Arnaudiella eucalyptorum sp.nov. (Dothideales, Ascomycetes), and its hyphomycetous anamorph *Xenogliocladiopsis* gen.nov., from *Eucalyptus* leaf litter in South Africa

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Received May 6, 1993

CROUS, P.W., and KENDRICK, W.B. 1994. ***Arnaudiella eucalyptorum*** sp.nov. (Dothideales, Ascomycetes), and its hyphomycetous anamorph ***Xenogliocladiopsis*** gen.nov., from *Eucalyptus* leaf litter in South Africa. Can. J. Bot. **72**: 59–64.

Arnaudiella eucalyptorum is described as a new saprobic Ascomycete from *Eucalyptus* leaf litter. Its anamorph, which developed in pure culture, is placed in a new hyphomycetous genus, ***Xenogliocladiopsis***. ***Xenogliocladiopsis eucalyptorum*** sp.nov. is known from collections of *Eucalyptus* leaf litter in the Transvaal and Cape provinces of South Africa, where it appears to be specific to this substrate.

Key words: *Arnaudiella eucalyptorum*, Ascomycetes, Dothideales, *Eucalyptus* leaves, hyphomycetes, *Xenogliocladiopsis eucalyptorum*.

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Les auteurs décrivent l'***Arnaudiella eucalyptorum*** comme un nouvel ascomycète saprotrophe venant sur les litières folaires de l'*Eucalyptus*. L'anamorphe, obtenu en culture pure, est attribué à un nouveau genre d'hyphomycètes, le ***Xenogliocladiopsis***. Le ***Xenogliocladiopsis eucalyptorum*** sp.nov. provient de litières foliaires d'*Eucalyptus récoltées* dans les provinces du Transvaal et du Cap en Afrique du Sud, où il semble être spécifique à ce substrat.

Mots clés : *Arnaudiella eucalyptorum*, Ascomycètes, Dothidéales, *Eucalyptus*, feuilles, hyphomycètes, *Xenogliocladiopsis eucalyptorum*.

[Traduit par la rédaction]

Introduction

During the course of a damp-chamber study of fungi growing on *Eucalyptus* leaves, an ascomycete with characteristic scutate ascomata developed on litter collected in the Transvaal and Cape provinces of South Africa. Preliminary observations of ascomata showed this fungus to be similar to *Microthyrium amygdalinum* Cooke & Massee, recently reported as a pathogen of several *Eucalyptus* species in Australia by Park and Keane (1982). However, the South African fungus was clearly saprobic, as opposed to the parasitic *M. amygdalinum*. Swart (1986), discussing several *Microthyrium*-like fungi, placed *M. amygdalinum* and several other species in synonymy with *Phaeothyriolum microthyrioides* (Winter) Swart and considered *Phaeothyriolum* to be distinct from similar taxa such as *Asterina* Lév. and *Arnaudiella* Petrak.

Single-ascospore isolations made from material collected in the Transvaal and Cape provinces produced a colorless hyphomycetous anamorph in culture. This anamorph had cylindrical to slightly curved conidia borne on penicillate conidiophores with several series of branches, terminating in whorls of phialides. Conidiophores occurred either singly or clustered in a sporodochium. Several genera have conidiophores similar to those encountered in the present study. These include *Acontiopsis* Negru, *Cylindrocarpon* Wollenw., *Cylindrocladiella* Boesewinkel, *Cylindrocladium* Morgan, *Cylindrodendrum* Bonorden, *Gliocladiopsis* Saksena, *Myrothecium* Tode:Link, *Setomyrothecium* Matsushima, *Uncigera* Saccardo, and *Volutella* Tode:Fries. The aim of the present study, therefore, was to find an appropriate taxonomic disposition for both teleomorph and anamorph of the fungus isolated from *Eucalyptus* leaf litter.

Materials and methods

Eucalyptus leaf litter was collected on Stellenbosch Mountain (Stellenbosch, Western Cape Province, South Africa) and at the Goudrivier Game Lodge (Transvaal Province, South Africa) on several occasions since 1990. Leaves were incubated in moist chambers at 25°C for 14 days under near-ultraviolet light. Single-ascospore isolations were made on 2% malt extract agar (MEA) (Biolab (PTY) Ltd., Fedlife Park, Tonetti St., Johannesburg, South Africa), using the method described by Crous et al. (1991). Single-ascospore cultures were transferred to carnation leaf agar (CLA) (Crous et al. 1992) and *Eucalyptus* leaf agar (ELA) (autoclaved pieces of *Eucalyptus* leaf placed on water agar) and incubated at 25°C for 14 days under near-ultraviolet light.

Results and discussion

Once the pathogenic *Phaeothyriolum* H. Sydow had been excluded, our fungus was compared with two other *Microthyrium*-like genera, *Asterina* and *Arnaudiella*, discussed by Swart (1986) and was satisfactorily disposed in the latter. Swart (1986) characterized *Arnaudiella* as being saprobic and having separate, scutate ascomata, with a subcuticular mycelium, no superficial hyphae, and no central column of paraphysoid tissue in the ascoma. The South African fungus

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...ted in Canada / Imprimé au Canada

matches these criteria well, except that sparse superficial mycelium is often present. This apparent discrepancy might be a result of the continuous high humidity in the damp chambers.

The thyriothecial ascomata of our fungus (Figs. 1 and 2) developed on both sides of the dead leaves, either individually or in groups of two to four. Where sparse superficial hyphae were observed, circular patches of cells approximately 20 μm in diameter were seen developing into ascoma initials. The peridium of mature ascomata consisted of small, dark brown cells of textura angularis arranged in rows radiating outward from a central ostiole. The rows ended at the smooth margin of the ascoma, and sparse, dark brown hyphae, 3–4 μm wide, extended beyond the periphery. The asci were bitunicate, obclavate, and stipitate, and the basal stipe was easily seen in detached asci (Fig. 3). The ascospores were fusoid, 1-septate, widest just above the slightly supramedian septum (as arranged in the ascus), and tapered toward both rounded ends. The lower cell was slightly longer than the upper cell, and the cell wall was fairly thick, and not constricted, or only slightly constricted, at the septum. The spores were invested in a firm, nongelatinous sheath, frequently appearing as only caplike appendages in older, larger spores (Figs. 4 and 5). Germinating ascospores found on the dead leaves produced germ tubes perpendicular to the long axis of the spore, and spores darkened with germination. This observation could not, however, be confirmed in culture.

As far as we have been able to establish, only one species of *Arnaudiella*, *A. bancroftii* Hansf., has been described from *Eucalyptus*, and it has slightly larger ascospores that lack a sheath. Our collections were quite distinct from *A. bancroftii*, which has in any case been reduced to synonymy with *Phaeothyriolum microthyrioides*. Since the South African isolate from *Eucalyptus* has many unique attributes, it is described here as a new species of *Arnaudiella*.

Arnaudiella eucalyptorum Crous et Kendrick sp.nov.

Figs. 1–12

Mycelium plerumque in substrato immersum, sparsum, ex hyphis hyalinis ad brunneis, septatus, parce ramosis, 3–4 μm crassis compositum. Ascomata thyriothecia, amphigena, orbicularia, 89–180 μm diametro, sparsa vel aggregata. Scutellum ex cellulis pallide brunneis oblongis textura angularis, 2–15 × 3–4 μm compositum. Ostiolum 15–25 μm diametro. Asci bitunicati, cylindrici ad obpyriformes, 30–45 × 13–16 μm, stipitati, octosporati. Ascosporae hyalinae, 1-septatae, fusiformes, 14–20 × 4–5 μm, guttulatae, vagina cum persistenti super ascospores extensa. Coloniae pallido- ad atrobrunneae, cum marginibus laevibus et mycelio sparso aerio.

Mycelium sparse, mostly immersed in substrate, composed of colorless, septate hyphae that become dark brown with age, sparingly branched, 3–4 μm wide. Ascomata thyriothecia, amphigenous, orbicular, 89–180 μm in diameter, scattered or in groups of 2–4. Scutellum composed of radiating rows of brown cells of textura angularis, individual cells 2–15 × 3–4 μm, with the marginal cells divided at their ends. Ostiole 15–25 μm in diameter. Asci bitunicate, cylindrical to obpyriform, 30–45 × 13–16 μm, stipitate, 8-spored. Ascospores hyaline, 1-septate, fusiform, 14–20 × 4–5 μm, upper cell 5–9 × 4.5–5 μm, lower cell 9–11 × 4–4.5 μm, guttulate, with a persistent sheath extending over the ends of the spore. Colonies 40–45 mm in diameter, light to dark brown (reverse), with smooth margins and sparse aerial mycelium on MEA after 14 days at 25°C under near-ultraviolet light. Colonies were sterile on MEA but sporulated on CLA and ELA. Chlamydospores absent.

HOLOTYPE: SOUTH AFRICA: TRANSVAAL: Goudrivier Game Lodge, *Eucalyptus* leaf litter, P.W. Crous, May 1991, PREM 51297. PARATYPE: WESTERN CAPE: Stellenbosch, Stellenbosch Mountain, *Eucalyptus* leaf litter, P.W. Crous, Sept. 1990, PREM 51298.

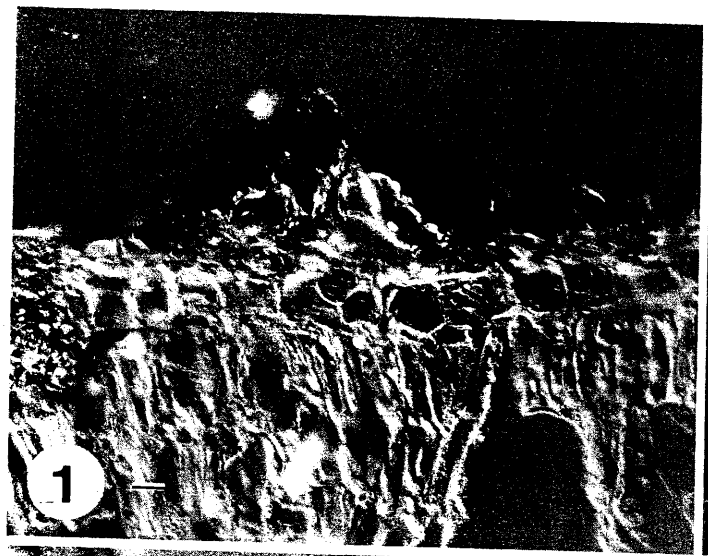
Single-ascospore isolates produced an anamorph with hyaline, cylindrical conidia on phialides with periclinal thickening, characteristics also observed in genera such as *Acontiopsis*, *Cylindrocarpon*, *Cylindrocladiella*, *Cylindrocladium*, *Cylindrodendrum*, *Gliocladiopsis*, and *Uncigera*. Conidiophores were also frequently aggregated in a sporodochium-like structure, resembling genera such as *Myrothecium*, *Septomyrothecium*, and *Volutella*.

The anamorph of *A. eucalyptorum* is distinguished from *Cylindrocarpon* by its more appressed and slender penicillate conidiophores, and the arrangement of its conidiophore branches (Crous and Wingfield 1993). A *Cylindrocarpon* synanamorph was recently reported for a species in a similar genus, namely *Cylindrodendrum album* Bonorden var. *paralion* (Summerbell et al. 1989). In the latter study, the genus *Pulvinotrichum* Gamundi, Arambarri & Giaiotti was shown to be congeneric with *Cylindrodendrum*, while evidence was also presented to show its similarity with *Uncigera*, both genera being characterized by an erect conidiogenous apparatus with a verticillate branching arrangement (Summerbell et al. 1989). The genus *Cylindrodendrum* is known to have conidiophores that can frequently terminate in sterile extensions, as was found for our isolate from *Eucalyptus* (Fig. 12). This characteristic was also observed for *Cylindrodendrum album* var. *paralion* by Summerbell et al. (1989), who described swollen cells from the ends of conidiophores. The lack of a definite penicillate conidiophore with more than one series of conidiophore branches, however, makes *Cylindrodendrum* an unsuitable genus for the anamorph of *A. eucalyptorum*.

Sterile stipe extensions have also been observed in the penicillate conidiophores of *Acontiopsis*, *Cylindrocladium*, and *Cylindrocladiella*. The taxonomic uncertainty surrounding the generic disposition of *Acontiopsis* has recently been considered by Crous and Wingfield (1993). They decided that *Acontiopsis* cannot be validated from the imprecise description and illustration and is therefore regarded as a nomen dubium.

The sterile stipe extensions of our isolates never had apical

FIGS. 1–8. *Arnaudiella eucalyptorum* and its anamorph *Xenogliocladiopsis eucalyptorum*. Fig. 1. Vertical section through a thyriothecial ascoma with central ostiole. Scale bar = 10 μm. Fig. 2. Mature ascomata on leaf surface (arrowheads). Scale bar = 90 μm. Fig. 3. Bitunicate, stipitate asci. Scale bar = 10 μm. Figs. 4 and 5. Ascospore invested in a firm sheath. Scale bars = 10 μm. Fig. 6. Penicillate conidiophore. Scale bar = 10 μm. Fig. 7. Apex of conidiophore proliferation surrounded by conidia. Scale bar = 10 μm. Fig. 8. Cylindrical to fusiform conidia with acutely rounded ends. Scale bar = 10 μm.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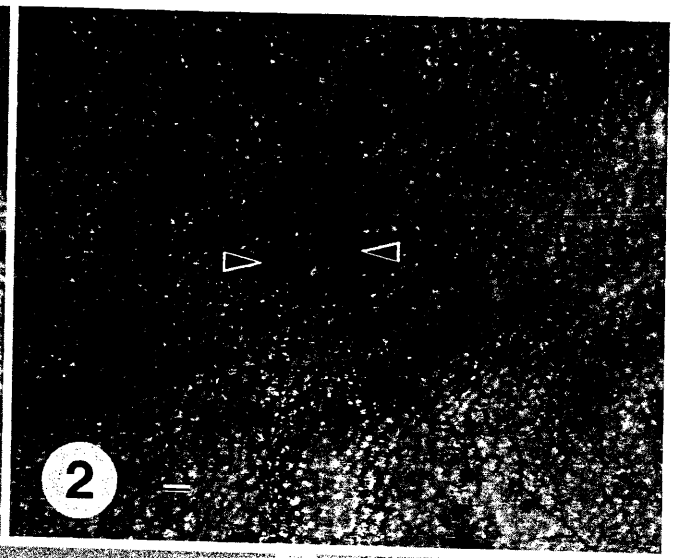
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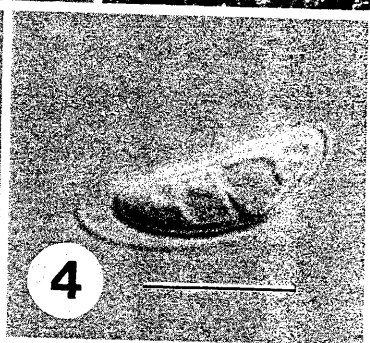
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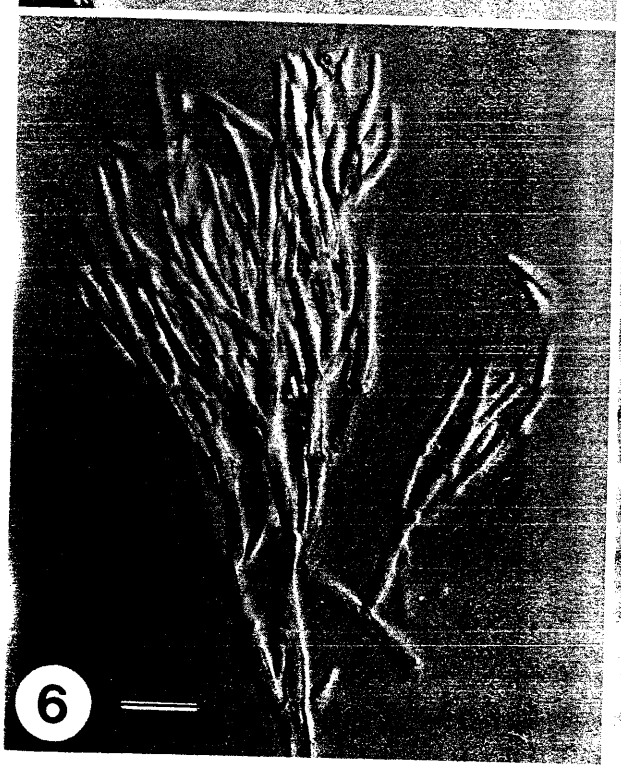
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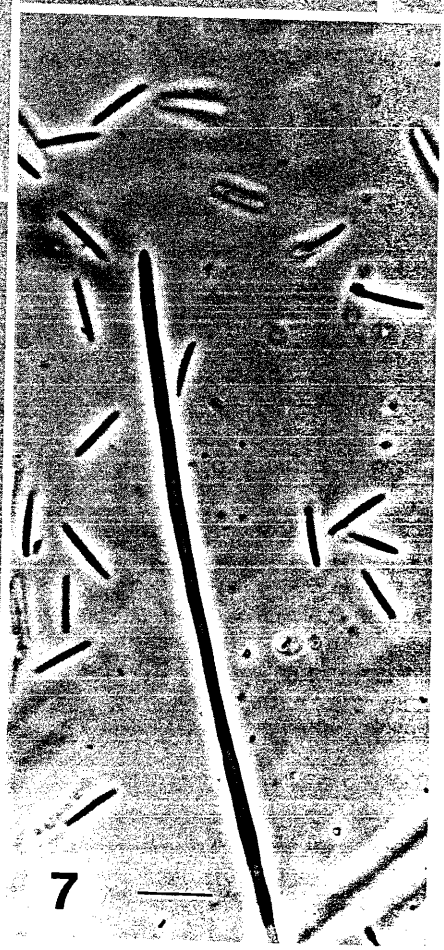
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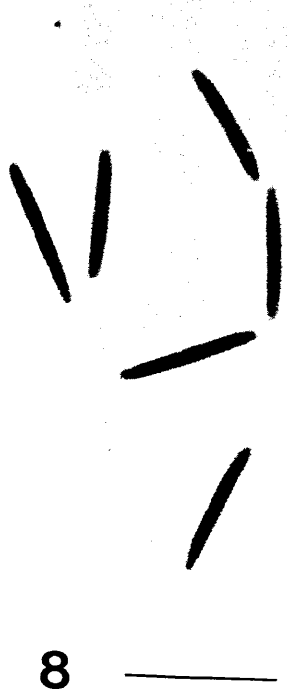
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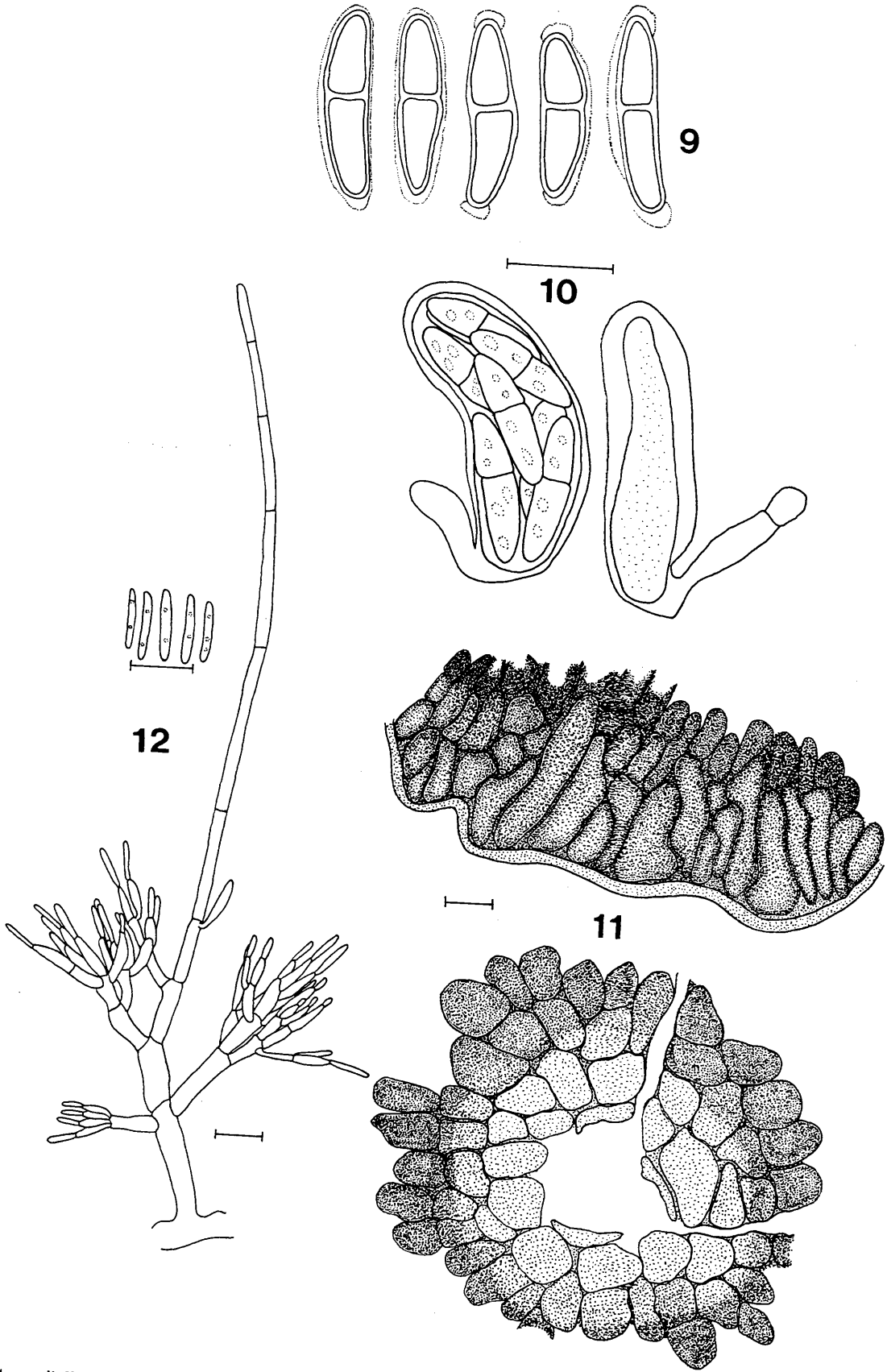

FIGS. 9–12. *Arnaudiella eucalyptorum* and its anamorph *Xenogliocladiopsis eucalyptorum*. Scale bars = 10 μm. Fig. 9. Ascospores invested in a sheath, or partly covered in a broken sheath. Fig. 10. Bitunicate, stipitate asci. Fig. 11. The peridia of mature ascomata consisting of radiating rows of cells of textura angularis, showing the smooth margin and ostiolar area. Fig. 12. Penicillate conidiophore and conidia.

vesicles (Fig. 7) and seemed to represent conidiophore proliferations rather than being analogous to stipe extentions typical of *Cylindrocladium* and *Cylindrocladiella*. The conidiophore proliferation frequently produces side branches, which develop into separate penicillate conidiophores. This results in a central rachis, with penicillate conidiophores arranged on each side, while each conidiophore can once again form a central proliferation as a basis for further development. In very young cultures, however, penicillate conidiophores appear as separate structures with determinate growth, a phenomenon commonly observed in *Cylindrocladium* and *Cylindrocladiella*. The continued growth observed as conidiophore proliferations in our fungus, the ability to form sporodochia, and a teleomorph in the Microthyriaceae clearly distinguish our collections from these two genera.

In young cultures of the *Eucalyptus* isolate, the penicillate conidiophores rarely formed a conidiophore proliferation, suggesting a similarity to *Gliocladiopsis*. Furthermore, conidiophores of both genera have been observed to accumulate conidia in yellow, slimy masses. However, in fresh collections of both genera, conidiophores produced colorless masses of conidia, suggesting that this character is variable.

The genus *Gliocladiopsis* is characterized by penicillate conidiophores with several series of branches, terminating in whorls of two to four phialides that are cylindrical to cymbiform in shape. These characteristics are also seen in the anamorph of *A. eucalyptorum*. However, penicillate conidiophores of *Gliocladiopsis* always terminated in phialides. In exceptional cases (Crous and Wingfield 1993, figure 21) one phialide was observed to extend slightly above the rest because it had an additional basal cell. No stipe extension or sterile conidiophore proliferation was ever observed. Furthermore, penicillate conidiophores were always formed singly, never in the sporodochial arrangement found in our fungus. Lastly, *Gliocladiopsis* has uniformly cylindrical, septate conidia, which do not become swollen with age, as frequently occurs in *Cylindrocladium* and *Cylindrocladiella*. All species currently known in these three genera are also known to have conidia with bluntly rounded ends (Crous and Wingfield 1993). The conidia of the *Eucalyptus* isolate are nonseptate, not uniformly cylindrical but frequently fusiform, and have acutely rounded ends. The distinct conidium and conidiophore morphology thus separate our fungus from *Gliocladiopsis*.

The frequent occurrence of sporodochia also suggests that the *Eucalyptus* fungus should be compared with genera such as *Myrothecium*, *Septomyrothecium*, and *Volutella*. Apart from differences in the nature of the setae, fructifications of these three genera always occur either as sporodochia or synnemata, and never as separate penicillate conidiophores. Furthermore, *Myrothecium* and *Volutella* have Nectriaceous teleomorphs (Tulloch 1972) and are thus genetically and morphologically far removed from the bitunicate *Arnaudiella*. Because the anamorph of *A. eucalyptorum* cannot be accommodated in any of the existing genera discussed above, we elect a new anamorph genus for it.

Xenogliocladiopsis Crous et Kendrick gen.nov.

Figs. 6–8, 12

Conidiophora hyalina, penicillata, separata, vel in sporodochiis aggregata, seriebus ramis aliquot in phialidibus cylindricis ad cymbiformibus 2–6 aggregatis. Collaretta absentia. Centralis stipes super massam conidiorum, extensus non vesiculatus. Conidia hyalina, non septata, cylindrica ad fusiformia apicibus acutis rotundatis.

Conidiophores hyaline, penicillate, separate or aggregated in sporodochia, having several series of branches, terminating in cylindrical to cymbiform phialides, arranged in whorls of 2–6 per branch. Collarettes absent. Central stipe of conidiophore frequently extending above conidial mass, apex nonvesiculate. Conidia hyaline, nonseptate, cylindrical to fusiform with acutely rounded ends.

TYPE: *X. eucalyptorum*.

Xenogliocladiopsis eucalyptorum Crous et Kendrick sp.nov.

Conidiophora hyalina, penicillata, separata, vel in sporodochiis aggregata; rami primarii non septati, 15–25 × 3.5–7 μm, rami secundarii non septati, 13–15 × 3.5–6 μm, rami tertiarii non septati, 11–15 × 2.5–3.5 μm, rami quaternari non septati, 10–14 × 2.5–3 μm, rami ceteri non septati, 7–10 × 2–2.5 μm, phialides cylindricae ad cymbiformes, 7–11 × 1.5–2 μm 2–6 aggregatae. Collaretta absentia. Centralis stipes super massam conidiorum, extensus non vesiculatus. Conidia hyalina, non septata, 7.5–11 × 1–1.5 μm, cylindrica ad fusiformia apicibus acutis rotundatis.

Conidiophores hyaline, penicillate, separate or aggregated in sporodochia, frequently having thickened conidiophore septa separating lower branches, primary branches nonseptate, 15–25 × 3.5–7 μm, secondary branches nonseptate, 13–15 × 3.5–6 μm, tertiary branches nonseptate, 11–15 × 2.5–3.5 μm, quaternary branches nonseptate, 10–14 × 2.5–3 μm, additional branches nonseptate, 7–10 × 2–2.5 μm, phialides cylindrical to cymbiform, 7–11 × 1.5–2 μm, arranged in whorls of 2–6 per branch. Collarettes absent. Central stipe of conidiophore frequently extending above conidial mass. Conidiophore proliferations septate, 30–220 × 1.5–2.5 μm, nonvesiculate. Conidia hyaline, nonseptate, 7.5–11 × 1–1.5 μm, cylindrical to fusiform with acutely rounded ends.

HOLOTYPE: SOUTH AFRICA: TRANSVAAL: Goudrivier Game Lodge, *Eucalyptus* leaf litter, P.W. Crous, May 1991, single-ascospore cultures sporulating on pieces of carnation leaf, PREM 51299. PARATYPES: WESTERN CAPE: Stellenbosch, Stellenbosch Mountain, *Eucalyptus* leaf litter, P.W. Crous, Sept. 1990, single-ascospore cultures sporulating on pieces of carnation leaf PREM 51300; Stellenbosch, Stellenbosch Mountain, *Eucalyptus* leaf litter, P.W. Crous, March 1992, PREM 51301.

Of all the genera of conidial fungi considered in this study, *Xenogliocladiopsis* is morphologically closest to *Gliocladiopsis*. It is interesting to note that isolates of *X. eucalyptorum* were obtained only from *Eucalyptus* and did not occur on litter of any other plants in the immediate vicinity. Isolates of *Gliocladiopsis*, however, have thus far been collected from soil and hosts totally unrelated to *Eucalyptus* (Crous and Wingfield 1993). Furthermore, although no teleomorph connection has yet been made for *Gliocladiopsis*, it seems distinctly possible that it is part of a Hypocrealean holomorph, since it is so similar to genera such as *Cylindrocladium* and *Cylindrocarpon*, with which it had already been considered congeneric by Kendrick and Carmichael (1973) and Agnihothrudu (1959), respectively. Although phenotypically similar to several genera discussed in this paper, *Xenogliocladiopsis*

seems genetically far removed in having a strong host specificity and a teleomorph in the Microthyriaceae.

Acknowledgments

The authors are grateful to Dr. Margaret Barr (Inverness Ave., Sidney, B.C.) and Dr. Keith Seifert (Agriculture Canada, Research Branch, Ottawa, Ont.) for their respective comments on the teleomorph and anamorph material examined in this study. The junior author is grateful to the Natural Sciences and Engineering Research Council of Canada for financial support in the form of an operating grant, and the South African Foundation for Research Development for research support during a visit to South Africa.

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