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Anamorphic fungi from French Guyana. *Septomyrothecium maraitiense* sp. nov. and *S. setiramosum* comb. nov. (anamorphic Hypocreales, Ascomycota)

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Abstract – *Septomyrothecium maraitiense sp. nov.,* isolated from a decaying leaf in French Guyana, South America, is described and illustrated. The species is compared to *Septomyrothecium uniseptatum* and *Myrothecium setiramosum*, and the latter is transferred into *Septomyrothecium*.

***Myrothecium* / anamorphic Hypocreales / phylogeny / systematics / South America**

Résumé – *Septomyrothecium maraitiense sp. nov*., isolée de litière forestière collectée en Guyane française est décrite et illustrée. Cette espèce est comparée à *S. uniseptatum* et *Myrothecium setiramosum*, cette dernière étant transférée dans *Septomyrothecium*. Une approche phylogénétique préliminaire permet de discuter brièvement des affinités de ces espèces et du genre *Septomyrothecium*.

***Myrothecium* / Hypocreales anamorphiques / phylogénie / systématique / Amérique du Sud**

INTRODUCTION

During a study of leaf litter anamorphic fungi from the rainforest in French Guyana (Crous *et al.,* 2001; Decock, 2005; Decock & Crous, 1998; Decock *et al.,* 2006), a *Myrothecium*-like collection was examined that could not be satisfactorily identified as any of the described species. In particular, it produces remarkable, thick-walled hyphoid extensions, once or twice dichotomously branched (Figs 4, 5-6) and apically slightly twisted, emerging through the green conidial mucoid mass (Fig. 1). Conidia are long (up to 23 µm long), narrowly cylindrical, 0- to 1-septate, straight or slightly curved (Fig. 3). This combination of characteristics is unique within *Myrothecium* and related genera (Agarwal, 1980; Bohn, 1993; Castañeda, 1986; Castañeda & Kendrick, 1991; Castañeda *et al*., 2008; Dicosmo *et al.,* 1980; Escalona, 1997; Matsushima, 1971, 1995; Nag Raj, 1993,

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1995a, b; Rao & de Hoog, 1983; Seifert *et al.,* 2003; Sullia & Padma, 1985; Sutton, 1985; Tulloch, 1972; Udagawa & Awao, 1984; Watanabe *et al.,* 2003). We therefore concluded that we were dealing with a species not yet described, and whose description is given below.

However, and although the gross morphological characteristics – sporodochia with slimy phialoconidia gathered in dark green mucilaginous mass – would straightforwardly point toward *Myrothecium* as defined by Tulloch (1972), its generic placement was questioned. The hyphoid, extensions and the conidial features could also indicate a possible relationship with the monotypic *Septomyrothecium* (type species: *S. uniseptatum*, Matsushima, 1971a), which distinctiveness from *Myrothecium* is uncertain.

The phylogenetic relationships of the French Guyana fungus and representatives of *Myrothecium* and *Septomyrothecium* were assessed from a parsimony analysis of a DNA data set from the nuclear ribosomal ITS regions.

These analyses evidenced that this French Guyana fungus is more closely related to *S. uniseptatum* than to *M. inundatum*, *Myrothecium* type species (Tulloch, 1972). They also established *Septomyrothecium* as a monophyletic clade, indirectly related to *Myrothecium*, and including in addition to *S. uniseptatum* and the French Guyana fungus, *M. setiramosum* (Castañeda, 1986) and two (or possibly three) additional species, still of uncertain identity, and originating from South America and Southeast Asia.

The new species *Septomyrothecium maraitiense* and the new combination *Septomyrothecium setiramosum* are proposed. The circumscription of *Septomyrothecium* is briefly outlined.

MATERIAL AND METHODS

Isolates were obtained from rain forest leaf litter collected in Feb. 1994 in French Guyana. Strains were isolated *in situ* from mass conidia plated on Malt Agar 2% to which 100 ppm chloramphenicol was added, and incubated at 25°C. Additional strains of various species were obtained from MUCL and CBS (Netherlands). For morphological examination, cultures were grown on V-8 juice agar (V8) and Banana leaf agar (BLA) (Untereiner *et al.* 1998) at 25°C, with a 12/12 hrs incident near UV light/dark cycle. Microscopic measurements were made of structures mounted in lactic acid cotton blue (Kirk *et al.,* 2001). In presenting the size range of several microscopic elements, 5% of the measurements at each end of the range are given in parentheses, when relevant. In the text, the following abbreviations are used: $\bar{X}$ = arithmetic mean; R = ratio of length/width of the conidia; $\bar{X}_R$ = arithmetic mean of the ratio R.

DNA was extracted from freshly collected mycelium grown in liquid malt extract at 25°C in the dark. Extractions were carried out using the QIAGEN Dneasy plant Mini Kit (QIAGEN Inc., Hilden, Germany), and later purified with Geneclean® III kit (Q-Biogene, USA), following the manufacturer's recommendations. The primer pairs NS7-ITS4 (White *et al.*, 1990) were used to amplify the ITS1-5.8S-ITS2 regions of the nuclear ribosomal operon. Successful PCR reactions resulted in a single band observed on an 0.8% agarose gel, corresponding to approximately 900 bps (ITS). Polymerase chain reaction products were cleaned using the QIAquick® PCR purification kit (250)

(QIAGEN Inc., Hilden, Germany), following the manufacturer's protocol. Sequencing reactions were performed using CEQ DTCS Quick Start Kit® (Beckman Coulter Inc., USA), according to the manufacturer's recommendations, with the primers ITS2, ITS3, ITS4 (White *et al*. 1990) for the ITS region. Nucleotide sequences were determined with a CEQ 2000 XL capillary automated sequencer (Beckman Coulter Inc., USA). Initially, nucleotide sequences were automatically aligned with Clustal X for MacIntosh (version 1.5b), then manually adjusted as necessary by the editor in PAUP* (version 4.0b10).

The final ITS data set comprised 35 sequences (Table 1) and 578 characters were confidently aligned and included in the analysis, including gaps (392 positions were constant, 113 were parsimony informative). 42 characters ambiguously aligned were moved. Phylogenetic analysis of the aligned sequences was performed using the maximum parsimony method of PAUP* version 4.0b10 (Swofford, 2002) with gaps treated as fifth base. The most parsimonious trees were identified using heuristic searches with random addition sequence (1000), max tree set to 100, and further evaluated by bootstrap analysis, retaining clades compatible with the 50% majority-rules in the bootstrap consensus tree. Analysis conditions were: tree bisection addition branch swapping (TBR), starting tree obtained via stepwise addition, steepest descent not in effect, MulTrees effective. *Stachybotrys nilagirica* MUCL 39210 was used as outgroup based on the results of Castlebury *et al.* (2004).

Sequences generated at MUCL (available at MUCL):

Myrothecium Tode ex Fr.: *Myrothecium cinctum* (Corda) Sacc., MUCL 50263; *Myrothecium gramineum* Lib., MUCL 39210; *Myrothecium inundatum* Tode, MUCL 47992; *Myrothecium lachastrae* Sacc., MUCL 47256; *Myrothecium roridum* Tode, MUCL 39102; MUCL 50132.

Didymostilbe Henn.: *Didymostilbe echinofibrosa* (Finley) Rossman (teleomorph: *P. spirostriata* (Rossman) Rossman), MUCL 39092; MUCL 40322; *Didymostilbe sundara* (Subram. & Bhat) Seifert (teleomorph: *P. sundara* Subram. & Bhat), MUCL 39093.

Septomyrothecium Matsushima: *Septomyrothecium setiramosum* (R.F. Castañeda) Decock, MUCL 48260; MUCL 41902; MUCL 41187; MUCL 48180; MUCL 48271; MUCL 48084; *Septomyrothecium uniseptatum* Matsushima, MUCL 41240; *Septomyrothecium maraitiense* Decock et al., MUCL 47202; *Septomyrothecium sp. 1*, MUCL 48121; MUCL 48165; MUCL 48282; *Septomyrothecium sp. 2*, MUCL 41.81.

Stachybotrys Matsushima: *Stachybotrys nilagirica* Subram., MUCL 39120.

Accession numbers of sequences downloaded from GenBank are directly noted on the phylogenetic trees, and the information not repeated.

RESULTS

Preliminary indications of the relationships of our fungus were obtained using a BLAST search at GenBank (Altschul *et al.* 1990). The search using the ITS (or partial 5' end of the LSU) sequences demonstrated homology with *M. setiramosum* (ITS), several unidentified collections for which only the ITS sequences are known, and some members of the Hypocreales, of which *Didymostilbe echinofibrosa* (teleomorph: *P. spirostriata*) and *D. sundara* (teleomorph: *P. sundara*) had the most similar sequences.

The ITS-based phylogenic inference under the parsimony hypothesis yielded 59 most parsimonious trees, 385 steps in length, CI = 0.686, RI = 0.805. These 59 trees present the same overall topologies, most of the variations lying within the *Septomyrothecium* clade, that has is internally poorly resolved. In our analyses, species of *Myrothecium* as currently circumscribed are not resolved into a monophyletic clade. The phylogeny confidently resolved a clade (bootstrap value 70%) containing *S. uniseptatum*, the French Guyana fungus, and several isolates of *M. setiramosum* from various geographic origins. It also includes two distinct subclades representing two (sp. 1 and sp. 2) and possibly three (sp. 1a and 1b) taxa of uncertain identity. *Myrothecium cinctum* DQ135998 and MUCL 50263 and *M. atrum* AY254160 do not cluster within the *Myrothecium* s.s. clade.

Within the *S. uniseptatum* clade, the French Guyana fungus appears isolated, and this supports its peculiar morphology and status of a distinct species.

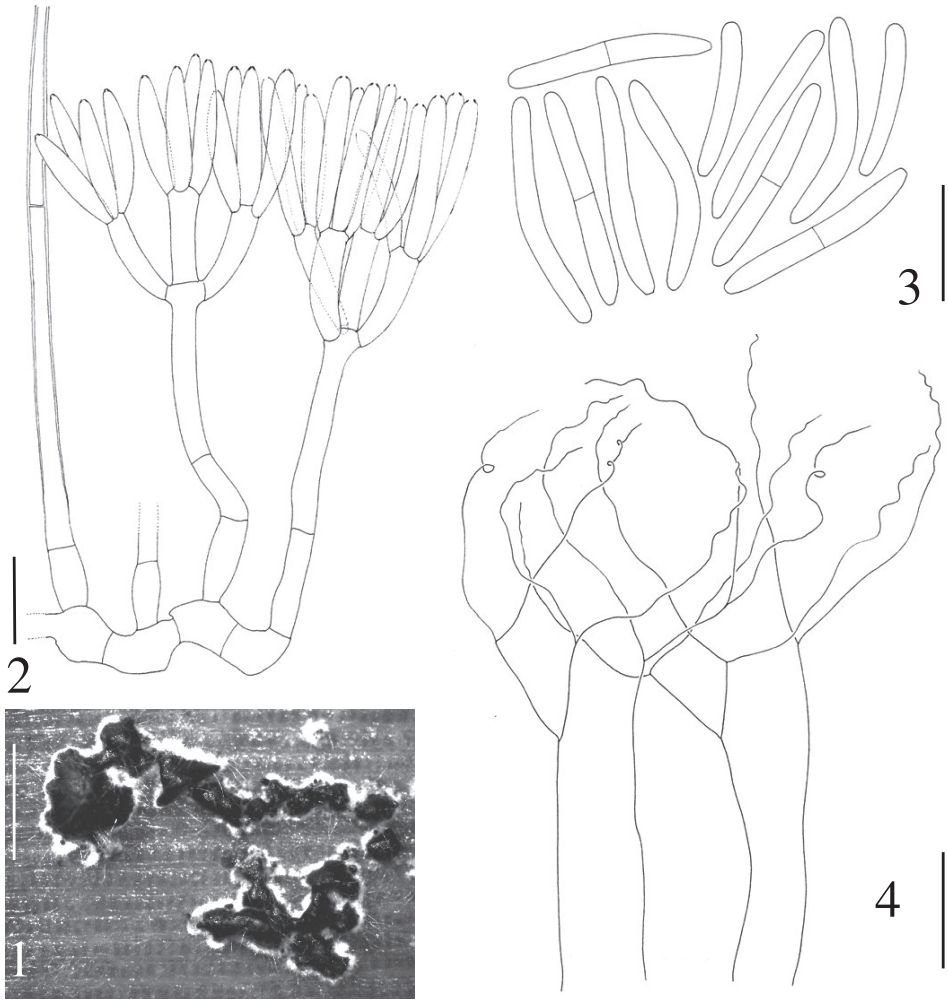
TAXONOMY

Myrothecium maraitiense Decock *sp. nov*. Figs 1-6

Typo generis Septomyrothecium uniseptatum affinis, sed steriles stipitis extensiones dichotomomiter ramosae et apice leviter tortiles adsunt, satis differt.

Etymology : named in honor of Prof. *Emeritus* Henri Maraite, former director of the *Mycothèque of the Université catholique de Louvain* (BCCM/MUCL), for his continuous support.

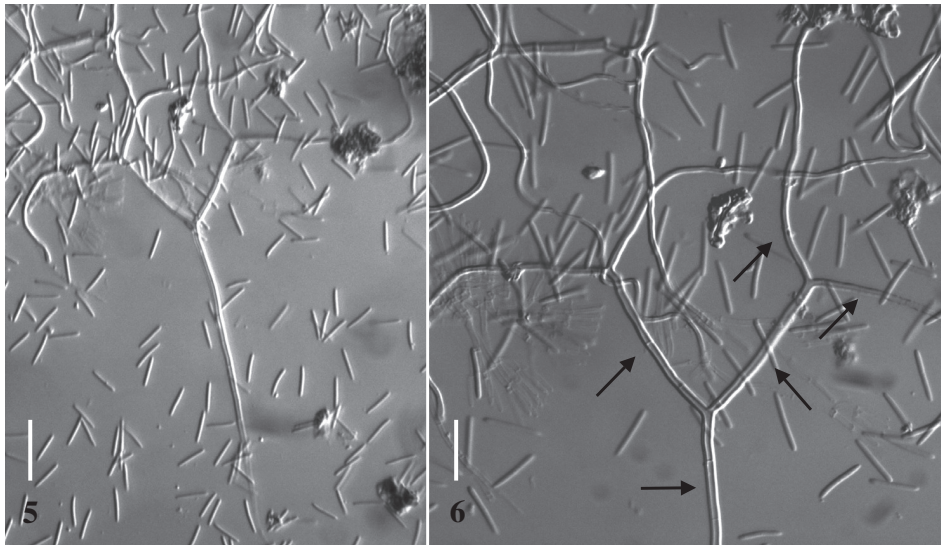
Sporodochia on natural substrate circular to ellipsoid or irregular, up to 1-2 × 1-1.5 mm, appearing wooly and with a superficial silvery tint due to a dense network of branched, sterile hyphoid extensions covering a deep green mucoid masses of conidia; margin distinct, white, densely wooly; *mycelium* mostly immersed, with little or no aerial mycelium; *hyphae* hyaline, mostly thin-walled, 2-4 µm wide; *marginal hyphae* made of a network of much branched, short, curling hyphae 1.5-2.5 µm wide, forming a white cottony fringe; individual *conidiophores* densely packed, mononematous, macronematous, erect, arising from basal, short hyphae running parallel to the substrate, composed of a basal stipe and an apical conidiogenous penicillus; *conidiophores stipe* hyaline, thin-walled, 1-2 septate, cylindrical (22-)25-30(-36) µm long ($\bar{X}$ = 28 µm long), 3.1-3.6 µm wide at the base ($\bar{X}$ = 3.4 µm) down to 2.2-2.8 µm wide at apex ($\bar{X}$ = 2.5 µm); *conidiogenous head* bi-verticillate, with 1 whorl of 3-4(-5) branches, each giving birth to 1 apical whorl of 3-5phialides; *branches*, cylindrical to clavate, or slightly bi-convex, 8.0-13.8 × 1.6-2.4 µm ($\bar{X}$ = 10.1 × 2.3 µm); *phialides* cylindrical, finger-like to slightly bi-convex, straight to very slightly incurved inward penicillus, with a narrow apex, dome-like, and with a small apical poroid conidiogenous locus, the wall of which thickens progressively by wall accumulation, 9.5-15.5 × 1.5-2.5 µm ($\bar{X}$ = 13.1 × 2.0 µm); *conidia* narrowly cylindrical, straight to slightly curved, base slightly truncate, apex rounded, hyaline, thin-walled, 0-1 septate, (17-)19-22(-23) × 2.0-2.5 µm, ($\bar{X}$ = 20.4 × 2.2 µm), R = 8.0-10.4, ($\bar{X}_R$ = 9.3); *sterile extensions* originating from the basal hyphae, protruding through, and extending well above conidial masses, hyaline, regularly septate (occasionally with secondary septa), thick-walled (except at the very bases and apices), smooth, apically dichotomously branched, all the extensions up to 480 µm long (8 = 340 µm); basal part straight, 95-260 µm long ($\bar{X}$ = 152 µm), 5.0-6.5 µm diam.; branching dichotomous,



Figs 1-4. *Myrothecium maraitiense*, type specimen. 1. Sporodochia (scale bar = 2 mm); 2. conidiophores, conidiogenous cells, and base of a hyphoid extension (scale bar = 20 µm); 3. conidia (scale bar = 20 µm); 4. sterile, hyphoid extensions (scale bar = 100 µm).

1-2(-rarely 3) levels, then with terminal branches 150-290 µm long, 3.0-4.5 µm wide at base progressively narrowing to 2.0-2.5 µm wide at the apices, sinuous, ending slightly twisted or coiled; when present, intermediate ramifications short, 10-70 µm long ($\bar{X}$ = 38 µm) and terminal branches 140-210 µm ($\bar{X}$ = 173 µm); *chlamydospores* absent.

Colonies on V-8 45-55 mm diam. in 7 days, with white aerial mycelium; *sporodochia* appearing after 2-3 weeks on V8 and BLA, scattered on the media or the banana leaf piece or in 1-2 concentric ring (V-8); sporodochia as in natural substrate but with fewer hyphoid extensions, with a reduced branching (mostly a single dichotomy) or completely unbranched.



Figs 5-6. *Myrothecium maraitiense*, type specimen. 5. Sterile, hyphoid extensions (scale bar = 40 µm; 6. Details of the sterile, hyphoid extensions, arrows indicate the dichotomous branching (scale bar = 20 µm).

Teleomorph not observed, neither *in vivo*, nor *in vitro*.

Substratum: on a decaying leaf of an unidentified angiosperm, in leaf litter.

Habitat: rainforest leaf litter.

Geographic distribution: so far known only from the type locality, French Guyana.

HOLOTYPE. FRENCH GUYANA: Cayenne Area, Matouri, *Sentier d'Interprétation de la Nature "Lamirande"*, from a decaying leaf of an unidentified angiosperm, in leaf litter, Feb. 1994, C. Decock, in MUCL herbarium as MUCL 47202; (living strain ex-holotype MUCL 47202).

DISCUSSION

Septomyrothecium was taken for a *Myrothecium*-like [*haud distinguibilis*] fungus differing from "typical" *Myrothecium* in having septate conidia (Matsushima, 1971a, b). However, all other characteristics being in accordance with *Myrothecium* as defined by Tulloch (1972), which is also the currently accepted concept (Seifert *et al.,* 2003), the pertinence of the conidial septation and the relationships of *Septomyrothecium* with *Myrothecium* needed to be evaluated.

The molecular data currently in hand would plead in favor of recognizing *Septomyrothecium*. *Septomyrothecium uniseptatum* forms a moderately resolved "*Septomyrothecium*" clade (bootstrap value 70%, Fig. 7), including the French Guyana fungus, several isolates (or ITS sequences) of uncertain identities (see below), and isolates of *M. setiramosum*. This clade is distinct from and indirectly

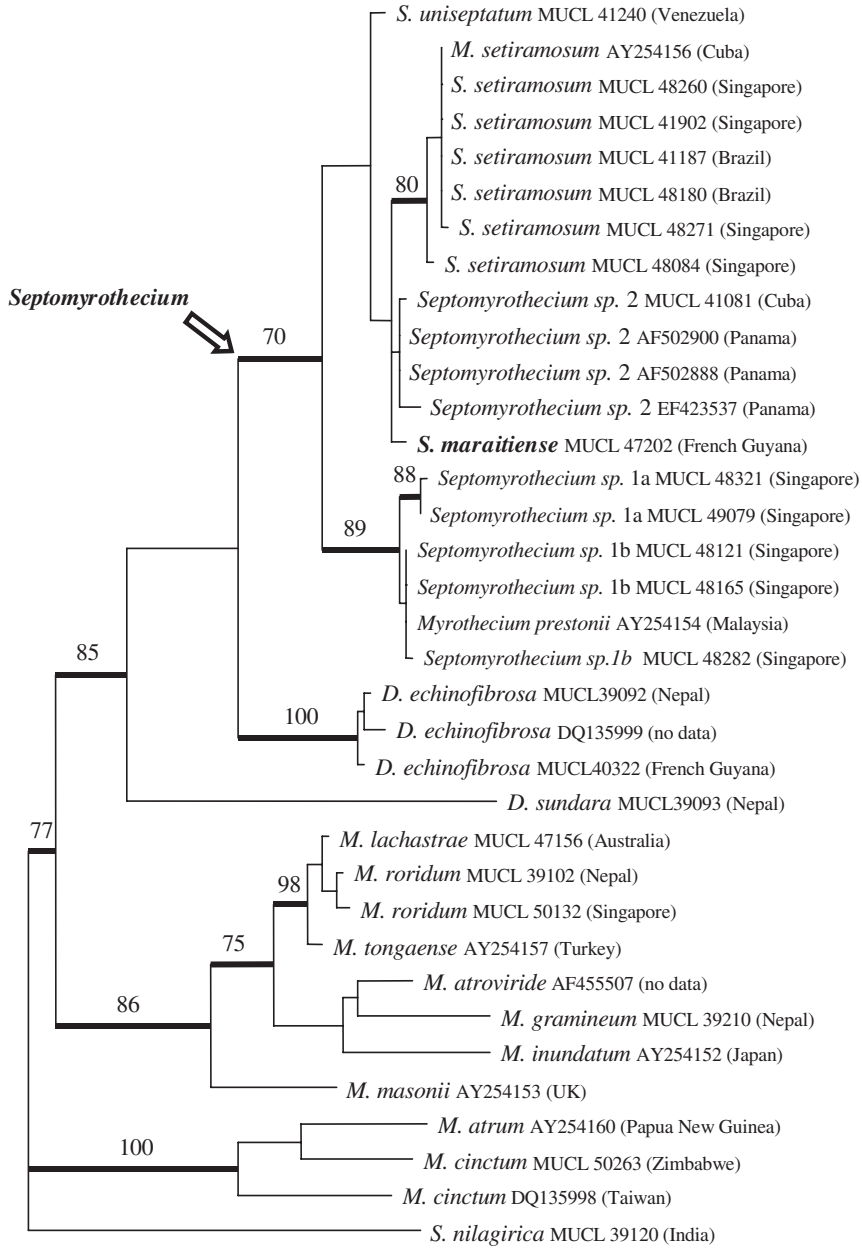


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Fig. 7. One of the 59 most parsimonious trees (see text for explanation). Branches in bold are supported by bootstrap value > 70% (number above branches are bootstrap value).

related to the *M. inundatum* clade (Fig. 7). The closest relatives of the *Septomyrothecium* clade appear so far to be members of the anamorphic/teleomorphic pair *Didymostilbe*/*Peethambara* (especially *D. sundara*/*P. sundara* and *D. echinofibrosa*/*P. spirostriata*) and not members of the *Myrothecium* s.s. clade (the *M. inundatum* clade). The affinities of *Septomyrothecium* (but also more globally of *Myrothecium s.l.*) with *Didymostilbe*/*Peethambara* have been discussed already (Castlebury *et al.,* 2004; Rossman *et al.,* 1999, 2001; Schroers, 2001; Seifert *et al.,* 2003). However, it is beyond the scope of this paper to dig more deeply into these relationships.

Morphologically, the critical features distinguishing *Septomyrothecium* from *Myrothecium* remains to us difficult to define. The conidial septation would be no longer critical, however; yet *M. setiramosum* (Castañeda, 1986) and the species represented by the clades *Septomyrothecium* sp. 1 and *Septomyrothecium* sp. 2 have aseptate conidia. The long hyphoid extensions stemming from basal hyphae next to the conidiophores, and extending far beyond the conidial masses could be apomorphic for the clade, and distinguish *Septomyrothecium* from other related genera. A sporodochial conidiomata, absence of setae or setae-like cells arising from marginal hyphae, penicillate conidiophores, apically verticillate phialidic conidiogenous cells, and 0 to 1-septate conidia accumulating in dark green mucoid masses add to the circumscription.

So far, a connection to a sexual state has been presumed only for the hypothetic *Septomyrothecium* anamorph of *Nectria septomyrothecii* Samuels (Samuels, 1988).

Septomyrothecium maraitiense is described on the basis of morphological characteristics and DNA sequence data. The species is morphologically unique within *Septomyrothecium* and related genera (*op. cit.* cf. introduction) in having one or twice dichotomously branched, hyphal-like extensions, born from basal hyphae, protruding through, and extending above the conidial masses (Figs 4, 5-6). On the original substrate, the density of extensions (Fig. 1) gives the sporodochia a superficial wooly aspect and a silvery tint. In *in vitro* culture, the degree of branching may be reduced or develop later. Seifert (2003) noted also that in some species of *Myrothecium* (e.g. *M. inundatum* or *M. gramineum*), setae might be scarcer or even absent *in vitro* while present *in vivo*. Conidia are long, ellipsoid, narrow, 0- to 1-septate, straight to slightly curved, and very similar to those of *S. uniseptatum*.

Septomyrothecium uniseptatum (Matsushima, 1971a, b) is the closest species from a morphological point of view; it differs in having unbranched hyphoid extensions. In a phylogenetic perspective, the internal relationships within the *Septomyrothecium* clade are not confidently resolved, however.

Myrothecium setiramosum shares with the other species of *Septomyrothecium* clade the sporodochial conidiomata, identical conidiogenous apparatus, and both morphologically related and ontogenetically homologous hyphal-like extensions. It differs in having the extensions unbranched for most of their length except at their apices that bear a crown of 3-6, short (up to 10 µm long) processes (Castañeda, 1986; Watanabe *et al.,* 2003). Given the phylogenetic and also the morphological proximity of *M. setiramosum* to *S. uniseptatum*, the new combination *Septomyrothecium setiramosum* (R.F. Castañeda) Decock *comb. nov*. is proposed [basionym: *Myrothecium setiramosum* R.F. Castañeda, Deuteromycotina de Cuba, Hyphomycetes IV [La Habana]: 10, 1986].

Septomyrothecium setiramosum was originally described from Cuba (Castañeda, 1986) and later reported from other tropical regions including Brazil,

Singapore (BCCM/MUCL, http://bccm.belspo.be/db/mucl_search_form.php), and Nigeria (Calduch *et al.,* 2002).

Myrothecium dimorphum Watanabe *et al.* (Watanabe *et al.,* 2003) could be considered too. This species is morphologically very similar to *M. setiramosum*, and differs in having the extensions apices turning into conidiogenous loci, producing small and globose conidia. No sequence of this species was available for inclusion in the phylogenetic studies. Further studies might prove that it belongs to the same clade.

Two (or possibly three) other *Septomyrothecium* species represented by 6 strains and several ITS sequences from unidentified "leaf litter ascomycetes", originating from Southeast Asia (*Septomyrothecium sp. 1a, sp. 1b*, Singapore) and the Neotropics (*Septomyrothecium sp*. 2, Panama and Cuba) are also evidenced, demonstrating diversity larger than presumed. They all have unbranched hyphoid extensions, overall very similar to those of *S. uniseptatum* but, comparatively, smaller conidia (about half the size).

The identity of these species is still uncertain, and more critical studies of several *Myrothecium* species would be needed to prove that they are related, if not identical, or of unclear concept. For instance, the ITS sequence registered at GenBank as AY254154 is 100 % identical to the ITS sequences of *Septomyrothecium sp*. 1b (= MUCL 48121, 48165, and 48282). These 3 strains have symmetrical and narrowly ellipsoid conidia. Their hyphoid extensions are slightly rough as if covered with dried drops of exudates. The sequence AY254154 belongs to the strain CBS 175.73 = IMI 160372, registered in both collections as the isotype of *M. prestonii* (originating from Malaysia). However, as illustrated by Tulloch (1972) and Nag Raj (1995), *M. prestonii* has ellipsoid and asymmetrical conidia, that bears an apical funnel, fan-shaped mucoid appendage, a feature not observed in *Septomyrothecium sp*. 1(a or b) and in all other taxa of the *Septomyrothecium* clade. Comparison of herbarium type material of *M. prestonii* (Nag Raj, 1995) with the presumed isotype culture is necessary. This will be treated separately.

Myrothecium lachastrae Sacc. has conidia, both in size and shape (Tulloch, 1972) rather similar to those of *S. uniseptatum* or *S. maraitiense* but this species lacks any hyphoid extensions or other kind of sterile "ornamental" hyphae. A strain of *M. lachastrae*, received from IMI (IMI 273160), nested within the *Myrothecium* s.s. clade (Fig. 7) . However, this strain has much smaller conidia than described in *M. lachastrae* (about half the size, pers. obs.) and most probably belongs to *M. carmichaelii* Grev.

Myrothecium, as currently expanded (*op. cit.* cf. introduction) represents an assemblage of taxa encompassing various morphologies and whose affinities remain still uncertain. The genus encompasses different conidiomatal anatomy (ontogeny), conidiogenous apparatus, conidiogenesis, marginal or tramal sterile "setae" or "setae"-like elements, and conidial morphology (Nag Raj, 1993, 1995a, b; Seifert *et al.,* 2003; Tulloch, 1972). In all probability, the present concept is not monophyletic. Its circumscription has been debated on several occasions (Ahrazem *et al.,* 2000; Nag Raj, 1993, 1995a, b; Samuels & Rossman, 1979, Schroers *et al.,* 1999; Seifert *et al.,* 2003), although with uncertain or sometimes opposite conclusions (*e.g.* Ahrazem *et al.,* 2000 *versus* Seifert *et al.,* 2003). The genus needs to be critically evaluated by combining sets of both morphological and non-morphological data. Morphologically more homogeneous and phylogenetically monophyletic entities would certainly emerge from the genus.

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