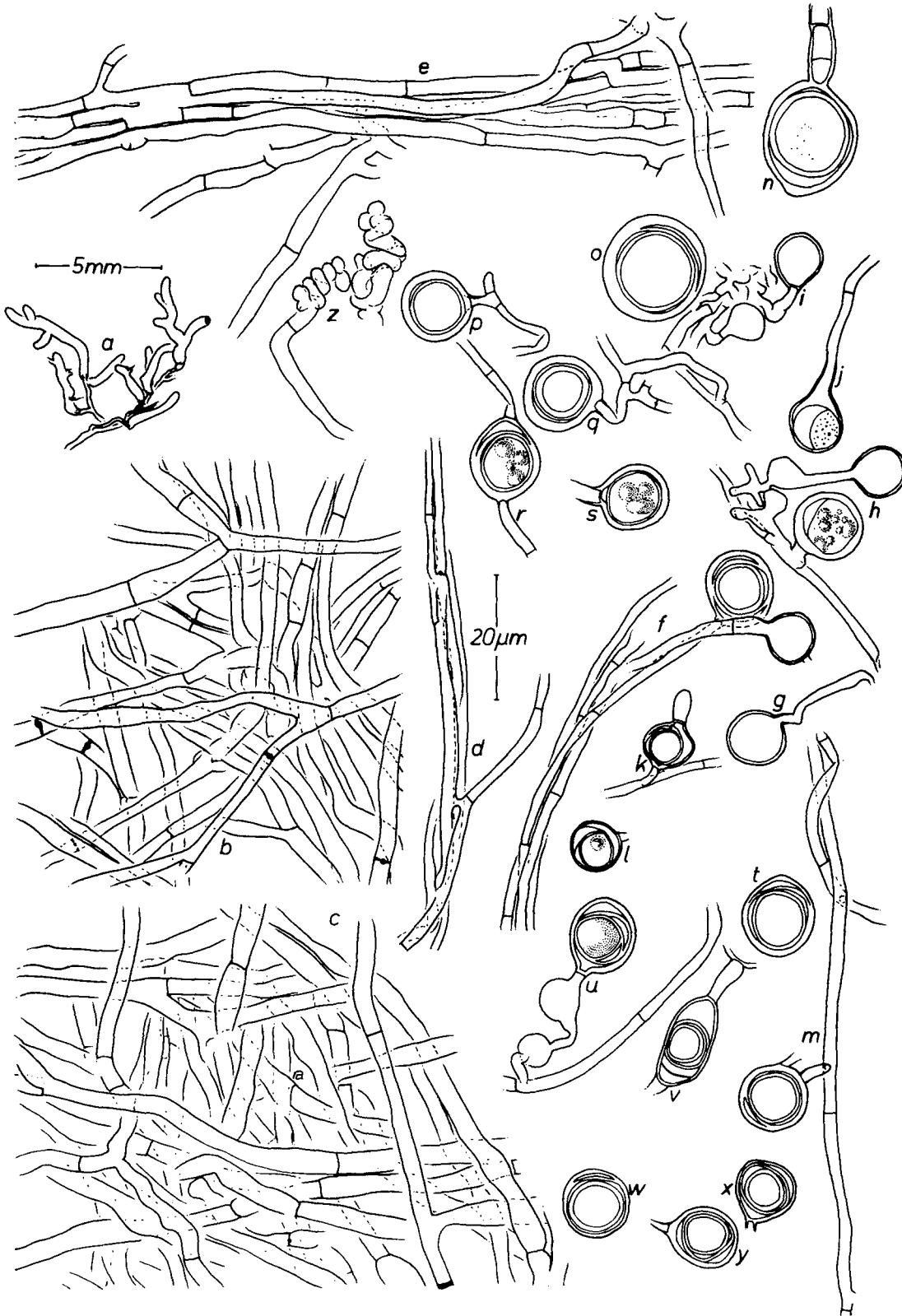
Ramification irregularly pinnate, but very often mycorrhizae occurring solitarily or infrequently with a single

side branch; mycorrhizae embedded in a dense complex net of hyphal fans and rhizomorphs which completely envelope soil particles; often mycorrhizal tips appearing through the dense mycelium; shorter unramified ends approximately straight, others slightly bent or seldom slightly tortuous, up to 2.8 (3) mm long and 0.32–0.39 mm in diameter; mycorrhizae carbonizing, i.e. mycorrhizal mantle lost rather early together with parts of the cortex, leaving a rather thin central part of the root 0.19–0.20 mm in diameter; axes 0.32–0.39 mm in diameter or, after breaking off of cortex, 0.20–0.26 mm in diameter; surface of unramified ends silvery, slightly grainy or sometimes slightly stringy, with a glistening (due to mineral soil particles glued to the surface), silvery-white appearance (due to trapped air between the hyphae) on a brown to blackish root, extreme tips whitish and glistening, older parts blackish with a yellow covering. Rhizomorphs very frequent but often enveloped by dense mycelium, white to yellowish, mostly growing off obliquely from the mantle, variable in diameter, dividing repeatedly into smaller filaments, often rather flat in cross-section, margin not definite.

### Anatomical characters of mantle in plan views (Figs. 1b, c, 2)

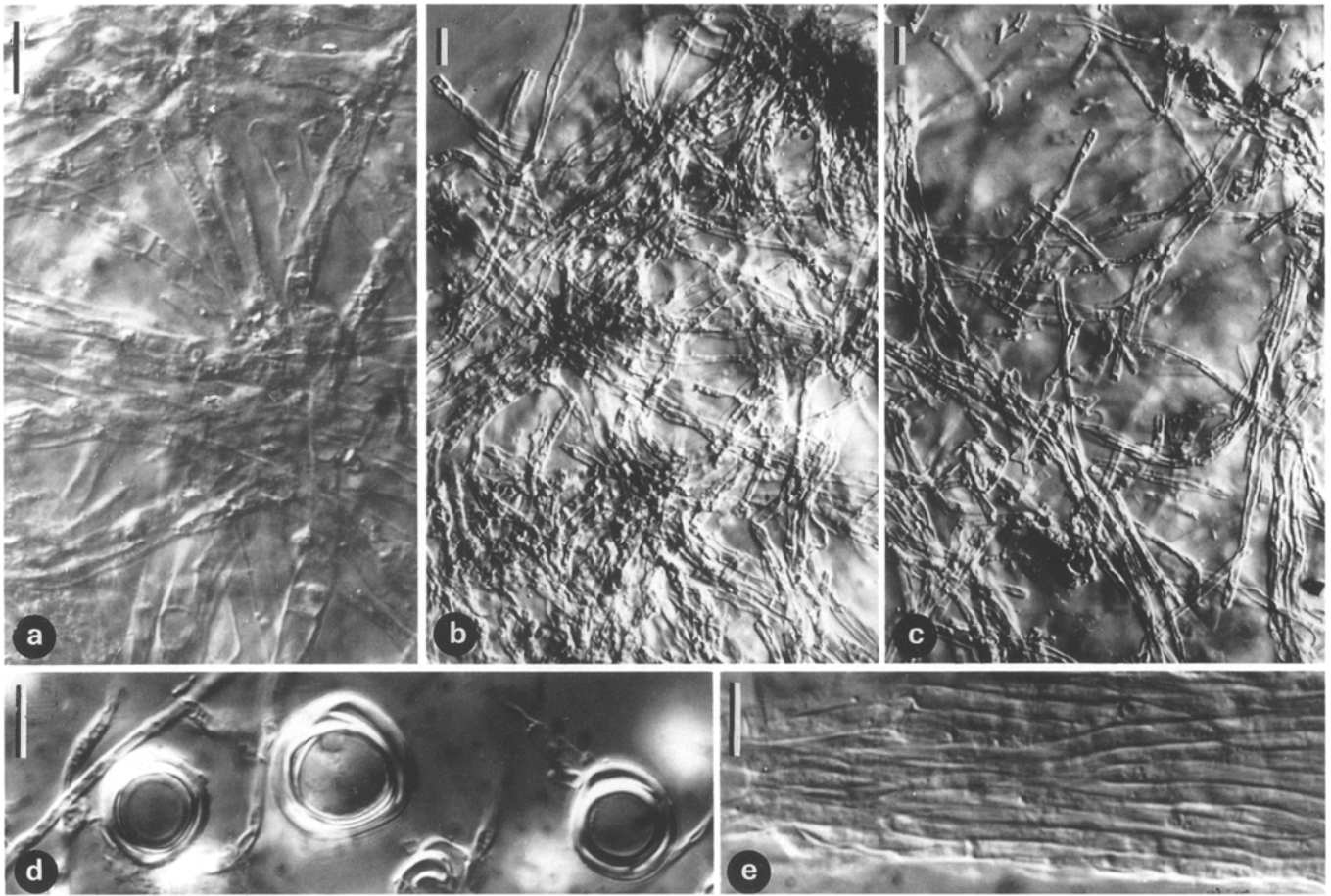
*Outer surface of mantle* (Figs. 1b, c, 2a–c) loosely plectenchymatous, net-like (Agerer 1991b: type A), hyphae 1.5–2 (2.5)  $\mu\text{m}$  in diameter, slightly thick-walled, up to 0.5  $\mu\text{m}$  thick, partially slightly gelatinous, hyphae slightly membranaceously brownish, clamps lacking, some septa very thick and bearing a central globular thickening. *Middle layers of mantle* more densely plectenchymatous than outer surface, hyphae embedded in a gelatinous matrix, hyphae star-like in arrangement, 2–3 (3.5)  $\mu\text{m}$  in diameter, slightly membranaceously brownish, slightly darker than the hyphae of the mantle surface, walls only slightly thick, less than 0.5  $\mu\text{m}$ . *Inner surface of mantle* densely plectenchymatous, distinctly star-like in arrangement, hyphae in part embedded in a gelatinous matrix, otherwise densely aggregated, slightly thick-

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**Fig. 1.** a Habit. b, c Plan view of mantle surface with net-like arrangement of hyphae, showing some thick septa with central globular thickenings. d, e Thin rhizomorphs. f Thin rhizomorph bearing a chlamyospore. g-y Ontology of chlamyospores with different stages of development. g-j Very young stages with only

an outer thicker wall or with one or two asymmetrical walls. k-l Chlamyospores with several asymmetrically eccentric and still rather thin walls. m-y Chlamyospores with a thick outer wall. z Cork-screw like distal ends of emanating hyphae. a-z from RA 11270



**Fig. 2.** **a** Plan view of mantle surface with a “star” formed by hyphae. **b** Plan view of mantle surface with stellate pattern of hyphal arrangement. **c** Plan view of mantle surface with thin emanating

hyphae. **d** Chlamydospores. **e** Plan view of a rhizomorph near its surface. **a–e** from RA 11270; bar = 10  $\mu$ m

walled, darker than hyphae of outer layers, (2) 2.5–4 (5)  $\mu$ m in diameter. *Surface of tip* distinctly gelatinous, no pattern in hyphal arrangement discernable. *Rhizomorphs* (Figs. 1d–f, 2e) at least up to 200  $\mu$ m in diameter, gelatinous, enveloped by many thin, loose hyphae, hyphae in rhizomorphs growing approximately in parallel, no differentiation discernable (Agerer 1991b; type A/B), very slightly brownish or colourless, hyphae 2–2.5 (3.5)  $\mu$ m in diameter, clampless, anastomoses open. *Emanating hyphae* (Fig. 2c) variably thick, even within a single hypha, (2) 2.5–3.5 (4.5)  $\mu$ m in diameter, surface smooth, if rough then caused by adhering mineral soil particles, septa 5–15  $\mu$ m apart, sometimes more than 100  $\mu$ m, septa variably thick, hyphae slightly thick-walled (up to 0.5  $\mu$ m), colourless or at most proximally at most slightly membranaceous brownish, in part with small outgrowths resembling incomplete clamps, some distal ends shaped like a cork-screw (Fig. 1z). *Cystidia* not found.

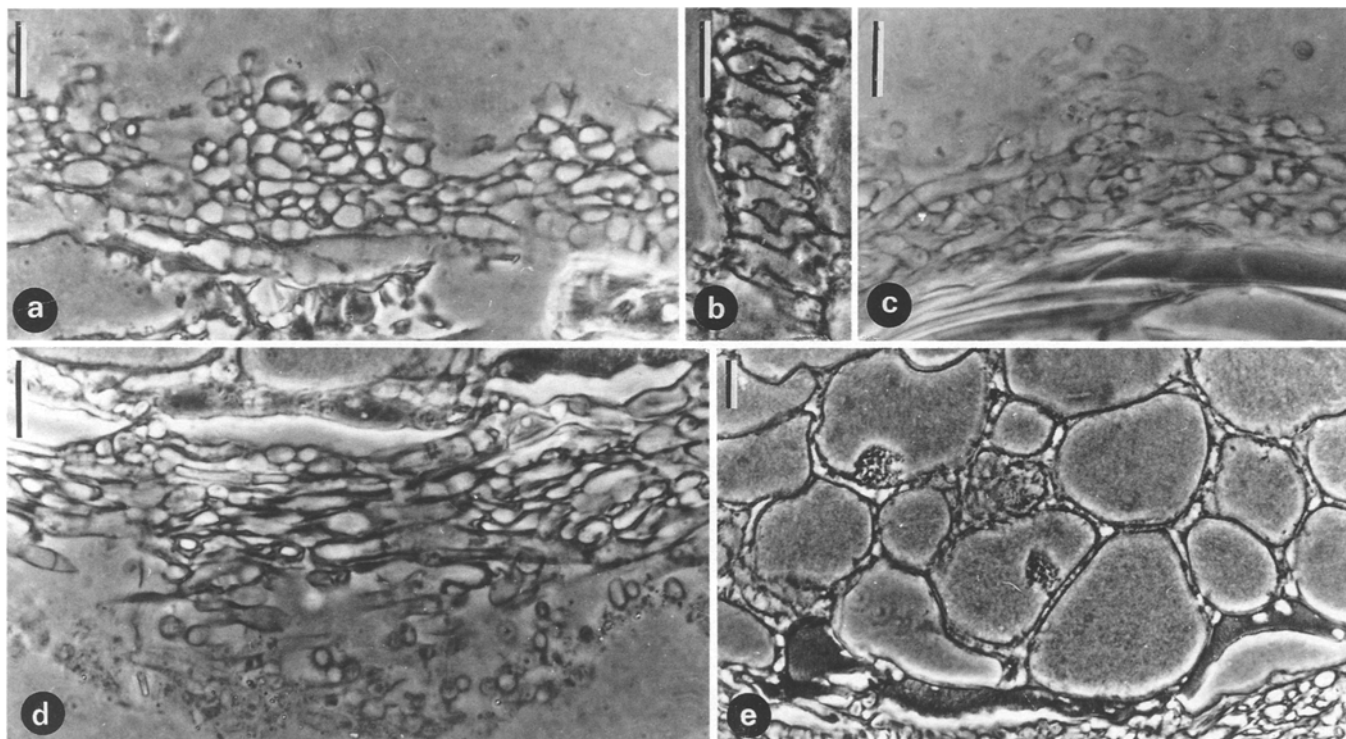
#### *Chlamydospores* (Figs. 1f–y, 2d)

Roundish, smooth, terminally, or mostly intercalarily formed; chlamydospore proper produced endogenously,

enveloped by several walls built alternately asymmetrically, later often splitting between consecutive walls; outer wall slightly expanding in lactic acid; chlamydospore proper slightly yellow, roundish, 8–10  $\mu$ m in diameter inclusive of a ca. 0.5- $\mu$ m-thick wall, diameter inclusive of enveloping walls 10–13.5  $\mu$ m; subtending hyphae of chlamydospores could be traced to rhizomorph and to mantle hyphae.

#### *Hyphae of stipe base*

Hyphae of stipe base close to substrate, 3–4  $\mu$ m in diameter, brownish, in KOH slightly greenish-brown; further up hyphae encrusted by black granules; hyphae of outer stipe base curled, loosely woven; in inner parts hyphae densely arranged in parallel; all hyphae clampless; no chlamydospores found. Apart from the black granules, inner stipe hyphae resemble those of inner mantle layers of older ectomycorrhizae in diameter, thickness of walls, and colour; no similarity between outer stipe base hyphae and outer mantle hyphae.



**Fig. 3.** **a** Longitudinal section, mantle composed of a series of heaps of densely aggregated hyphae with loosely arranged hyphae in between. **b** Longitudinal section, Hartig net in plan view. **c** Longitudinal section, mantle of very tip. **d** Cross-section, mantle

with heap-like aggregated hyphae and covered by loosely arranged emanating hyphae. **e** Cross-section from inner mantle layers to cortex cells close to endodermis. **a-e** from RA 11270; *bar* = 10  $\mu\text{m}$

#### *Anatomical characters, cross-section (Fig. 3d-e)*

**Mantle** (Fig. 3d) plectenchymatous throughout, 20–30  $\mu\text{m}$  thick; close to root surface with conical heaps of densely aggregated, rather thick hyphae, radially 3–4 (6)  $\mu\text{m}$  in diameter, tangentially (3) 5–10 (12)  $\mu\text{m}$  long, walls distinct; hyphae between the heaps rather loosely arranged, embedded in a gelatinous matrix, radially (1) 1.5–2.5 (4)  $\mu\text{m}$  in diameter, tangentially 3–10 (20)  $\mu\text{m}$ , that type of hyphae also covering the conical heaps, radially 1.5–2.5 (3)  $\mu\text{m}$ , tangentially 3–15 (30)  $\mu\text{m}$ , embedded in a gelatinous matrix. **Tannin cells** (Fig. 3e) tangentially oval, 0–1 rows, tangentially (15) 23–47 (56)  $\mu\text{m}$ , radially (2) 5–18 (25)  $\mu\text{m}$ ,  $\text{TCt} = 32.7 \mu\text{m}$ ,  $\text{TCq} = 4.1$ , often not densely filled by tannin granules; calyptra cells often prominent, hardly distinguishable from tannin cells in cross-sections (but see longitudinal section), in part embedded in mantle; Hartig net 2–3  $\mu\text{m}$  thick, composed mostly of one row of hyphal cells; hyphal cells in section oval. **Cortical cells** (Fig. 3e) tangentially or radially oval to elliptical, tangentially (11) 17–35 (45)  $\mu\text{m}$ , radially 10–37 (45)  $\mu\text{m}$ ,  $\text{CCt} = 25.4 \mu\text{m}$ ,  $\text{CCq} = 1.1$ ; Hartig net 2–3 cortex cell layers deep (tannin cells included), mostly not reaching endodermis, often innermost cortex cell row free of Hartig net; Hartig net (1.5) 2–2.5  $\mu\text{m}$  thick, composed of one row of hyphal cells; Hartig net cells cylindrical, of variable length.

#### *Anatomical characters, longitudinal section (Fig. 3a-c)*

**Mantle** (Fig. 3a) corresponding to cross-section; mantle of mycorrhizal tip 20–25  $\mu\text{m}$  thick, hyphae of inner two-thirds with distinct walls, outer one-third with thin walls, outer hyphae embedded in gelatinous matrix, heaps distinct; hyphal sections of variable dimensions, radially (1.5) 2–3  $\mu\text{m}$ , tangentially 2–15 (25)  $\mu\text{m}$ . **Tannin cells** irregularly cylindrical to oval, slightly oblique to root surface, not densely filled with tannin granules, with distinct rather thick residues of calyptra cells, tangentially (20) 31–70 (79)  $\mu\text{m}$ , radially 5–15 (30)  $\mu\text{m}$ ,  $\text{TCt} = 50.4 \mu\text{m}$ ,  $\text{TCq} = 4$ . **Cortical cells** cylindrical to oval, slightly oblique to root surface, tangentially (14) 20–73 (91)  $\mu\text{m}$ , radially (10) 14–35 (39)  $\mu\text{m}$ ,  $\text{CCt} = 46.5 \mu\text{m}$ ,  $\text{CCq} = 2.2$ . Hartig net (Fig. 3b) in plan view of a weakly ramified palmetti type, lobes rather broad (1.5) 2–3  $\mu\text{m}$ .

#### *Colour reaction in different reagents*

**Mantle and rhizomorph preparations:** acetic-fuchsin: --; aniline: walls distinctly pink; brilliant cresyl blue: --; cotton blue: --; ethanol 70%: --; guaiac: --; iron sulphate: --; KOH 15%: hyphae in thick layer of young ectomycorrhizae reddish-brown; Melzer's reagent: --; phenol: --; phenol-aniline: --; Sudan-glycerine: --; sulpho-vanillin: brownish, walls not dissolving. *Whole ec-*

*tomycorrhizae*: in KOH 15%: black with ochre-brownish covering.

#### Autofluorescence

*Whole mycorrhiza*: UV 254 nm: --; UV 366 nm: --. *Mantle and rhizomorphs*: UV-filter 340–380 nm: slightly bluish-green, in part contents of chlamydospores autofluorescing bluish; blue filter 450–490 nm: slightly reddish-yellow (rhizomorphs), slightly reddish-brown (mantle); green filter 530–560 nm: slightly reddish.

#### Staining of nuclei

Aceto-carmin: two nuclei per cell, up to 10 µm apart, round, ca. 1.5 µm in diameter; central thickenings of septa stain similarly to nuclei; siderophilous granules not found.

#### Material studied and method of identification

Only the reference specimen could be studied. Identification was done by tracing several rhizomorphs from the ectomycorrhizae to the fruit body stipe base. For stipe base hyphae: Germany, Baden-Württemberg, Ulm-Wiblingen, in Gögglinger Wäldchen, in moist spruce forest, leg. Enderle, 26 September 1982, det. Maas Geesteranus (in M).

#### Discussion

*P. niger* ectomycorrhizae were described by Otto (1989), who found monopodial greyish-black or black *Picea* ectomycorrhizae with similar dimensions and with identical appearance covered with a greyish-white mycelium composed of hyaline clampless, ca. 2.5-µm-thick hyphae. In addition, he found black but stouter ectomycorrhizae, which he argued also belonged to *P. niger*. In these samples he found the typical thicker hyphae (ca. 5 µm) on the root surface, enveloped by thinner ones as described above for the reference specimen. These ectomycorrhizae were probably only older specimens that lacked rhizomorphs. Unlike our observations, Otto neither described the ectomycorrhizae as carbonizing, nor did he mention chlamydospores.

An extensive discussion of the ectomycorrhizae of Thelephoraceae and Bankeraceae described to date is given by Agerer (1991a, 1992). Therefore, only the more important characteristics of *P. niger* ectomycorrhizae are discussed in comparison to those species which have already been described in detail.

The chlamydospores are the most striking feature of *P. niger* ectomycorrhizae. Chlamydospores have already been described from *Bankera fuligineo-alba* (Danielson 1984), *Sarcodon imbricatus* (Agerer 1991a), *Boletopsis leucomelaena* (Agerer 1991a), *Hydnellum peckii* (Agerer 1992), and from *Gomphidius roseus* in ectomycorrhizae

of *Suillus bovinus* (Agerer 1990, 1991c). Only the chlamydospores of *P. niger* show the characteristic asymmetrically concentric walls around the chlamydospore proper. The chlamydospores of *B. fuligineo-alba* are only 5–6 µm in diameter and have only slightly thickened walls (Danielson 1984); those of *S. imbricatus* possess hollow outgrowths (Agerer 1991a), whereas *H. peckii* shows an inner wall and an outer thick wall which splits radially into warts (Agerer 1992). *B. leucomelaena* is characterized by an oidia-like formation of cells each containing a chlamydospore (Agerer 1991a), whereas those of *G. roseus* are smooth, and characterized by a thick yellow wall; they are formed within the mantle of *S. bovinus* ectomycorrhizae (Agerer 1990, 1991c).

The ectomycorrhizae of *T. terrestris* lack chlamydospores and, in contrast to those of *P. niger*, possess thick-walled cystidia. *S. imbricatus* ectomycorrhizae differ not only in the type of chlamydospores but also in the presence of clamps and the occurrence of septated anastomoses. Ectomycorrhizae of *Tomentella*, described by Danielson (Danielson et al. 1984; Danielson and Pruden 1989) possess either prominent cystidia or an epidermoid mantle in surface view. The rhizomorphs of *P. niger* differ from both *T. terrestris* and *S. imbricatus* by their gelatinous matrix and by the lack of clamps, from that of *H. peckii* by the lack of clamps and by the absence of inflated hyphal parts.

Very characteristic of *P. niger* ectomycorrhizae is their carbonized appearance. The mantle and cortex cells of older parts of the ectomycorrhizae break off and only a thin central part of the root remains, whereas the ectomycorrhizal tip is still turgescient and alive. This feature is only known so far from *P. niger* and *H. peckii* (Agerer 1992).

Unlike *H. peckii* (Agerer 1992), *P. niger* ectomycorrhizae do not possess the peculiar characteristic of forming blue granules in the inner mantle surface after treatment for several weeks in lactic acid.

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