

## Sooty moulds from European Tertiary amber, with notes on the systematic position of *Rosaria* ('Cyanobacteria')

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Sooty moulds are described and illustrated from European amber dating back to 22–54 Myr. All the fossils are fragments of superficial subicula composed of brown moniliform hyphae with markedly tapering distal ends. The subglobose cells are identical to those of extant *Metacapnodium* (*Metacapnodiaceae*, *Capnodiales*) species. Also other preserved features, like the type of apical growth, wide-angled branching and the production of two distinctive conidial states, supports a placement in this genus. The fossils demonstrate that *Metacapnodium* hyphae have remained unchanged for tens of millions of years. This confirms that hyphal morphology and conidial states should be accorded considerable classificatory significance in this group of fungi. The following nomenclatural change is made: *Metacapnodium succinum* comb. nov. (syn. *Rosaria succina*). The type specimen was initially described as a filamentous cyanobacterium, due to similarities with *Rosaria ramosa*. Also the systematic position of this attribute is shortly discussed.

### INTRODUCTION

The term sooty mould has been used in a general sense for many saprophytic fungi, usually with dark-coloured hyphae, which produce colonies superficially on living plants. Many sooty moulds derive their nutrients mainly from insect excretions and live closely associated with aphids, scale insects and other producers of honeydew. Other species associate with plant exudates. For example in New Zealand, where sooty moulds form a conspicuous element in the mycoflora, the branches and trunks of trees can sometimes be almost covered, and even stones and the forest floor can support a thick carpet of these fungi (Hughes 1976).

Several species of *Metacapnodium* (*Metacapnodiaceae*, *Capnodiales*) form extensive, spongy subicula on plant or insect exudates on trunks and branches of woody plants (Hughes 1972, 1976, Reynolds 1997). These fungi are distinguished from other families of sooty moulds by their moniliform, noticeably tapering hyphae. The cells are brown, more or less globose and deeply constricted at the septa. The subiculum easily disintegrates into fragments, which may function as vegetative propagules. In addition, one or more of three distinctive categories of hyphomycetous conidial states are produced by the different species. *Metacapnodium* ascostromata are subglobose to broadly ellipsoidal, ostiolate at maturity and usually bear

tapering hyphal appendages of varying lengths around their apex. Asci are bitunicate and 8-spored, and the ascospores are ellipsoidal and usually 3- or 5-septate (Hughes 1972, 1976).

Here we report the first fossil evidence of *Metacapnodium*. Several well preserved specimens were found in pieces of European amber dating back to approx. 40 Myr ago. The excellent preservation permits a detailed comparison with modern analogues.

### MATERIAL AND METHODS

The fossilised hyphae are contained in six fragments of Baltic and Bitterfeld amber. The age of Baltic amber varies from early to late Eocene (ca 55–35 Myr) (Ganzelewski 1997). The amber-producing tree was *Pinus succinifera*. This species grew in northern Europe in the region of present Fennoscandia. Over millions of years large amounts of resin were transported by rivers to marine Baltic deposits south of the amber forests. Bitterfeld amber (Saxon amber) was mined in brown coal pits near Bitterfeld in Germany (commercial mining stopped in 1992). Based on stratigraphic evidence, the age of Bitterfeld amber is around 22–24 Myr (Barthel & Hetzer 1982, Krumbiegel 1991, Blumenstengel, Volland & Krutzsch 1999). However, many similarities between the fossil biota in Baltic and

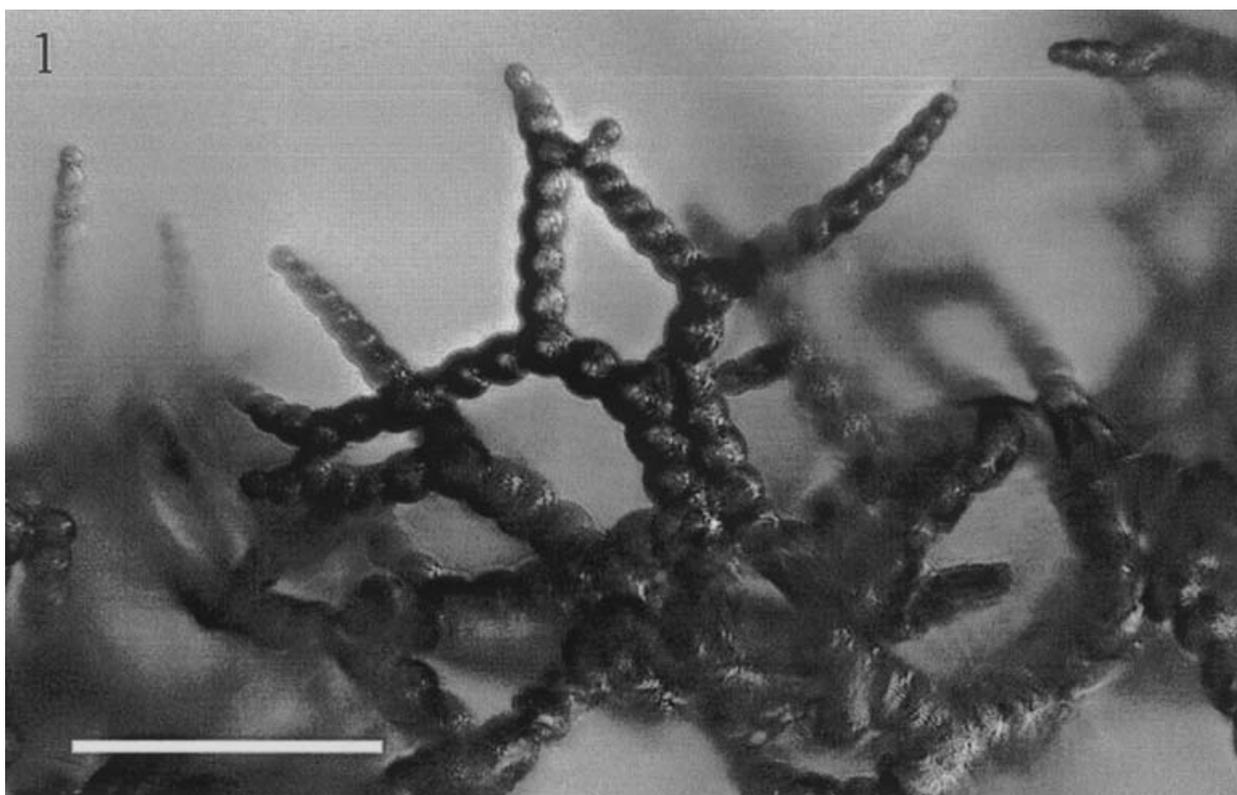


Fig. 1. *Metacapnodium succinum* (*V. Arnold*, no. 1371). Moniliform anastomosing hyphae with tapering tips. Bar = 50  $\mu$ m.

Bitterfeld amber strongly suggest that the Bitterfeld deposits originated by transport and re-deposition of Baltic amber in the Miocene (Wunderlich 1983, Poinar 1992, Weitschat 1997, Grolle 1999, Rikkinen & Poinar 2000, 2002, Frahm 2000, 2001).

The amber specimens have been polished to facilitate screening for inclusions. All measurements and photographs were taken from intact specimens under transmitted and/or incident light. Optical distortions were neutralised by coating the specimen in glycerine or vegetable oil. The deep fields of focus in Figs 1, 5 and 7 were achieved by combining several optical sections into composite pictures.

## RESULTS

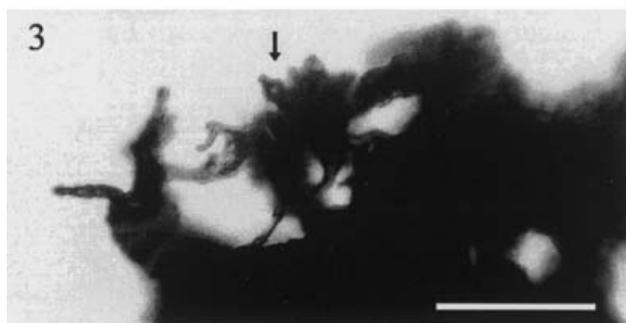
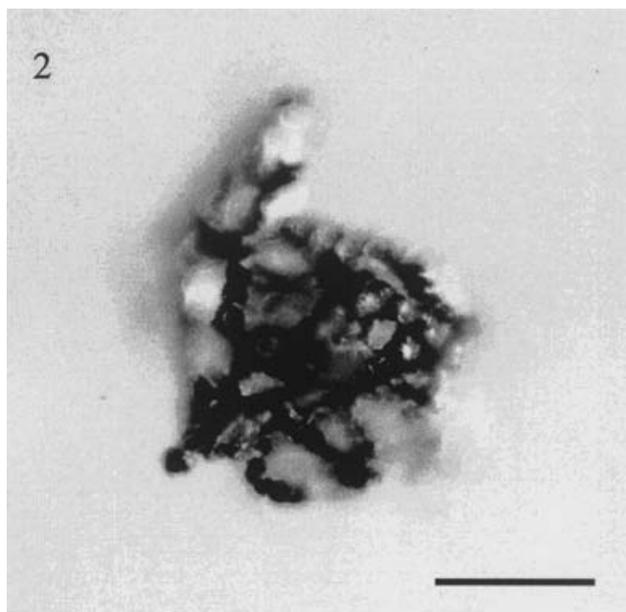
### *Preservation of specimens*

*Metacapnodium* specimen 1 (*J. Wunderlich*, no. F70/BB/PL/CYA/CJW, type specimen of *Rosaria succina*), Baltic amber (Figs 1–7 in Dörfelt, Schmidt & Wunderlich 2000). Fragment of superficial, spongy subiculum, ca 2.0  $\times$  0.9  $\times$  0.5 mm. Hyphae brown to dark brown, moniliform, much branched, with tapering distal ends. Branching wide-angled, with most branches arising at more or less right angles and curving upwards. Cells widely cylindrical to subglobose, deeply constricted at the septa, usually 9–13  $\times$  8–12  $\mu$ m diam; oldest cells up to 15  $\mu$ m wide, with smooth and relatively thick walls. Preserved together with insect (*Diptera*), stellate hairs (from flowering plant) and detritus.

*Metacapnodium* specimen 2 (*V. Arnold*, no. 1371), Baltic amber (Fig. 1). Fragment of superficial, spongy subiculum, ca 0.4  $\times$  0.5 mm. Hyphal morphology as in previous specimen, with occasionally anastomosing hyphae. Preserved together with insects (chironomid, fungus gnat), fragments of hardened resin and detritus.

*Metacapnodium* specimen 3 (*V. Arnold*, no. 1900), Bitterfeld amber (Figs 2–3). *Capnophialophora* conidial state of the fossilised fungus. Small fragment of superficial, spongy subiculum, ca 0.25  $\times$  0.20 mm. Hyphal morphology as in previous specimens. *Capnophialophora* conidiogenous cells developing singly or in groups of 2–4 on subterminal sections of hyphal branches. Phialides pale brown, more or less spherical or obpyriform with a flattened base, the distal end with a single subhyaline collarette. Phialoconidia not seen. Preserved together with insect (springtail) and stellate hairs.

*Metacapnodium* specimen 4 (*V. Arnold*, no. 1368), Baltic amber (Figs 4–5). *Capnosporium* conidial state of the fossilised fungus. Small network of hyphae, ca 500  $\times$  200  $\mu$ m, accompanied by detached hyphal fragments. Hyphal morphology as in previous specimens. Detached hyphal segments straight, 2–5-septate, 15–35  $\mu$ m long, cylindrical to obovoid, with smaller cells at both ends, similar to poroconidia of extant *Metacapnodium* species. The conidia were probably produced at the apexes of erect hyphal branches. The fossils are enclosed in a small fragment of dark resin (1.2  $\times$  0.75 mm) which had become embedded in younger exudate (Fig. 4). The clear amber matrix contains an insect (chironomid), stellate hairs, and pollen grains.



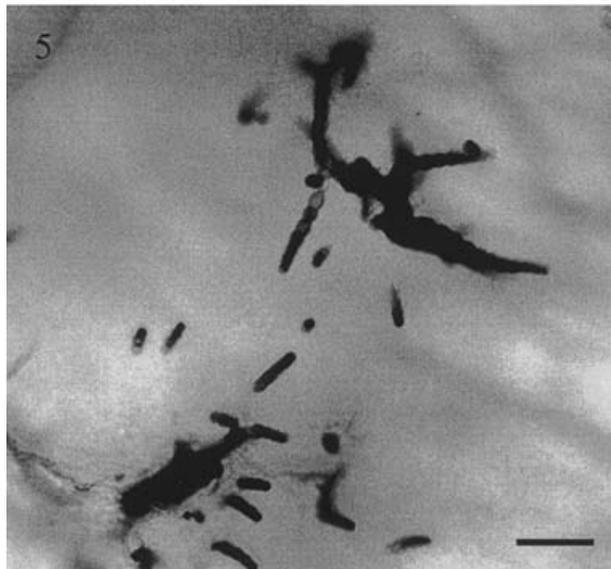
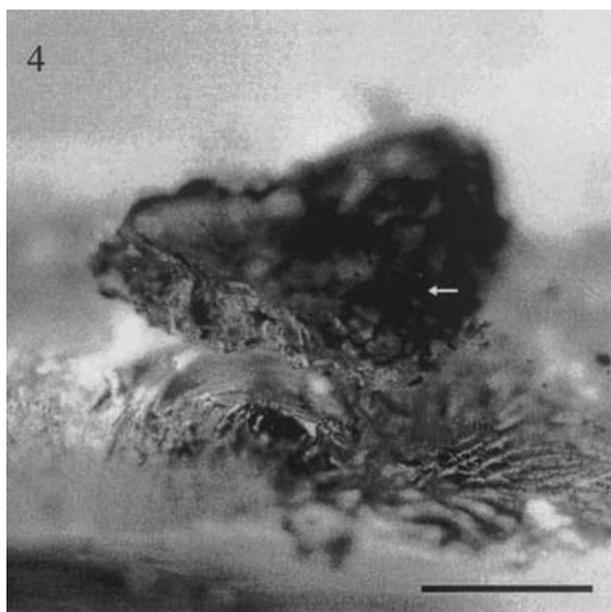
**Figs 2–3.** *Metacapnodium succinum* (V. Arnold, no. 1900). *Capnophialophora* conidial state of the fossilised fungus. **Fig. 2.** Fragment of superficial, spongy subiculum embedded in clear amber. Bar = 100  $\mu$ m. **Fig. 3.** Conidiogenous cell (arrow). Bar = 50  $\mu$ m.

*Metacapnodium* specimen 5 (M. Oschin, no. 5/01), Baltic amber. Tapering hyphal tip, 70  $\mu$ m long, consisting of five cells. Cells as in previous specimens, oldest cell 20  $\mu$ m wide. Preserved together with detritus, several types of fungal hyphae, foliose lichen (*Anzia*), bryophyte (cf. *Grimmia*), and insects (Rikkinen & Poinar 2002).

*Metacapnodium* specimen 6 (V. Arnold, no. 1908), Bitterfeld amber, bark imprint with foliose lichen (Figs 6–7). Networks of hyphae, growing over bark and lichen, accompanied by detached hyphal fragments. Hyphal morphology as in previous specimens. Cells usually 10–13  $\times$  7–10  $\mu$ m diam, some with distinctly ornamented walls. Preserved together with detritus, the foliose lichen, and several types of fungal hyphae.

### Taxonomy

The vegetative hyphae of most fungi are impossible to identify with any precision, but those of some sooty moulds are diagnostic. The tapering hyphae and subglobose cells of the fossilised fungi are identical to those of modern *Metacapnodium* species. Also the

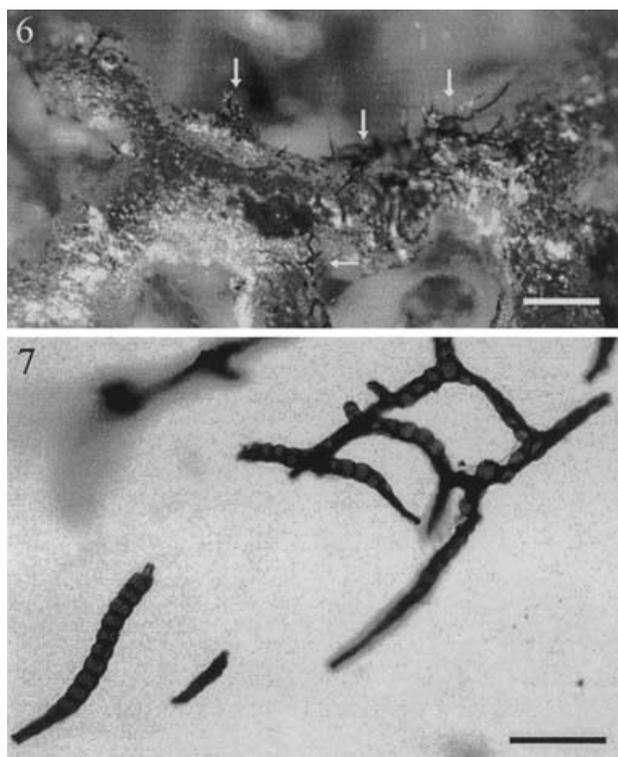


**Figs 4–5.** *Metacapnodium succinum* (V. Arnold, no. 1368). *Capnosporium* conidial state of the fossilised fungus. **Fig. 4.** Dark resin fragment embedded in clear, younger resin. Location of fossilised fungus shown by arrow. Bar = 0.5 mm. **Fig. 5.** Detached poroconidia and vegetative hyphae. Bar = 50  $\mu$ m.

combination of other preserved features, like the type of apical growth, the branching pattern and production of two distinctive conidial states, supports a placement in *Metacapnodium*. All the fossils may belong to the same taxon, originally described as *Rosaria succina* (Dörfelt, Schmidt & Wunderlich 2000). Accordingly, the following nomenclatural change is made:

***Metacapnodium succinum*** (Dörfelt, A. R. Schmidt & Wunderl.) Rikkinen, Dörfelt, A. R. Schmidt & Wunderl., **comb. nov.**

Basionym: *Rosaria succina* Dörfelt, A. R. Schmidt & Wunderl., *J. Basic Microbiol.* **40**: 330 (2000). Type: J. Wunderlich, no. F70/BB/PL/CYA/CJW.



**Figs 6–7.** *Metacapnodium succinum* (V. Arnold, no. 1908). Ecology. **Fig. 6.** Hyphae growing on the upper surface of a foliose lichen (arrows). Bar = 200  $\mu$ m. **Fig. 7.** Detached hyphae. Bar = 50  $\mu$ m.

## DISCUSSION

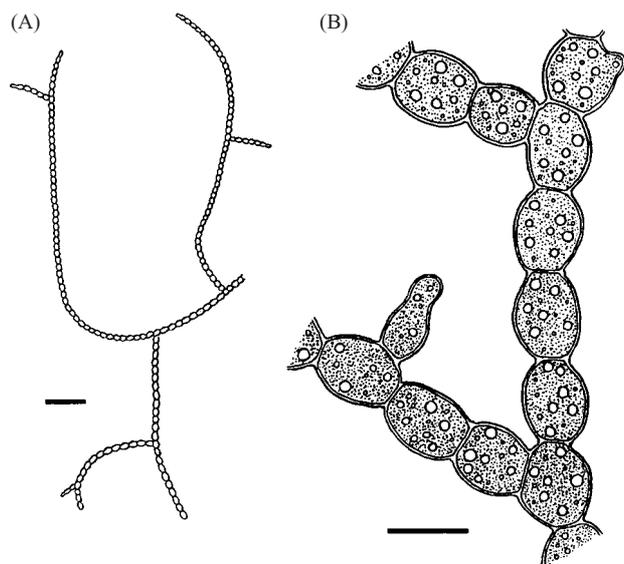
*Metacapnodium succinum* is very similar to modern species of the genus. The fossils demonstrate that distinctive features in the hyphal morphology of metacapnodiaceous sooty moulds have remained unchanged for at least tens of millions of years. As the fossilisation events were probably not preceded by a short period of more rapid evolution, the initial divergence of these fungi must have happened much earlier, probably in the Mesozoic. The stasis in the evolution confirms that hyphal morphology and conidial states should be accorded considerable phylogenetic and classificatory significance in this group of fungi (Hughes 1972, 1976, Reynolds 1985, 1997, Sugiyama & Amano 1987).

Several groups of ascomycetes seem to show a conservative maintenance of adaptations to successful ecological niches. For example, a variety of epiphyllous ascomycetes, most comparable with modern forms, have been described from Middle Eocene and Miocene angiosperm leaves (Dilcher 1965, Carlie *et al.* 2001). The divergence of some sooty mould lineages may even have been linked to the initial development of sucking herbivorous insect guilds producing liquid excretes rich in carbohydrates. Piercing-and-sucking mouthparts evolved in several insect groups in the Late Carboniferous and also honeydew production may have originated as early as in the Permian (Labandeira & Phillips

1996, Zherikhin 2002). Aphids and scale insects were already quite diverse in the Cretaceous (Shcherbakov & Popov 2002, Zherikhin 2002). Zherikhin (2002) suggested that sucking herbivorous insects may have been the dominant primary consumers in all Mesozoic land ecosystems. In contrast, most chewing insects fed on reproductive organs of plants which constituted a relatively minor fraction of total plant production. If so, the sucking herbivores must have produced huge amounts of honeydew which provided an attractive food source for fluid-feeding insects and many other organisms, including yeasts and sooty moulds.

Without the preservation of ascocata and ascospores, the amber fossils cannot be safely assigned to any modern *Metacapnodium* species. There are relatively few extant species of *Metacapnodium* and the number of species in the Northern Hemisphere is even more restricted (Barr 1955, 1972, Hughes 1972, Hawksworth *et al.* 1995). *M. juniperi*, the type species of the genus, produces spongy subicula composed of loosely interwoven hyphae, very similar to those of the fossils. It lives in Europe on twigs and branches of *Juniperus communis* (Hughes 1972, Reynolds 1985). Another close analogue is *M. spongiosa* which lives on the bark of *Calocedrus decurrens*, *Chamaecyparis lawsoniana* and other conifers in western North America (Barr 1955). Also the assimilative hyphae of *Capnobotrys neesii* are very similar to those of the fossils. This anamorph species grows on a variety of mainly coniferous trees in Europe and Japan (Sugiyama & Amano 1984, 1987).

Like modern metacapnodiaceous sooty moulds, also *M. succinum* was epiphytic. One of the fossils (V. Arnold, no. 1908) represents an imprint of an irregular bark surface with a well preserved epiphytic foliose lichen. Small networks of *Metacapnodium* hyphae had grown over the bark and the upper surface of the lichen (Fig. 6). The lichen and associated organisms were entrapped in their original microhabitat, probably by viscous resin running down a tree trunk or dripping from the canopy. One of the specimens (V. Arnold, no. 1368) may have grown and produced conidia on semi-solid resin (Fig. 4). Surface cracking indicates that the resin had dried before it and the adhering fungus were submerged in fresh exudate. The ability to tolerate resinous exudates would have made *M. succinum* a likely candidate for preservation in amber (Rikkinen & Poinar 2000). However, as amber fossils of *Metacapnodium* are not common, the resin itself was probably not the primary substrate of *M. succinum*. Most of the fossils are detached fragments surrounded by clear, massive amber. This indicates that the fungi were not entrapped in place, but either transported by flowing resin or deposited into stationary exudate, probably after first becoming detached from the upper canopy or neighbouring trees. The preferred hosts of several extant *Metacapnodium* species belong to the Cupressaceae, and thus also *M. succinum* may have grown on this group of conifers. Microfossils of several genera,



**Fig. 8.** *Rosaria ramosa*, redrawn from Carter (1922). A, Habit. Bar = 100  $\mu$ m. B, Moniliform cells with several small guttules in each cell. Bar = 20  $\mu$ m.

including *Calocedrus*, *Chamaecyparis*, and *Juniperus*, are known from European amber (Poinar 1992).

The type specimen of *M. succinum* was initially described as a filamentous cyanobacterium (Dörfelt *et al.* 2000) due to many similarities with *Rosaria ramosa* (syn. *Nelliecarteria ramosa*), a monotypic 'cyanobacterium' from New Caledonia. Carter (1922) depicted *R. ramosa* as a peculiar and beautiful organism, looking like a string on glistening pearls under low magnification (Fig. 8). The species was epiphytic and formed a thin mucous film over excrescences on the bark of a tree. The branching was sparse, irregular, at more or less right angles, the branches arising in all directions, with some second order branching (Fig. 8A). Cells were barrel-shaped, clearly constricted at the septa, thick and smooth walled, 13–19  $\times$  19–22  $\mu$ m diam, with several small globules in each cell (Fig. 8B). All cells were similar to each other and become simply more slender towards the apex. Growth and branching was achieved through a peculiar budding of cells (Carter 1922).

Most cyanobacteriologists have since regarded *R. ramosa* as a doubtful species and probably not a cyanobacterium at all (Geitler 1932, 1942, Friedmann 1955, Anagnostidis & Komárek 1990). Indeed, on the basis of the original description and illustrations it seemed possible that also *R. ramosa* would have been described on the basis of a sooty mould, rather than a cyanobacterium. The moniliform, repent growth, type of apical growth and branching, and the size and shape of cells all have parallels in sooty moulds. Some of these fungi also produce yellowish or brown hyphae containing several small guttules in each cell. Furthermore, sooty moulds are common and abundant in the southern Pacific Basin, including New Caledonia. However, an examination of the type (BM 3353) indicated that *Rosaria ramosa* most probably represents a green

alga. Francis Drouet had annotated the specimen as *Physolium monilia* (*Trentepohliales*). However, considering the fragmentary nature of the specimen (absence of aplanospores or -sporangia, identifiable chloroplasts and/or haemochromic particles) this identification remains tentative (Printz 1939, Davis, Rands & Lachapelle 1989, Rands & Lachapelle 1993, Rindi & Guiry 2002, López-Bautista, Waters & Chapman 2002).

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