Megaconidia as an additional taxonomic character in *Cylindrocladium*, with a note on *Cylindrocladiopsis*

Pedro W. Crous[1*] **and Keith A. Seifert**[2]

[1]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa; * email: pwc@land.sun.ac.za
[2]Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Research Branch, Ottawa, Ontario, K1A 0C6 Canada

Megaconidia are defined as a third conidial type in *Cylindrocladium*. They are characteristically formed singly from reduced conidiophores that occur either on or in water agar. Megaconidia are multi-septate, widest in the middle, straight to bent at right angles, and significantly larger than macroconidia. The genus *Cylindrocladiopsis* is reduced to synonymy with *Cylindrocladium*, with its type species *Cylindrocladiopsis lagerstraemiae* becoming a synonym of *Cylindrocladium heptaseptatum*.

Introduction

The genus *Cylindrocladium* Morgan is typified by *C. scoparium* Morgan (teleomorph *Calonectria morganii* Crous, Alfenas and M.J. Wingf.), and characterised as having species with penicillate conidiophores that bear cylindrical, septate conidia in parallel spore packets, and have stipe extensions terminating in vesicles of characteristic shape. The known teleomorphs of *Cylindrocladium* species are classified in *Calonectria* De Not., while the teleomorphs of morphologically similar genera such as *Cylindrocladiella* Boesew., *Gliocladiopsis* Saksena, *Falcocladium* Silveira, Alfenas, Crous and M.J. Wingf., *Xenocylindrocladium* Decock, Hennebert and Crous and *Curvicladium* Decock and Crous are either in the *Nectria sensu lato* complex, or are unknown (Crous and Wingfield, 1993; Crous *et al.*, 1994b; Crous, Kendrick and Alfenas, 1997; Decock, Hennebert and Crous, 1997; Decock and Crous, 1998). Generic synonyms of *Cylindrocladium* (Crous and Wingfield, 1994) include *Candelospora* Hawley apud Rea and Hawley (Boedijn and Reitsma, 1950), and *Tetracytum* Vanderwalle (Subramanian, 1971), while the status of the apparently similar genus, *Cylindrocladiopsis* J.M. Yen (Yen, 1979), remains to be considered.

Several features are used to distinguish *Cylindrocladium* species. Other than macro- and microconidial shape, septation and dimensions, characters such as phialide morphology, conidiophore branching pattern, length and width of the stipe extensions and vesicle shape are also important (Crous and Wingfield, 1994). Diagnostic features of the teleomorph include perithecial dimensions, anatomy, wall-roughening, presence/absence/disposition on a stroma, colour reaction in 3 % KOH (Rossman, 1979, 1983), as well as ascus and ascospore morphology. Cultural characteristics are important to distinguish some taxa, especially the abundance of chlamydospores on malt extract agar, which in turn largely determines colony colour, as well as features such as sporulation on aerial mycelium and cardinal temperature requirements for growth.

Cylindrocladium species produce several types of asexual propagules, namely macroconidia, microconidia and chlamydospores (which aggregate to form microsclerotia, and act as infection propagules). The terminology for macroconidia and microconidia is broadly similar to that used in the related hypocrealean genera such as *Fusarium* Link and *Cylindrocarpon* Wollenw. Macroconidia in *Cylindrocladium* are 1-9-septate, cylindrical with rounded ends, and are produced on doliiform to reniform or allantoid to cylindrical phialides. In contrast to species of *Fusarium* and *Cylindrocarpon*, where macroconidia are typically produced on conidiomata, macroconidia of *Cylindrocladium* species are produced from phialides on macronematous, mononematous conidiophores. These phialides have divergent collarettes, causing successively produced conidia to push older conidia to the side (Crous, Wingfield and Lennox, 1994a). The mucous that covers the macroconidia ensures that they adhere to each other, forming parallel bundles similar to those seen in species of *Chaetopsina* Rambelli (Onofri and Zucconi, 1991), but distinct from those of *Cylindrocladiella* species, which have convergent collarettes (Crous *et al.*, 1994a). Although typical phialides of *Cylindrocladium* produce several conidia, variations of the typical pattern have been observed in some cultures using scanning electron microscopy. Such conidiogenous cells proliferate percurrently once or twice, then resume typical phialide ontogeny (*sensu* Sutton, 1980) with periclinal thickening.

Although in most species macroconidia are straight and cylindrical, some species have curved conidia (i.e. *C. hawksworthii* Peerally), whereas others have conidia that appear slightly swollen at the basal septum (i.e. *C. variabile* Crous, B.J.H. Janse, D. Victor, G.F. Marais and Alfenas). In *Cylindrocladium* species, microconidia are generally borne on conidiophores without stipe extensions, which vary from being penicillate to slightly more divergent in the arrangement of their conidiogenous cells. Microconidia are cylindrical, straight or curved, and

1-septate. Recently, however, 3-septate microconidia have also been observed in *C. rumohrae* El-Gholl and Alfenas and *C. multiseptatum* Crous and M.J. Wingf. (El-Gholl *et al.*, 1997; Crous *et al.*, 1998). In the *Fusarium* species that produce them, microconidia are typically produced from the aerial mycelium and are independent of the conidiomata. In *Cylindrocladium*, microconidiophores occur intermingled with macroconidiophores, from which they are distinguished only with difficulty.

In a recent study dealing with the taxonomy of the genus *Fusarium*, Pascoe (1990a) coined the new term, mesoconidium, for a third conidial type that he recognized in some species of the genus. Mesoconidia are intermediate in size between micro- and macroconidia, and are produced singly and holoblastically in the aerial mycelium from sympodially proliferating conidiogenous cells, producing single, dry, spindle-shaped 0-3-septate conidia at each locus. Pascoe (1990b) suggested that mesoconidia were specialized for wind dispersal, with their production stimulated by weak nutrient environments, dry conditions and in the presence of fairly intense light. Although some authors (e.g. Nirenberg, 1990) have dismissed the term mesoconidium as superfluous, we find the concept useful for differentiating apparently ecologically specialized conidia from other types of conidia produced on the aerial mycelium.

An additional type of conidium, larger than macroconidia, exists in several species of *Cylindrocladium*, but has only been sporadically documented (e.g. Sobers, 1971; Alfieri *et al.*, 1972; Uchida and Aragaki, 1992). The purpose of the present paper, therefore, is to characterise this new conidial type, defined here as megaconidia. As an appendix, the similarity between *Cylindrocladium* and the recently described genus *Cylindrocladiopsis* is considered.

Materials and methods
Megaconidial formation
Strains examined for megaconidial production in this study were: *C. multiseptatum* (STE-U 1602), *C. heptaseptatum* Sobers, Alfieri and Knauss (UFV 9A, 216, 217, Uchida 2456), *C. rumohrae* (UFV 218), *C. theae* (Petch) Subram. (Uchida 1459-6, 1825, 1962, 1963-10, 1999, UFV 16A), *C. pteridis* F.A. Wolf (UFV 43, STE-U 1181), *C. spathiphylli* Schoult., El-Gholl and Alfieri (UFV 178, 180), *C. pseudogracile* Crous (AR 2677, STE-U 1556, 1586), *C. ovatum* El-Gholl, Alfenas, Crous and T.S. Schub. (STE-U 1440), *C. reteaudii* (Bugn.) Boesew. (STE-U 1484), *C. colhounii* Peerally var. *colhounii* (STE-U 1339), *C. colhounii* var. *macroconidiale* Crous, M.J. Wingf. and Alfenas (STE-U 1237), *C. quinqueseptatum* Boedijn and Reitsma (STE-U 759, UFV 64A), *C. variabile* (STE-U 1449), and a *Cylindrocladium* sp. (STE-U

1603). Single-macroconidial isolates were cultured on 2 % malt extract agar (MEA) (Biolab), plated onto divided plates containing carnation-leaf agar (CLA) (Fisher *et al.*, 1982; Crous, Phillips and Wingfield, 1992), in one half, and tap water agar (WA) in the other. Plates were incubated at 25 C under near UV light, and examined weekly for megaconidial formation.

Effect of osmotic potential on megaconidium formation

To determine the effect of osmotic potential on megaconidium production, strains were transferred onto WA plates emended with 0, 4, 6, 8 and 10 g KCl per litre agar. Each strain was grown on two plates at each concentration, and incubated as outlined above.

Results

Species with megaconidia

Although several large-spored species of *Cylindrocladium* were investigated in the present study for megaconidial formation, this conidial type could be confirmed from only five species, namely *C. heptaseptatum* (Figs. 9, 13), *C. multiseptatum* (Figs. 1-4, 10), *C. rumohrae* (Figs. 6, 11), *C. theae* (Figs. 7, 8, 14) and an as-yet undescribed taxon (Figs. 5, 12; Table 1). Not all strains of these five species readily formed megaconidia.

### Megaconidiophore and megaconidium morphology

Megaconidiophores were never borne on aerial mycelium, but occurred on the WA surface, or within the agar. Stipe extensions were only once observed in *C. multiseptatum*. Conidiophores were unbranched with a single phialide, or had a terminal whorl of 1-2 phialides, or sometimes had a single subterminal phialide. Phialides were straight to curved and seemed to mostly produce a single mesoconidium; periclinal thickening and an inconspicuous divergent collarette were sometimes visible. Primary conidia formed holoblastically, and subsequent conidia, when produced, were enteroblastic. Conidia frequently remained attached to phialides for extended periods. Conidia were characteristically multiseptate, widest in the middle, straight to curved or bent at right angles, significantly larger than macroconidia and had a truncate base and rounded apical cell. In several species, the megaconidia were often bent near the centre, and looked like boomerangs.

Macroconidiophore and macroconidium morphology

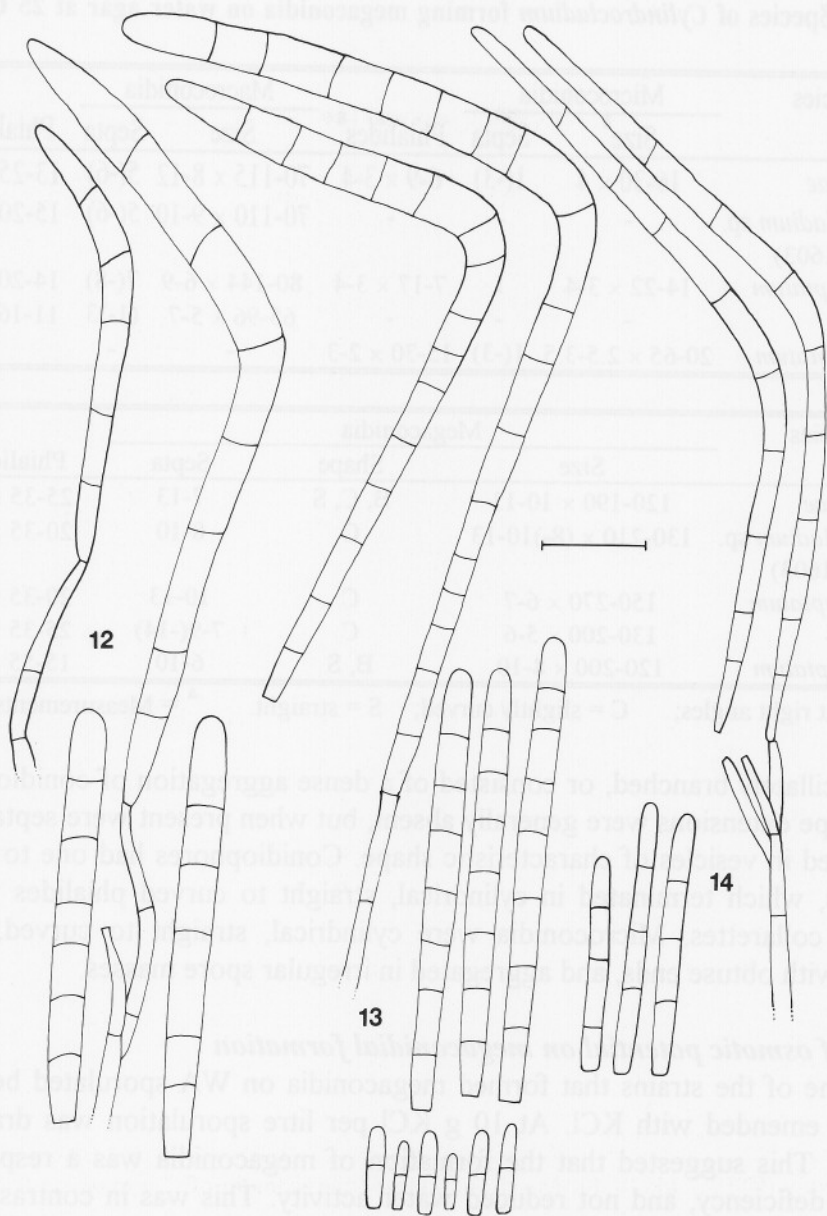Macroconidiophores were formed on the aerial mycelium, on agar and on

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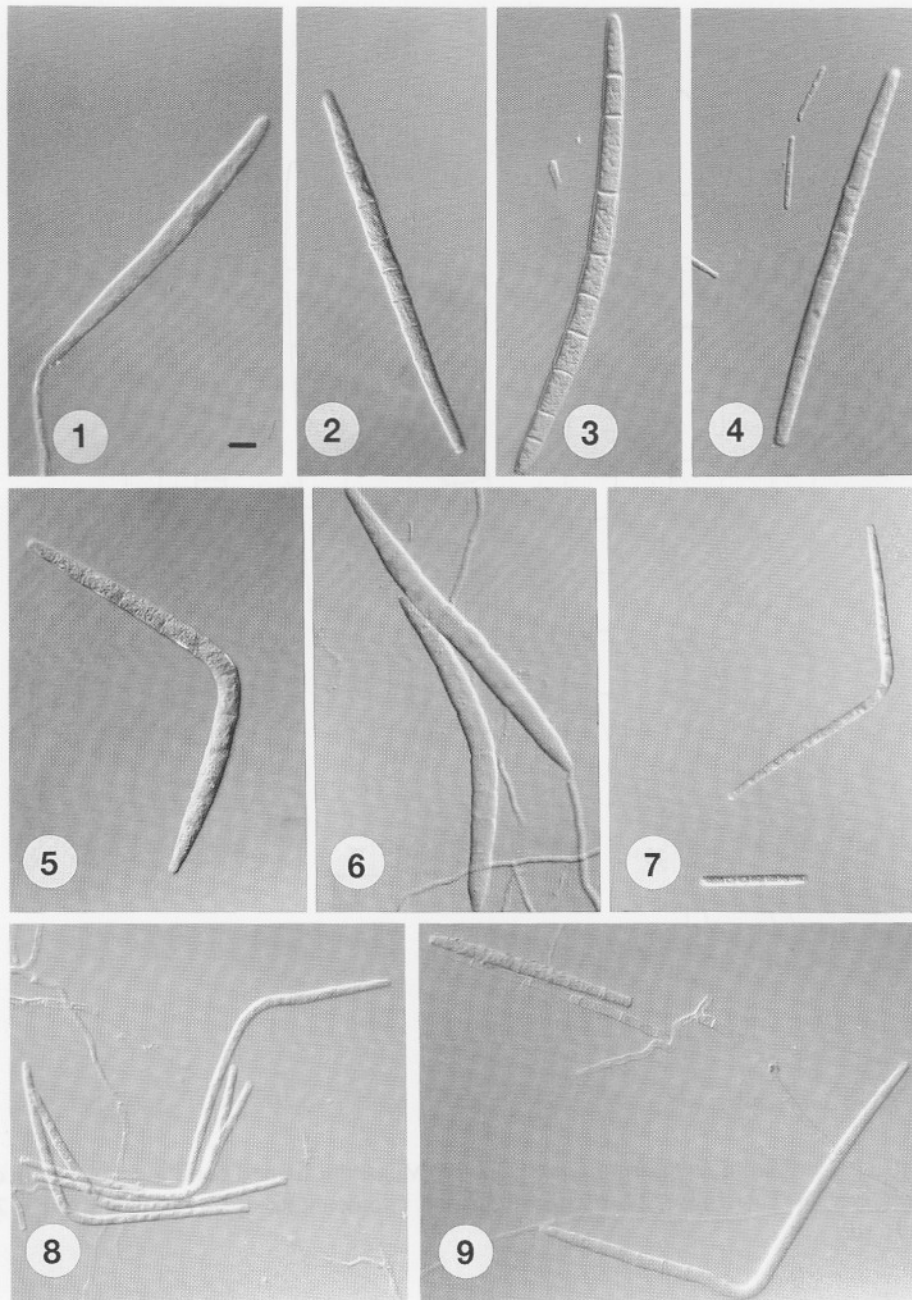
Figs. 1-9. Conidia of *Cylindrocladium* spp. **1-4.** *C. multiseptatum* (STE-U 1602). 1-3. Megaconidia. 4. Megaconidium and microconidia. **5.** Megaconidium of a *Cylindrocladium* sp. from *Adiantum* (STE-U 1603). **6.** Megaconidia of *C. rumohrae* (UFV 218). **7, 8.** Curved megaconidia and 3-septate macroconidium of *C. theae* (Uchida 1459-6). **9.** Curved megaconidium and straight macroconidium of *C. heptaseptatum* (UFV 9A). Bars = 10 μm.
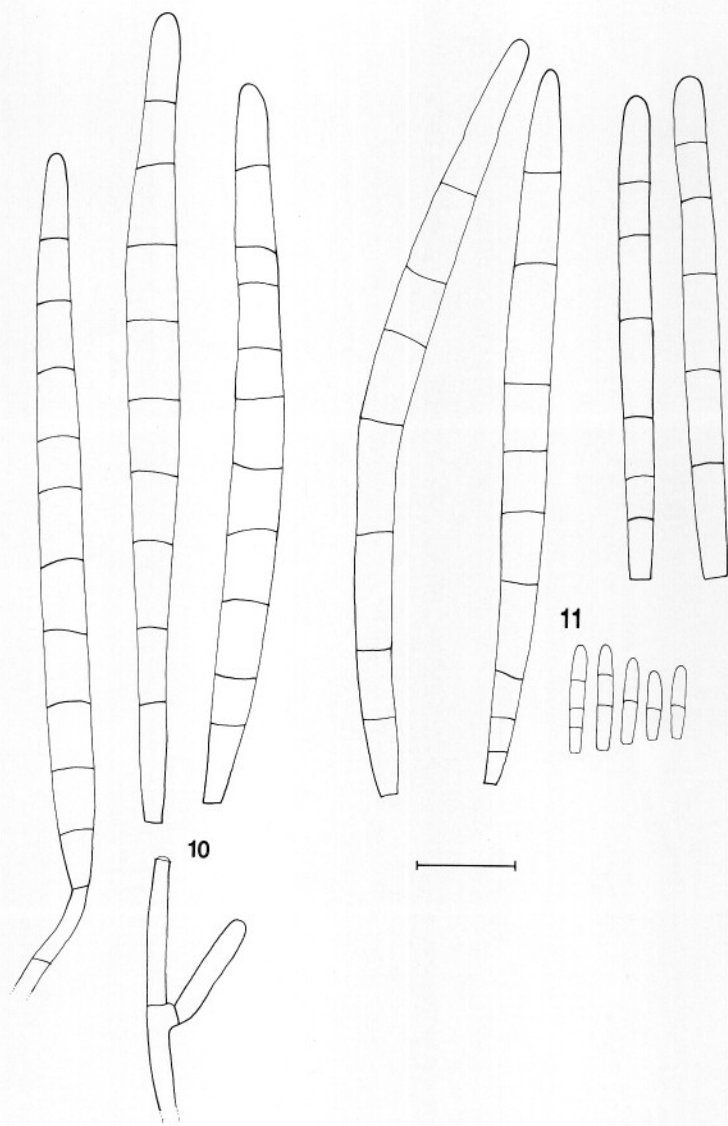
Figs. 10, 11. Conidia of *Cylindrocladium* spp. **10.** Megaconidia and conidiogenous cells of *C. multiseptatum* (STE-U 1602). **11.** Megaconidia and microconidia of *C. rumohrae* (UFV 218). Bars = 10 μm.

CLA. The conidiophores were usually penicillately branched, arose at right angles to the substrate, and had septate stipe extensions that terminated in vesicles of characteristic shape. Conidiophores had 1-6 levels of branches that gave rise to doliiform, reniform or cylindrical to allantoid phialides that occurred in terminal groups of up to six. Macroconidia were typically cylindrical with obtuse ends, straight to curved, 1-9-septate, widest near the base, and borne in cylindrical packets.

Figs. 12-14. Conidia of *Cylindrocladium* spp. **12.** Macroconidia, megaconidia and megaconidiogenous cells of a *Cylindrocladium* sp. (STE-U 1603). **13.** Megaconidia, macroconidia and microconidia of *C. heptaseptatum* (UFV 9A). **14.** Macroconidia, megaconidia and megaconidiogenous cells of *C. theae* (Uchida 1459-6). Bars = 10 µm.

Microconidiophore and microconidium morphology

Microconidiophores were formed on the aerial mycelium, on the agar surface as well as on CLA. The conidiophores were penicillately or

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Table 1. **Species of *Cylindrocladium* forming megaconidia on water agar at 25 C under nuv.**

Species	Microconidia			Macroconidia		
	Size[a]	Septa	Phialides[a]	Size	Septa	Phialides[a]
C. rumohrae	16-30 × 4	1(-3)	6-9 × 3-4	70-115 × 8-12	5(-6)	13-25 × 4-6
Cylindrocladium sp. (STE-U 1603)	-	-	-	70-110 × 9-10	5(-6)	15-20 × 4-5
C. heptaseptatum	14-22 × 3-4	1	7-17 × 3-4	80-144 × 6-9	7(-8)	14-20 × 4-5
C. theae	-	-	-	65-96 × 5-7	(1-)3	11-16 × 3-4
C. multiseptatum	20-65 × 2.5-3.5	1(-3)	15-30 × 2-3	-	-	-

Species	Megaconidia			
	Size	Shape	Septa	Phialides[a]
C. rumohrae	120-190 × 10-13	B, C, S	7-13	25-35 × 4-5
Cylindrocladium sp. (STE-U 1603)	130-210 × (8-)10-13	C	8-10	20-35 × 5-6
C. heptaseptatum	150-270 × 6-7	C	10-13	20-35 × 4-6
C. theae	130-200 × 5-6	C	7-9(-14)	25-35 × 4-5
C. multiseptatum	120-200 × 8-10	B, S	6-10	15-55 × 4-5

B = bent at right angles;   C = slightly curved;   S = straight.   [a] = Measurements in μm.

subverticillately branched, or consisted of a dense aggregation of conidiogenous cells. Stipe extensions were generally absent, but when present were septate, and terminated in vesicles of characteristic shape. Conidiophores had one to several branches, which terminated in cylindrical, straight to curved phialides with or without collarettes. Microconidia were cylindrical, straight to curved, 1(-3)-septate with obtuse ends, and aggregated in irregular spore masses.

Effect of osmotic potential on megaconidial formation
None of the strains that formed megaconidia on WA sporulated better on the WA emended with KCl. At 10 g KCl per litre sporulation was drastically reduced. This suggested that the formation of megaconidia was a response to nutrient deficiency, and not reduced water activity. This was in contrast to the mesoconidia of *Fusarium* species, where 8 g KCl per litre SNA seemed to be optimal for sporulation (Pascoe, 1990b).

Discussion
Crous and Wingfield (1994) monographed *Cylindrocladium* and described conidia using the standard macroconidiophore, macroconidia, microconidiophore and microconidia terminology employed for anamorphs of

the Hypocreales. In standardising methods for *Cylindrocladium* identification, Crous *et al.* (1992) stated that only conidia on carnation leaf agar should be examined, thus excluding the larger conidial form of *C. theae* (Figs. 8, 9, 15) first observed by Sobers on water agar and glycerol-water agar. The same megaconidia were described in detail by Alfieri *et al.* (1972). Uchida and Aragaki (1992) later isolated strains of this species in Hawaii, and induced megaconidial production on either water agar, or water agar emended with 0.5 % glycerol (Figs. 1-14).

The description of *C. rumohrae* (El-Gholl *et al.*, 1997; Figs. 6, 11) demonstrated that this larger conidial form also occurred in other species of *Cylindrocladium*. Furthermore, *Crous et al.* (1998) described *C. multiseptatum* (Figs. 1-4, 10) from *Eucalyptus* exhibiting branch die-back and leaf spot symptoms in Indonesia. When the fungus was grown on CLA, however, only the *Calonectria* teleomorph developed, while the *Cylindrocladium* state was only formed on or in WA. Crous *et al.* (1998) commented that the species had reduced conidiophore morphology and peculiar conidia that were frequently curved and widest in the middle. These conidia were most frequently formed from solitary phialides on unbranched conidiophores. Recognition of the megaconidium as a distinct conidial type allowed reconsideration of the morphology of *C. multiseptatum*. Cultures of this species had apparently lost the ability to form true macroconidia, and only formed the teleomorph, microconidia, megaconidia and chlamydospores in culture.

It is important that megaconidia be distinguished from macroconidia to ensure that mycologists compare conidia of homologous conidial types when studying *Cylindrocladium* isolates. Uchida and Aragaki (1997), who also discussed this conidial type, suggested that the term "macroconidium" be used for conidia characteristically distinct from "normal" conidia and microconidia. The latter proposal is, however, in contrast with the terminology currently employed in other hypocrealean genera.

The observation of megaconidia in five species of *Cylindrocladium* suggests that it is a more common phenomenon than previously realized. The fact that only some strains of these species form megaconidia suggests that their value as an additional taxonomic character may be limited. Their morphology apparently remains constant within species, suggesting that their presence may be helpful for some species identifications. Because some species only form this conidial type, the recognition of megaconidia is obviously an important concept in *Cylindrocladium* taxonomy. Unfortunately, some conidia do show overlap between typical megaconidial and macroconidial characters. However, the distinction can always be made if conidiophores, position on or in the agar, and
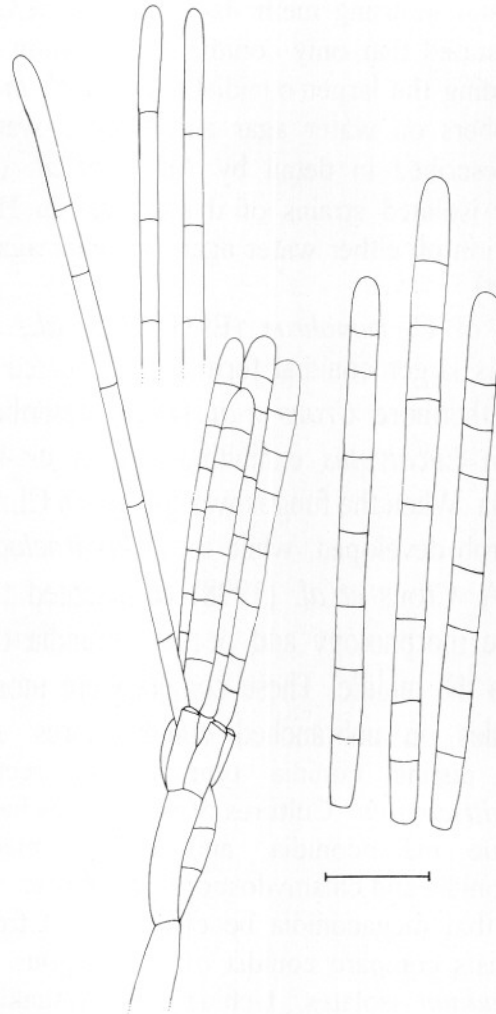
Fig. 15. Penicillate conidiophore, vesicles and 1-7-septate macroconidia of *Cylindrocladiopsis lagerstroemiae* (= *Cylindrocladium heptaseptatum*) (MUCL 20465). Bar = 10 μm.

and conidial shapes are taken into consideration.

The Taxonomic Status Of **Cylindrocladiopsis**
Cylindrocladiopsis J.M. Yen, Mycotaxon 8: 236 (1979).

To investigate the apparent similarity between *Cylindrocladium* and the genus *Cylindrocladiopsis*, the isotype specimen of the latter was obtained from MUCL (Yen, 1979). Slide preparations were mounted in clear lactophenol, and wherever possible 30 measurements were made to derive the mean dimensions of each structure, with the extremes given in parentheses.

The genus is typified by *C. lagerstraemiae* J.M. Yen, a hyaline hyphomycete associated with leaf spots of *Lagerstroemia flos-reginae* Retz.

collected in Malaysia. In the original description Yen (1979) distinguished *Cylindrocladiopsis* from *Cylindrocladium* by the production of cylindrical holoblastic conidiogenous cells that gave rise to single conidia, and the absence of stipe extensions. Braun (1995) regarded this genus as similar to others in the cercosporoid complex, and considered it related to *Thedgonia* B. Sutton. Our examination of the isotype (MUCL 20465) demonstrates that *C. lagerstraemiae* is a species of *Cylindrocladium*. The conidiophores have stipe extensions that terminate in narrowly clavate vesicles (4-5 µm diam.), while conidia are cylindrical with rounded ends, (1-)7-septate, (45-)80-100(-120) × 6-7(-8) µm, and are formed enteroblastically. Conidiogenous cells are cylindrical, 5-22 × 3-4 µm, with minute, divergent collarettes at their apices (Fig. 15). The latter characters are very similar to *C. heptaseptatum*, which has narrowly clavate vesicles 3-5 µm diam., cylindrical phialides, and (1-)7-septate conidia 80-144 × 6-9 µm. We thus consider *Cylindrocladiopsis* a synonym of *Cylindrocladium*.

Braun (1995) also assigned a cercosporoid fungus, *Cercosporella hurae* Linder and Whetzel to *Cylindrocladiopsis*. This taxon appears to be a true cercosporoid fungus, and will be considered elsewhere once its type specimen has been re-examined.

Acknowledgements

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