Costiferous Hyphae and Related Cell Structures in *Paxillus* (Boletales)

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Introduction

Microscopic examination of some hundred European collections of *Paxillus* (belonging to the section *Paxillus*) revealed the existence of an uncommon hyphal type characterized by transverse ribs located at the inner surface of the wall. The present paper describes possible development and the occurrence of these and related structures in *Paxillus*.

Material and methods

The collections studied are deposited in Leiden (L), Munich (M), Stockholm (S), Kew (K) and Lausanne (LAU) under the names *Paxillus filamentosus, P. leptopus, P. rubicundulus* (including type specimen) and *P. involutus*. For ease of reference to the herbarium collections all these names will be used here, despite the fact that the epithets *"leptopus"* and probably also *"rubicundulus"* are synonyms of *filamentosus*.

A small fragment of the gills is soaked for 5 to 15 minutes in concentrated ammonia or preferably in a solution called "Kanamoa" (KOH-NaCl buffer 55 ml; glycerol 15 g; ethylene glycol monomethyl ether, Merck 859 25 ml).

The KOH-NaCl buffer is prepared by dissolving 3.6 g of KOH and 3.8 g of NaCl in 420 ml of distilled water. Merck 859 is called 2-methoxyethanol, glycol monomethyl ether or methyl cellosolve by some workers. Final pH is about 12.5 to 12.6.

The soaking process is necessary because the trama of *Paxillus* species becomes subgelatinous in age and then gets rather hard on drying. It is not necessary when working with fresh material.

It is most recommended to stain the soaked or fresh fragment thoroughly by immersing it for a few minutes in a 1% solution of Congo red in weak ammonia. It is then crushed between slide and coverglass in a drop of colourless ammonia or better in "Kanamoa". Costiferous hyphae may escape detection in unstained or poorly squashed preparations. Phase contrast is essential for detection of spuriously ribbed costiferous hyphae, which may appear structureless in bright field, even after staining.

Morphology, properties and possible origin of the costiferous hyphae in *Paxillus*

Mature costiferous hyphae are empty, clamped, septate, cylindrical with a slight constriction at the septa, and bear characteristic ribs at the inner surface of the thin wall. These ribs consist of open

or closed, often fragmented, roughly transverse rings of unknown chemical composition. They are 1–2 μm wide and up to 1–2 μm high, and they may be present over most of the hyphal cell or may cover only a limited part of it.

The ribs are strongly stained with Congo red and calcofluor white M2R, but only weakly with cotton blue. They are not stained with toluidine blue, eosin, iron-aceto carmine, alcian blue and ruthenium red. The ribs are dissolved instantly by concentrated sulphuric acid, but they resist 1% chromic acid and 5% KOH applied at 70° C for 24 hours. They are equally resistant in concentrated chloral hydrate solution and in lactophenol.

The thickness, and therefore the visibility of the ribs varies greatly from one hypha to the other. The faintest ribs are visible only in phase contrast, whereas fully developed ribs can easily be seen with medium power objectives and without any staining.

From my observations it is possible to speculate on the origin of the costiferous hyphae by arranging the different supposedly ontogenetic steps into a logical sequence. It seems that costiferous hyphae derive from hyphae with a homogeneous, brownish content that stains with iron-aceto carmine but does not stain with Congo red. Their wall is thin and devoid of internal ribs. Occasionally some cells of such hyphae show a faint transversal striation in phase contrast over a limited region. This striation is interpreted as primordial ribs being deposited at the internal side of the hyphal wall. As the ribs grow thicker and get more visible, the aspect of the hyphal content becomes more and more heterogeneous, and irregular cavities form near the hyphal axis. While the ribs thicken gradually, the hypha looses its brownish content, so that the material of the ribs seems to be modified hyphal content. The modification becomes manifest not only in the more condensed aspect of the substance, which has visibly a higher refractive index and becomes birefringent, but also in a change of the staining properties, as already indicated above.

When herbarium material is strongly crushed it happens sometimes that the brownish hyphae break neatly and almost exactly at a right angle to the cell axis. When this happens, the hyphal content does not immediately dissolve in the mounting medium, but swells moderately (figure 1). Brownish hyphae with primordial ribs often break in the same way, and the breaking line is always parallel to the ribs. This suggests that the hyphal wall has a transversal, submicroscopic texture, and that the ribs are formed along it.

The brownish hyphae originate from vegetative hyphae. In some collections, e. g. in *Paxillus involutus* [leg. Bresinsky, Sept. 4, 1968, at Landsberg, Bayern (M)], the transition of vegetative hyphae into

hyphae with brownish content can be seen within about 100–200 μm in a single hyphal cell.

Occurrence and distribution of costiferous hyphae

Most costiferous hyphae are found in the gill trama. I have never seen them in the pileipellis or the context of the cap, and only rarely in the stipitipellis and in the context of the stipe.

In the following table a distinction is made between spurious, weak and strong ribs. Spurious ribs are visible only in phase contrast (figure 2), weak ribs are visible in bright field after staining but they are under ¼ μm thick (figure 3), and strong ribs are visible without staining at medium power already (figure 4).

Roughly one out of seven collections has costiferous hyphae. Within those a general trend can be observed. Spurious ribs are more common than weak ribs, and strong ribs are rather rare. With regard to individual fruit bodies, it can be noted that basidiomata with many strong ribs (collections 7, 9, 13) also have many weak ribs but have no or only infrequent spurious ribs. On the other hand, collections 2, 3, 6, 10 with many spurious ribs have no strong ribs

Table 1: Costiferous Hyphae in *Paxillus* (gill trama)

Nr	Collection	spurious ribs	weak ribs	strong ribs
	P. involutus			
1	Bas 5329 (L)	infrequent	–	–
2	Bas 5807 (L)	frequent	–	–
3	Stangl 911/70 (M)	frequent	–	–
4	Fungi exsiccati Suecici no 23 (S)	–	frequent	–
	P. filamentosus			
5	Hakelier 1979 (S)	infrequent	infrequent	–
6	Suber 1966 (S)	frequent	frequent	–
7	Daams 7306 (S)	–	frequent	frequent
8	Einhellinger 1962 (M)	infrequent	–	–
9	HC 82/177 (LAU)	infrequent	frequent	frequent
10	Fungi exsiccati Suecici no 408 (S)	frequent	frequent	–
	P. leptopus			
19	Bresadola 1899 (S)	infrequent	–	–
	P. rubicundulus			
12	Bas 4760 (L)	infrequent	–	–
13	Bas 5325 (L)	–	frequent	frequent
14	Bas 7306 (L)	infrequent	–	–
15	Orton 2905, Type (K)	–	frequent	–

and only occasionally many weak ribs. Collections 1, 8, 11, 12 and 14 with infrequent spurious ribs lack other ribs. Finally, collections 4 and 15 have only weak ribs, but in great quantities.

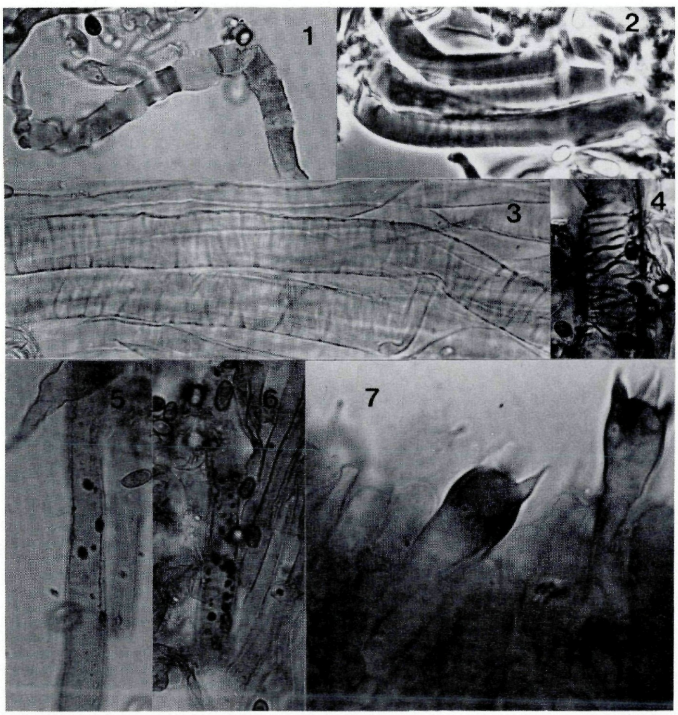
It is possible that fruit bodies begin tardively to deposit ribs, and that most have been collected before the ribs become visible or strongly developed. It is also possible that rib formation is present already in young fruit bodies in some strains, but that it is constantly absent in others. Our samples did not allow to decide on that matter.

Related cell structures

The content of the brownish hyphae may be deposited homogeneously at the inner surface of the wall. This leads to thick walled hyphae with a large central cavity. In other instances the condensing material is deposited in small to large lumps forming irregularly punctate to spotted hyphae (figures 5, 6).

It is interesting to note that the cystidia of these fungi are characterized by a content appearantly identical to that of the brownish hyphae, but only rarely a "condensation" occurs in these cells. It must be remembered that the brownish hyphae do not necessarily bear cystidia.

One is tempted to consider the brownish hyphae as a kind of excretory system akin to gloeovessels or oleifera. The aspect of the cystidia and the occasional local, internal, congophilous wall thickenings of the cystidia point in the same direction. By some mycologists excretory hyphae are considered fundamentally different from the generative hyphae, although both are derived from the same undifferentiated cell. It is therefore striking to see that many basidia of *Paxillus filamentosus* [leg. STANGL 479/69 (M)] behave in the same way as the brownish hyphae do. Many basidioles and young basidia of this collection have a brownish content, and many mature basidia have internally thickened walls in the upper part of the cell (figure 7). It seems, therefore, that the brownish hyphae and their derivates, the costiferous hyphae, the thick walled hyphae and the spotted hyphae, may be considered generative hyphae better than excretory hyphae. This view is directly supported by the observed transition of generative hyphae to brownish hyphae, as cited above.



Figures 1–7: Costiferous hyphae and related cell structures in *Paxillus* 500 : 1. 1. Brownish hypha fractured at right angle to the cell axis; *P. leptopus,* leg. BRESADOLA 1899 (S); bright field. – 2. Spurious ribs in *P. filamentosus;* transition of a brownish hypha to a spurious costiferous hypha; Fung. Exsicc. Suecici, no 408 (S); phase contrast. – 3. Weak costiferous hyphae in *P. rubicundulus,* leg. BAS, 5325 (L); Congo red, bright field. – 4. Costiferous hypha with strong ribs; *P. filamentosus,* leg. DAAMS (L); Congo red, bright field. – 5, 6. Hyphae spotted by internal deposit homologous to ribs; *P. filamentosus,* leg. BRESINSKY, 9. 10. 1965 (M); Congo red, bright field. – 7. Basidia with internal deposits homologous to ribs; *P. filamentosus,* leg. STANGL, 479/69 (M); Congo red, bright field.