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GLIOCLADIOPSIS IRREGULARIS SP.NOV. AND NOTES ON *CYLINDROCLADIUM SPATHIPHYLLI*

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ABSTRACT

A new species of *Gliocladiopsis* with penicillate and subverticillate conidiophores and irregularly curved 1-septate conidia is described from soil as *G. irregularis*. The variation present in the vesicle morphology of *Cylindrocladium spathiphylli* is discussed, and a previously unknown microconidial state reported.

INTRODUCTION

The genus *Gliocladiopsis* Saksena (1954) was erected for species with hyaline, cylindrical conidia borne in slimy masses on whorls of phialides on penicillate conidiophores. This genus is presently monotypic, and the type species, *G. sagariensis* Saksena was shown to be synonymous with the earlier described *Cylindrocarpon tenue* Bugn. (Agnihothrudu, 1959). Barron (1968), however, regarded the genus as a later synonym of *Cylindrocladium* Morgan. Crous & Wingfield (1993) stated that *Gliocladiopsis* should be retained for species with penicillate conidiophores lacking stipe extensions and terminal vesicles as commonly found in *Cylindrocladium* and *Cylindrocladiella* Boesewinkel. Based on the converging branching pattern of the penicillate conidiophores, the genus *Cylindrocarpon* was also found unsuitable. *C. tenue* was subsequently placed in *Gliocladiopsis* as *G. tenuis* (Bugn.) Crous & M.J. Wingf., with *G. sagariensis* as synonym. Watanabe (1994) stated, however, that because several species of *Cylindrocladium* and *Cylindrocladiella* lose their ability to produce stipe extensions in culture, this is not a stable criterium to separate

these genera. *Cylindrocarpon tenue* was subsequently transferred to *Cylindrocladium*. Other studies on *Cylindrocladium* and *Cylindrocladiella* (Crous & Wingfield, 1993; 1994) showed that all species in these two genera do in fact produce stipe extensions on penicillate conidiophores.

Watanabe (1994) also regarded *Cylindrocladium intermedium* Matsushima to be a synonym of *Gliocladiopsis tenuis*. However, the latter species was described with conidia being 20-30 x 1.6-2.8 µm (Matsushima, 1971), which is larger than any conidial measurement ever published for *G. tenuis* (Crous & Wingfield, 1993), or those obtained in the present study (Table 1). Although *C. intermedium* is probably better accommodated in *Gliocladiopsis* than *Cylindrocladium*, such a decision will have to be based on a re-examination of the type specimen.

As part of an ongoing study baiting soil samples for isolates of *Cylindrocladium* spp. (Crous *et al.*, 1994), several strains of *Gliocladiopsis tenuis* were also obtained (Table 1). One such a collection proved to be distinct from *G. tenuis*. Although similar in general morphology, this species is unique in having smaller conidia and phialides. Furthermore, conidia are variously curved, and frequently slightly swollen in the apical cell. Because this collection proved distinct from the type strain of *G. tenuis* and all other strains studied, it is described as new below:

Gliocladiopsis irregularis Crous & Peerally sp. nov. Fig. 1.
Vesiculae et stipites absentiae. *Conidiophorae penicillatae*, rami primarii rare 1-septati, (15-)17(-25) x (3-)3.5 µm, rami secondarii non septati, (15-)18(-20) x 2.5(-3) µm, rami tertiarii non septati, (9-)11(-14) x 2(-2.5) µm; phialides doliiformes ad reniformes ad cymbiformes, (10-)13 (-16) x (2-)3 µm, in verticillis terminalibus, ad sex in quoque ramulo; collaretae praesentes. *Conidiophorae subverticillatae* paucae, plerumque in mycelium aerium; phialides cylindricae, (20-)25(-35) x 2.5(-3) µm; collarettae praesentes. *Conidia* 1-septata, hyalina, laevigata, cylindrica, recta ad varie curvata, apice obtuso, base subtruncata, cellula apicali saepe parum tumida, (11-)13(-14) x 2.5(-3) µm. *Teleomorphus* ignotus.

HOLOTYPUS. INDONESIA. Northern Sumatra, soil, 22 Nov. 1993, A.C. Alfenas, PREM 51870, ex type culture STE-U 718).

Table 1 Morphological features and temperature requirements for growth of *Gliocladiopsis* spp. studied.

Species	Accession No	Conidial dimensions (um)[a] length x width	Conidial septation	Cardinal temps. for growth (°C)[b] min	opt	max	Substrate	Origin	Collector
G. tenuis	IMI 300 597	(15-)18(-22) x 1.5(-2)	1	<10	30	>35	*Psidium guajava*	India	IMI
	CBS 199.55	(12-)19.5(-23) x (1.5-)2	1	<10	30	>35	Soil	India	S.B. Saksena
	CBS 978.73	(14-)17(-21) x 1.5(-2)	1	<10	25	>35	Soil	Brazil	C.S. Hodges
	STE-U 636	(16-)22(-26) x 1.5(-2.5)	1(-3)	<10	30	>35	*Chamaedorea elegans*	USA	N.E. El-Gholl
	STE-U 706	(15-)17(-19) x (1.5-)2(-2.5)	1	<10	30	>35	Soil	Hong Kong	P.W. Crous
	STE-U 707	(15-)19(-21) x (1.5-)2	1	<10	30	>35	Soil	Thailand	P.W. Crous
	STE-U 714	(14-)17(-22) x 2(-2.5)	1	<10	25	>35	Soil	Indonesia	P.W. Crous
	STE-U 715	(13-)17(-20) x (1.5-)2(-2.5)	1	<10	25	>35	Soil	Indonesia	P.W. Crous
	STE-U 716	(15-)17(-19) x (1.5-)2(-2.5)	1	<10	30	>35	Soil	Indonesia	P.W. Crous
	STE-U 717	(15-)19(-21) x (1.5-)2	1	<10	30	>35	Soil	Indonesia	P.W. Crous
	STE-U 731	(12-)17(-20) x (1.5-)2(-2.5)	1	<10	25	>35	Soil	Columbia	P.W. Crous
G. irregularis	STE-U 718	(11-)13(-14) x 2.5(-3)	1	<15	25	>35	Soil	Indonesia	P.W. Crous

[a] Averages are representative of 30 observations on CLA, with extremes listed in parentheses.

[b] Radial growth assessed on MEA after 7d in the dark at 5-35°C in 5° intervals. Three plates were used per isolate at each temperature, and the experiment repeated.

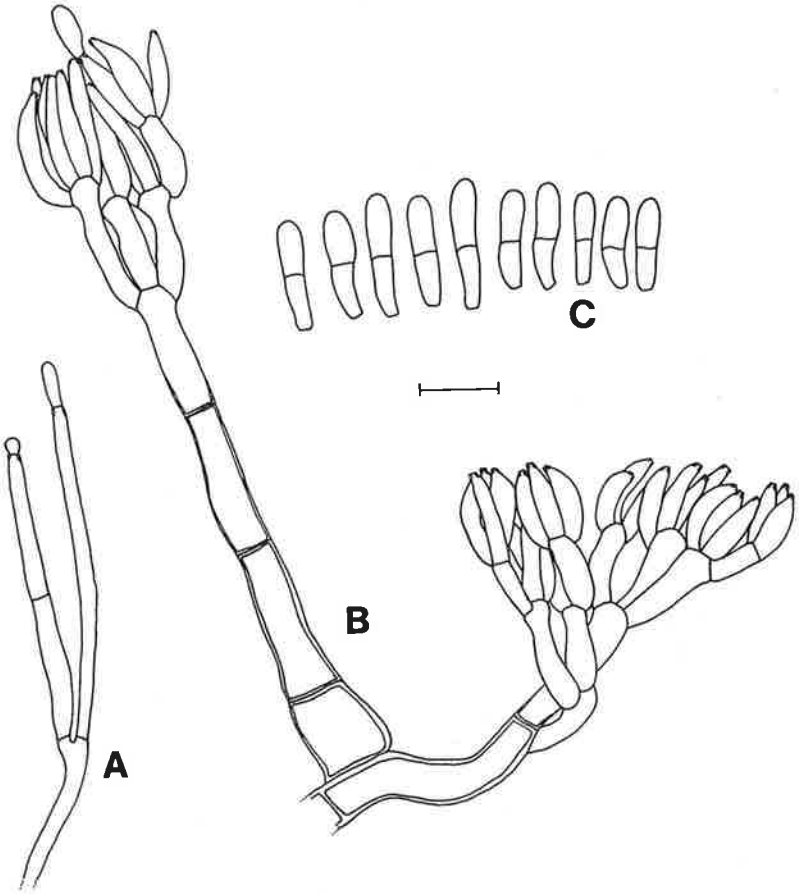

Fig. 1A-C. *Gliocladiopsis irregularis*. A. Subverticillate conidiophore. B. Penicillate conidiophore. C. Irregular 1-septate conidia (bar = 10 µm).

Colonies on 2 % malt extract agar (MEA) (Biolab) plates incubated at 25°C in the dark for 7 d were cinnamon in colour (15"b, Rayner, 1970). Chamydospores formed in sparse numbers, and frequently formed a stroma on which several conidiophores were situated. Cardinal temperature requirements for growth in 5° increments were: below 15°C (minimum), 25°C (optimum), and above 35°C (maximum).

All morphological observations were made on carnation-leaf agar (CLA) plates incubated for 7 d at 25°C in the dark. Vesicles and stipes absent. *Penicillate conidiophores*, primary branches rarely 1-septate, (15-)17(-25) x (3-)3.5 µm, secondary branches non-septate, (15-)18(-20) x 2.5(-3) µm, tertiary branches non-septate, (9-)11(-14) x 2(-2.5) µm; phialides doliiform to reniform to cymbiform, (10-)13(-16) x (2-)3 µm, arranged in terminal whorls of up to six per branch; collarettes present. *Subverticillate conidiophores* sparse in number, mostly present in aerial mycelium; phialides cylindrical, (20-)25(-35) x 2.5(-3) µm; collarettes present. *Conidia* 1-septate, hyaline, smooth, cylindrical, straight to variously curved, apex obtuse, base subtruncate, apical cell frequently slightly swollen, (11-)13(-14) x 2.5(-3) µm. *Teleomorph* unknown.

SUBSTRATE. Soil.

KNOWN DISTRIBUTION. Indonesia (northern Sumatra), known only from type collection.

One strain (STE-U 636) isolated from *Chamaedorea elegans* in the USA has conidia which are (16-)22(-26) x 1.5(-2) µm and 1(-3)-septate. Although these conidia have more septa and are slightly longer than those of the other strains sporulating on CLA, we are hesitant to describe this as a new taxon, and are of the opinion that additional collections or molecular techniques would be required to support such a decision. Although conidial dimensions and septation is a good indication of species limits in *Cylindrocladium*, some strains of species such as *C. ovatum* El-Gholl *et al.* and *C. spathiphylli* Schoulties *et al.* have been collected that form only one conidial septum, whereas others, as in the case of *Gliocladiopsis* strain STE-U 636, can form up to three.

All teleomorphs with *Cylindrocladium* anamorphs are presently placed in *Calonectria* De Not., while those with *Cylindrocladiella* anamorphs are accommodated in *Nectria* Fr. subsection *Dialonectria* Sacc. Although all

Gliocladiopsis strains (Table 1) were mated in all possible combinations using the methods described by Crous *et al.* (1993a), no teleomorph was obtained. This suggests, therefore, that a molecular approach would probably be required to elucidate relatedness amoung the genera *Gliocladiopsis*, *Cylindrocladium* and *Cylindrocladiella*.

Cylindrocladium spathiphylli Fig. 2.

In 1972 the second author collected a *Cylindrocladium* species from *Camellia sinensis* L. in Mauritius, which was subsequently lodged at IMI. In an examination of this specimen (Crous *et al.*, 1991), globose vesicles and 3-septate conidia were observed. Several years after this isolate was collected, Schoulties *et al.* (1982) described a species with 1-septate conidia and similar vesicle morphology from *Spathiphyllum* as *C. spathiphylli*. Uchida & Aragaki (1992) reported, however, that this species could have 1-3-septate conidia with sphaeropedunculate, ellipsoid or spathulate vesicles. El-Gholl *et al.* (1992) subsequently described the heterothallic teleomorph as *Calonectria spathiphylli* El-Gholl *et al.* Furthermore, additional hosts such as *Heliconia*, *Ludwigia* and *Strelitzia* were reported for this species, while Crous & Wingfield (1994) also listed it on *Araucaria*.

The Mauritian isolate (IMI 167983) has conidia which are (1-)3-septate, (55-)90(-120) x (6-)6.5(-7) µm. El-Gholl *et al.* (1992) reported that cultures of *C. spathiphylli* were primarily 1(-3)-septate, 39-148 x 4.5-8 µm, thus corresponding with our isolate. Furthermore, IMI 167983 also produced a distinct microconidial state on CLA. Conidia are 1-septate, (32-)39(-50) x (3.5-)4(-5) µm, and form on straight cylindrical to allantoid phialides, 10-26 x 3-3.5 µm. No microconidial vesicles were, however, observed. This is the first report of a microconidial state for *C. spathiphylli*, and a new host and geographic record for the species.

Isolate IMI 167983 was mated with several predetermined tester strains of *C. spathiphylli* (El-Gholl *et al.*, 1992), namely P87-0167 (+, from *Heliconia*), P91-7693 (+, from *Ludwigia*), P86-0210 (-, from *Heliconia*) and P91-1735 (-, from *Strelitzia*). A few strains of undetermined mating type from *Heliconia* spp. were also included, namely P89-0823, P89-1685, P88-0321, as well as an isolate from Switzerland, K2C (from *Spathiphyllum*).

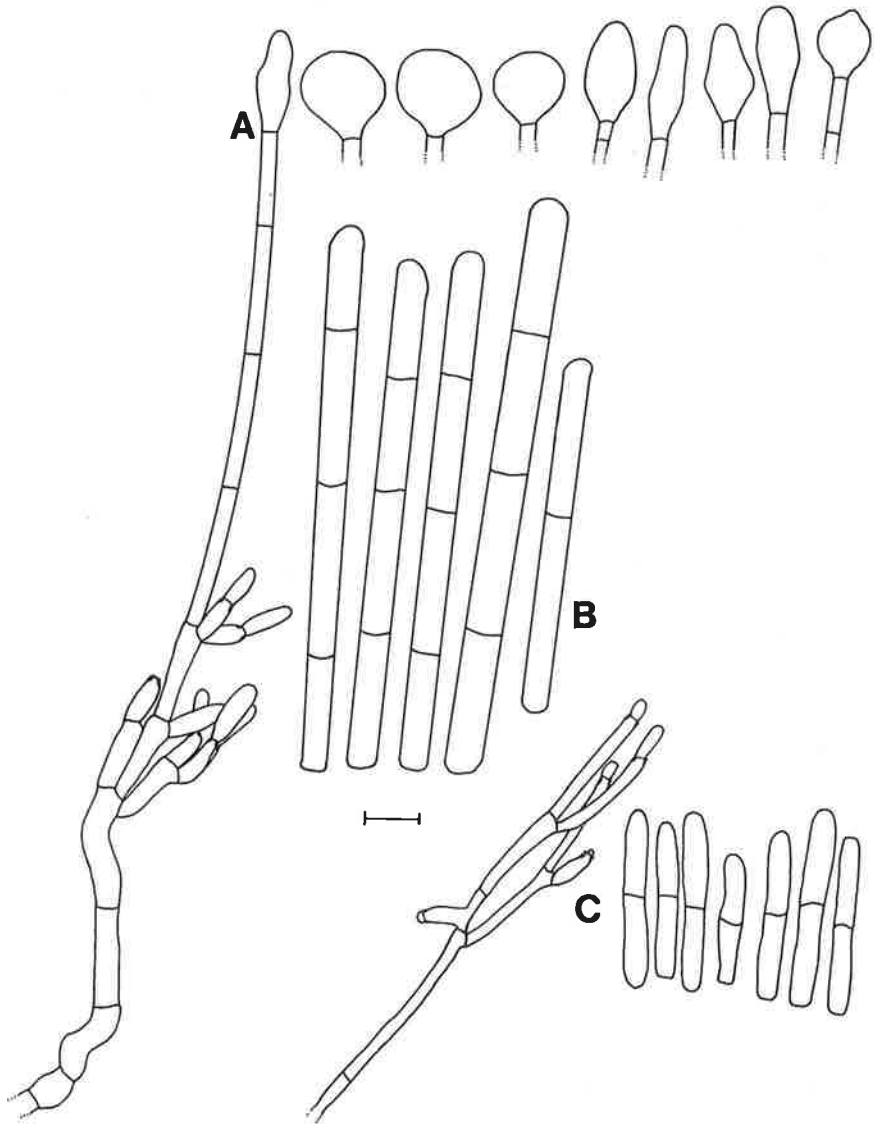

Fig. 2A-C. *Cylindrocladium spathiphylli*. A. Penicillate macroconidiophore with vesicles. B. Cylindrical 1-3-septate macroconidia. C. Microconidiophore with 1-septate microconidia (bar = 10 µm).

Perithecia with viable ascospores were obtained within 2 mo at 25°C on the laboratory bench. Isolate IMI 167983 mated with (-) strains, namely P91-1735, P86-0210, P89-1685, P88-0321, P89-0823 and K2C, confirming it to be a (+) mating type, and a strain of *C. spathiphylli*. It can therefore be accepted that although vesicle morphology is characteristic of the various species of *Cylindrocladium*, some species are characterized by a certain amount of variation in their vesicles, as has also been reported for *C. variabile* Crous *et al.* (Crous *et al.*, 1993b). Further studies on the various species complexes using a combination of molecular techniques and sexual compatibility are required, therefore, to determine the amount of variation acceptable within each biological species.

The formation of microconidial states in *Cylindrocladium* is poorly understood (Crous & Wingfield, 1994). Although these states are much less common than in morphologically similar genera such as *Cylindrocarpon* Wollenw. and *Fusarium* Link, some species of *Cylindrocladium* are nearly always isolated with both conidial types (*C. ovatum*, *C. pteridis* Wolf, *C. variabile*), whereas this is rarely observed in others (Peerally, 1991; Crous & Wingfield, 1994). In the case of strain IMI 167983 of *C. spathiphylli*, it would seem that long term preservation induced the ability to form both conidial states, as has also been observed for strain CBS 190.50 of *C. parasiticum* Crous *et al.* (Crous *et al.*, 1993c). This suggests, therefore, that the formation of microconidia in some species of *Cylindrocladium* may only be due to unfavourable conditions, and be of little value as an additional taxonomic feature.

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