

Pleurostomophora, an anamorph of *Pleurostoma* (*Calosphaeriales*), a new anamorph genus morphologically similar to *Phialophora*

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Abstract: *Pleurostoma ootheca* (*Calosphaeriales*) was newly collected, and found to produce a *Phialophora*-like anamorph in culture, which was morphologically similar to *Phialophora* (*Ph.*) *repens* and *Ph. richardsiae*. The close relationship among these three species was confirmed on the basis of sequence analysis of the 5.8S nuclear ribosomal RNA gene and its flanking internal transcribed spacers (ITS1 and ITS2). Furthermore, molecular data from partial 18S small subunit (SSU) DNA, revealed these three species were not phylogenetically closely related to the type of *Phialophora*, *Ph. verrucosa*, which was nested in the *Chaetothyriales* clade. A new anamorph genus, *Pleurostomophora* (*P.*), was therefore proposed to accommodate these species. The formation of perithecia from single-ascospore isolates of *Pleurostoma* (*Pl.*) *ootheca* also showed it to have a homothallic mating system.

Taxonomic novelties: *Pleurostomophora* D. Vijaykrishna, L. Mostert, R. Jeewon, W. Gams, K.D. Hyde & Crous gen. nov., *Pleurostomophora ootheca* D. Vijaykrishna, R. Jeewon & K.D. Hyde sp. nov., *Pleurostomophora richardsiae* (Nannf.) L. Mostert, W. Gams & Crous comb. nov., *Pleurostomophora repens* (R.W. Davidson) L. Mostert, W. Gams & Crous comb. nov.

Key words: *Calosphaeriales*, ITS, *Phaeoacremonium*, phylogeny, *Pleurostomophora*, *Pleurostoma*, SSU, systematics, *Togninia*, 18S.

INTRODUCTION

The history and diagnostic characteristics of the *Calosphaeriales* M.E. Barr (*Calosphaeriaceae* Munk) were extensively reviewed by Barr (1985). Through a combination of features of ascomata and asci, the following eight genera were included: *Calosphaeria* Tul. & C. Tul., *Enchnoa* Fr., *Graphostroma* Piroz., *Jattaea* Berl., *Pleurostoma* Tul. & C. Tul., *Romellia* Murrill, *Scoptria* Nitschke, and *Togninia* Berl. Barr (1985) restricted the *Calosphaeriales* to those fungi that had broad and tapered paraphyses, asci that lined the entire inner region of the perithecium, and hyaline ascospores that were often allantoid. Barr (1993) proposed a second family to the *Calosphaeriaceae*, namely *Graphostromataceae* M.E. Barr, J.D. Rogers & Y.M. Ju, to accommodate the genus *Graphostroma*. The *Calosphaeriaceae* was circumscribed to contain *Calosphaeria* (= *Wegelina* Berl.), *Enchnoa*, *Jattaea*, *Pachytrype* Berl. ex M.E. Barr, J.D. Rogers & Y.M. Ju and *Pleurostoma* (= *Romellia*, *Togninia* and *Erostella* (Sacc.) Sacc.) (Barr 1993). *Pleurostoma* (*Pl.*) contained two species, the type species *Pleurostoma candollei* Tul. & C. Tul., and *Pleurostoma ootheca* (Berk. & M.A. Curtis) M.E. Barr (Barr 1985). The

genus *Wegelina* was again reinstated and distinguished from *Calosphaeria* by Barr (1998).

Of the genera originally placed in synonymy with *Pleurostoma* by Barr (1993), *Erostella* proved to be a synonym of the distinct genus *Togninia* (Hausner *et al.* 1992, Mostert *et al.* 2003). *Romellia*, which is currently still regarded as synonym of *Pleurostoma* for lack of fresh collections and data, has species with eight-spored asci; therefore the genus *Pleurostoma* accommodates both polysporous and octosporous species.

Only a few anamorphs are known for genera in the *Calosphaeriales*, ranging from *Nodulisporium*-like (conidiogenous cells proliferating sympodially) to *Acremonium*-like (conidiogenous cells phialidic) (Barr 1990). *Graphostroma platystroma* (Schwein.) Piroz. has a *Nodulisporium* Preuss anamorph (Glawe & Rogers 1986). Recently the anamorph of *Togninia* was confirmed to be *Phaeoacremonium* W. Gams, Crous & M.J. Wingf. (Mostert *et al.* 2003), a genus morphologically intermediate between *Phialophora* and *Acremonium* (Crous *et al.* 1996). Other anamorph connections within the order remain unknown.

Phialophora has been a poorly defined, little differentiated and highly polyphyletic genus of more or less pigmented hyphomycetes (Gams 2000). Gen-

erally *Phialophora* is characterized by flask-shaped phialides with a collarette and one-celled slimy conidia (Schol-Schwarz 1970). Taxa formally classified in *Phialophora* have, however, proven to belong to several distinct genera, namely *Cadophora* Conant, *Catenulifera* Hosoya (Hosoya 2002), *Coryne* Nees (Groves & Wilson 1967), *Harpophora* W. Gams (Gams 2000), *Lecythophora* Nannfeldt (Gams & McGinnis 1983), *Margarinomyces* Laxa (Gams 2000) and *Phaeoacremonium* (Crous *et al.* 1996). With these segregations not all known anamorph taxa are yet satisfactorily reallocated.

A fresh collection of *Pl. ootheca* was recently obtained from degrading wood. Single-ascospore isolates were found to produce a *Phialophora*-like anamorph in culture. The aim of the present study was to resolve the generic placement of the anamorph, as well as the higher-order phylogeny of *Pl. ootheca*. The fungus was compared to morphologically similar taxa, particularly *Ph. repens* and *Ph. richardsiae*, and their 5.8S nuclear ribosomal RNA gene and the flanking internal transcribed spacer (ITS1 and ITS2) regions sequenced to resolve species differences and placement. Higher order phylogeny was investigated by sequencing the small subunit (SSU) of the ribosomal DNA gene of *Pl. ootheca* and related taxa.

MATERIALS AND METHODS

Isolates

Pleurostoma ootheca (CBS 115329, HKUCC 10126) was isolated from perithecia found on degrading wood on the forest floor in Thailand. Single-spore cultures of this taxon were made on artificial media following the protocols outlined by Choi *et al.* (1999) and Goh (1999). Teleomorph morphology was studied on fresh material and on potato-dextrose agar (PDA, Oxoid 4 g potato extract, 20 g glucose, 15 g agar in 1000 mL water). Cultures produced a *Phialophora*-like anamorph when incubated at 22 °C under a 12 h fluorescent white light/dark regime on PDA. For microscopical investigation, material was mounted in water or lactic acid. Vertical sections (4 µm) of fruiting bodies were cut with a Zeiss HM505E freezing microtome. All measurements were made in water. Cultures are maintained in the Hong Kong University Culture Collection (HKUCC) and representative strains have been deposited at the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands. The type cultures of *Ph. repens* (R.W. Davidson) Conant (CBS 294.39) and *Ph. richardsiae* (Nannf.) Conant (CBS 270.33) were included for comparison purposes. Strains were plated onto 2 % malt extract agar (MEA, 20 g Oxoid malt extract, 15 g Difco agar in 1000 mL water), and incubated at 5 °C temperature intervals from 10 to 40 °C to determine the cardinal temperatures for growth. Colony growth was deter-

mined by calculating the mean of two perpendicular colony diameter measurements of three repeats for each isolate at each temperature after 8 d in the dark at 20 °C. The colony color was determined on MEA after 8 d in the dark at 25 °C (a more pronounced colony colour was observed at 25 °C than at 20 °C) using the charts of Kornerup & Wanscher (1978).

DNA isolation and amplification

Genomic DNA was extracted (Lee & Taylor 1990), and two gene regions were amplified. A fragment of approximately 550 base pairs including the 5.8S nuclear ribosomal RNA gene and the flanking internal transcribed spacers (ITS1 and ITS2) was amplified using primers ITS1 and ITS4 (White *et al.* 1990). The ITS sequence for the ex-type strain of *Ph. repens* was obtained from GenBank (AF083195). To determine the higher-order phylogeny, a fragment of approximately 1000 base pairs of the 5' end of the 18S ribosomal DNA (SSU) was amplified using primers NS1 and NS4 (White *et al.* 1990). The reaction mixture contained 5 µL of diluted DNA, 1× PCR buffer (Bio-line), 2.5 pmol of each primer, 200 µM of each of the dNTP's, 0.5 U of Taq DNA polymerase (Bio-line), 1.5 mM MgCl₂, and each reaction was made up to a final volume of 25 µL with sterile water. The following PCR amplification cycles were run on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California, U.S.A.): 96 °C for 5 min, followed by 36 cycles of denaturation (94 °C for 30 s), annealing (50 °C for 30 s), elongation (72 °C for 90 s), and a final extension step (72 °C for 7 min). PCR products were analyzed by electrophoresis as described by Mostert *et al.* (2003). PCR products were purified according to the manufacturer's instructions using a commercial kit (Nucleospin Extract 2 in 1 Purification Kit, Machery-Nagel GmbH & Co., Germany). Sequencing reactions were carried out with the PCR primers using a DYNAMIC ET Terminator Cycle Sequencing Kit (Amersham Biosciences, Roosendal, the Netherlands) according to the manufacturer's recommendations, and the resulting products were analyzed on an ABI Prism 3700 DNA Sequencer (Perkin-Elmer, Norwalk, Foster City, CA). A consensus sequence was computed from the forward and reverse sequences with SeqMan from the Lasergene package (DNASTar, Madison, WI). The ITS and SSU sequences of the isolates were lodged in GenBank: *Ph. richardsiae* CBS 270.33 (AY729811, AY729812); *Pl. ootheca* CBS 115329 (AY725469, AY725470), and *Ph. repens* CBS 294.39 (AY729813). Alignments and trees were lodged in TreeBASE (SN2004, SN2005).

Phylogenetic analyses

Sequences were manually aligned in Sequence Alignment Editor v. 2.0a11 (Rambaut 2002) by inserting gaps. Phylogenetic analyses using parsimony for

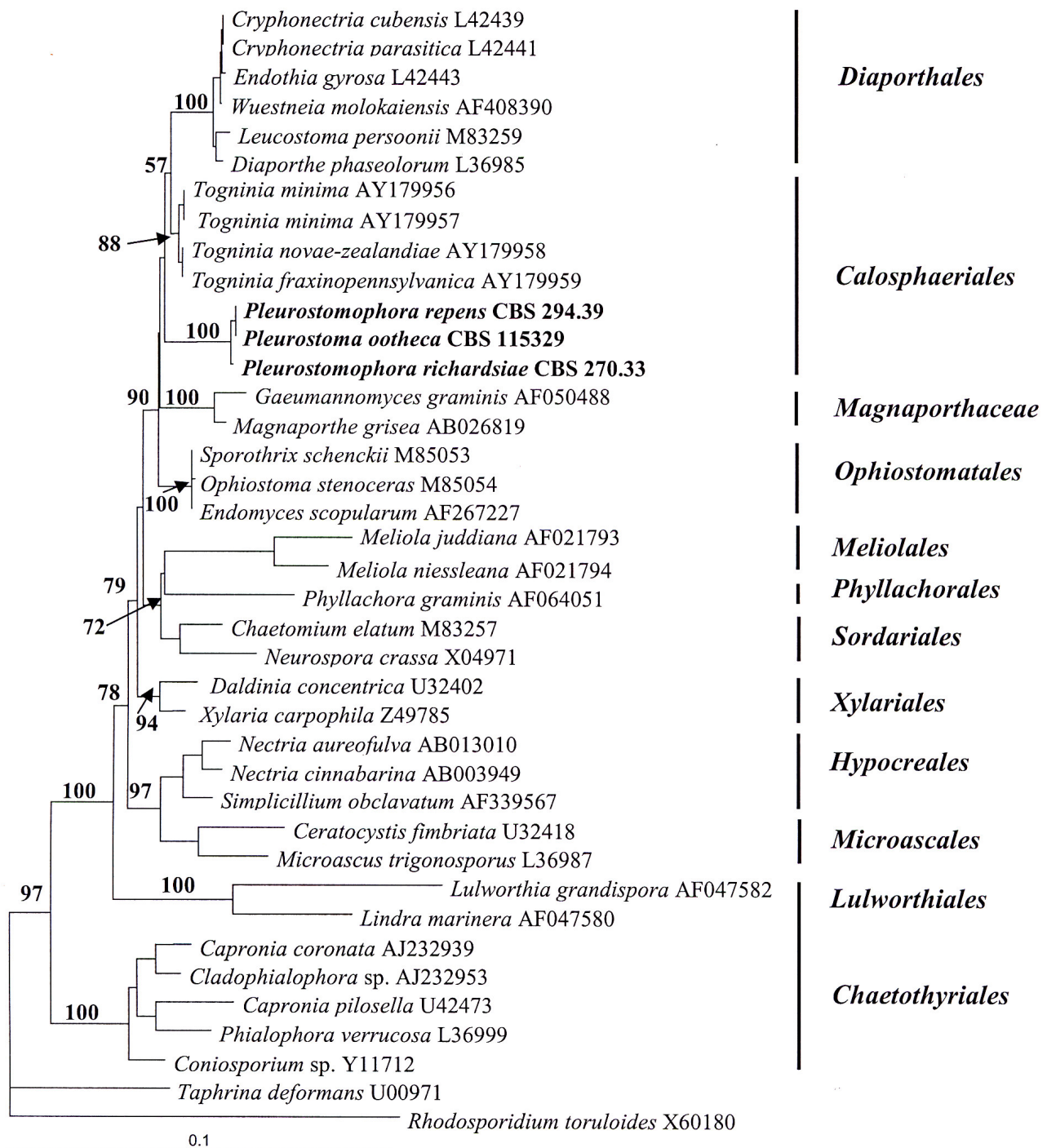


Fig. 1. Neighbour-joining phylogenetic tree obtained from partial 18S rDNA sequences. Bootstrap support values (1000 replicates) are shown above the nodes. Bootstrap values for subclades within an order are not given. *Taphrina deformans* and *Rhodosporidium toruloides* were used as outgroups.

the ITS analysis were conducted with PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002). *Cercospora apii* Fresen. (CBS 119.25, GenBank ITS = AY179949) was used as outgroup in the ITS analysis. Gaps were treated as a fifth character and all characters were unordered and of equal weight. Maximum parsimony analysis was performed using the heuristic search option with 1000 random taxon additions and tree bisection and reconstruction (TBR) as the branch swapping algorithm. Bootstrap support values for the analysis were calcu-

lated from 1000 heuristic search replicates and 100 random taxon additions. Tree length, consistency index (CI), retention index (RI) and the rescaled consistency index (RC) values were also calculated. Small subunit sequences were added to the alignment obtained from TreeBASE (SN1269-3617). Neighbour-joining analyses of the SSU alignment (using uncorrected "p") were performed. *Rhodosporidium toruloides* Banno and *Taphrina deformans* (Berk.) Tul. were used as outgroups for the neighbour-joining analysis of the SSU alignment.

RESULTS

Phylogenetic analysis

The SSU phylogenetic analysis (Fig. 1) showed that *Pleurostoma ootheca* CBS 115329, *Ph. repens* CBS 294.39 and *Ph. richardsiae* CBS 270.33 clustered together with a high bootstrap support (100 %). This cluster had as neighbours *Diaporthales*, *Calosphaeriales*, *Magnaporthaceae*, and *Ophiostomatales*. The *Pleurostoma* clade did not merge with the *Togninia* clade. The *Togninia* clade grouped with the *Diaporthales* clade, but with low bootstrap support (57 %). Presently the *Pleurostoma* and *Togninia* clades have been included in the *Calosphaeriales* based on morphology. The ITS rDNA consisted of 524 characters, out of which 167 were parsimony informative. Maximum parsimony analyses based on the ITS sequences resulted in two most parsimonious trees (Fig. 2). The alignment of the ITS sequences was difficult due to many insertions and deletions in the ITS1 and ITS2 regions when *Togninia*, *Pleurostoma*

and *Diaporthales* were compared. Higher order relationships obtained with the ITS analysis should therefore be inferred with caution. The ITS phylogenetic analysis showed that *Pleurostoma ootheca*, *Ph. repens* and *Ph. richardsiae* clustered together with high bootstrap support (99 %). *Pleurostoma ootheca* and *Ph. repens* were obviously related (99 % bootstrap support), though still having 29 nucleotide differences between them.

Morphology

Species of the *Pleurostomophora* cluster can be distinguished from the true *Phialophora* species by their mostly slender, subcylindrical phialides, flaring or inconspicuous, but not long collarettes and cylindrical to allantoid conidia. *Phialophora richardsiae* has distinct flaring collarettes and produces both oblong ellipsoidal hyaline, and (sub)globose pigmented conidia. Other morphological differences among the three species of *Pleurostomophora* are discussed below.

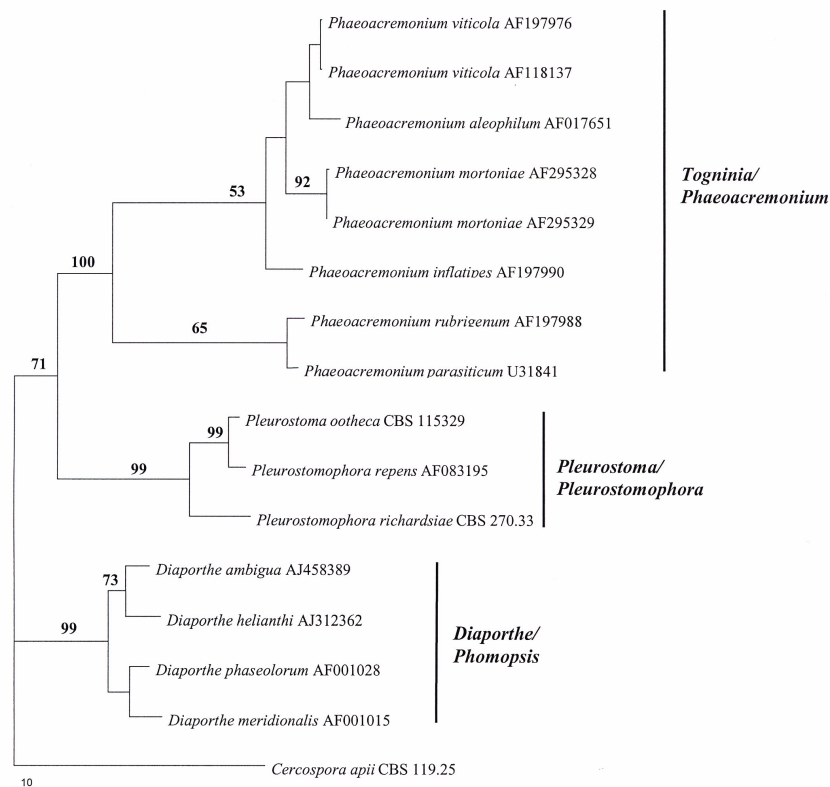


Fig. 2. One of two most parsimonious trees obtained from heuristic searches of the 5.8S rRNA gene and flanking ITS1 and ITS2 regions (length = 480 steps, CI = 0.790, RI = 0.817, RC = 0.645 and HI = 0.210). Relevant bootstrap support values (1000 replicates) are shown at the nodes. *Cercospora apii* was used as outgroup.

Pleurostomophora D. Vijaykrishna, L. Mostert, R. Jeewon, W. Gams, K.D. Hyde & Crous, **gen. nov.** MycoBank MB500095.

Genus anamorphicum. Conidiophora singula, separata, *Phialophorae* similia, hyalina vel pigmentata, plerumque monophialidica, brevia, collari inconspicuo vel expanso. Conidia in capitulis mucidis aggregata in summa phialide,

hyalina, levia, plerumque dimorphica, seu oblonga vel allantoidea, seu breviora ellipsoidea.

Typus: *Pleurostomophora ootheca* D. Vijaykrishna, R. Jeewon & K.D. Hyde, sp. nov.

Genus asexual; conidiophores single, separate, resembling those of *Phialophora*, hyaline to pigmented; mostly monophialidic, short, with inconspicuous or

flaring collarettes. *Conidia* aggregated in slimy masses at the apices of conidiogenous cells, hyaline, smooth, mostly dimorphic, being either straight to allantoid, or shorter and more ellipsoid.

Pleurostoma ootheca (Berk. & M.A. Curtis) M.E. Barr, *Mycologia* 77: 564. 1985. Figs 3–18.

Basionym: *Sphaeria ootheca* Berk. & M.A. Curtis, *Grevillea* 4: 108. 1876. Figs 3–18.

≡ *Coronophora ootheca* (Berk. & M.A. Curtis) Sacc., *Syll. Fung.* 1: 106. 1882.

≡ *Neoarcanelia ootheca* (Berk. & M.A. Curtis) Berl., *Icon. Fung.* 3: 6. 1900.

Anamorph: *Pleurostomophora ootheca* D. Vijaykrishna, R. Jeewon & K.D. Hyde, sp. nov.

Ascomata mostly aggregated, partially immersed to superficial, black, with apical papilla, apex oblique or even horizontal, eccentric or parallel to the host surface; venter globose, 210–330 µm high and 170–275 µm diam at the central region; peridium consisting of two layers of cells, an inner 10–15 µm thick *textura angularis*, of three to four layers of dark brown flattened or compressed cells, and an outer 25–30 µm thick *textura epidermoidea* of pale brown with smaller rounded cells; necks very short, straight or curved. *Paraphyses* not observed. *Asci* 20–27 × 11–15 µm, arising from the ascogenous hyphae in clusters with spicate appearance, subglobose or obpyriform, polysporous, pedicellate, a hair-like structure is visible upon release from the ascogenous hypha; apex complex, with bulging apical thickening on the concave side of the asci. *Ascospores* 2–3 × 1–2 µm, aseptate, hyaline, allantoid, strongly curved, smooth-walled.

Pleurostomophora ootheca D. Vijaykrishna, R. Jeewon & K.D. Hyde, sp. nov. MycoBank MB500096. Figs 3–10.

Hyphae ramosae, septatae, hyalinae vel deinde fusciscentes, tuberculatae, 1.8–3.3 µm latae. Chlamydosporae absentes. Conidiophora plerumque singula, ex hyphis aeriis vel submersis oriunda, erecta, cylindrica, hyalina, tuberculata vel leviter, recta vel flexuosa, 0–2-septata, (12–)14–24(–35) × (1.5–)2.5(–3) µm. Phialides terminales, raro laterales, monophialidicae, raro polyphialidicae, leviter, hyalinae, cylindricae, 6–19 µm longae, prope basim 2–4.5 µm latae, sursum 0.8–4.5 µm, collari brevi, saepe inconspicuo, ad 1.5 × 1.5 µm. *Conidia* in capitulis mucidis aggregata, hyalina, leviter, guttulate an non, dimorphica: seu recta oblonga vel allantoida, (4–)4.5–5.5(–16) × (1–)1.5(–4) µm, seu breviora, ellipsoidea, (3–)4–5(–8) × (1–)2(–3) µm.

Typus: CMU (Herbarium) 23858, ad lignum putridum in solo silvestri, Chiang Mai in Thailandia, 6 Jul. 2003, D. Vijaykrishna.

Mycelium consisting of branched, septate, hyaline hyphae, older hyphae becoming brown, tuberculate, 1.8–3.3 µm wide. *Chlamydospores* absent. *Conidiophores* mostly single, arising directly from aerial or submerged hyphae, erect, cylindrical, hyaline, tuberculate or smooth, straight or flexuous, 0–2-septate, (12–)14–24(–35) × (1.5–)2.5(–3) µm. *Conidiogenous cells* terminal rarely lateral, monophialidic, rarely polyphialidic, smooth, hyaline, cylindrical, 6–19 µm long, 2–4.5 µm wide at base, 0.8–2.5 (–4.5) µm at apex, with short, frequently inconspicuous collarettes up to 1.5 µm wide and 1.5 µm long. *Conidia* aggregated in round, slimy heads at the apices of the conidiogenous cells, hyaline, smooth, guttulate or not, dimorphic, being either oblong, straight to allantoid, (4–)4.5–5.5(–16) × (1–)1.5(–4) µm, or shorter and more ellipsoid, (3–)4–5(–8) × (1–)2(–3) µm.

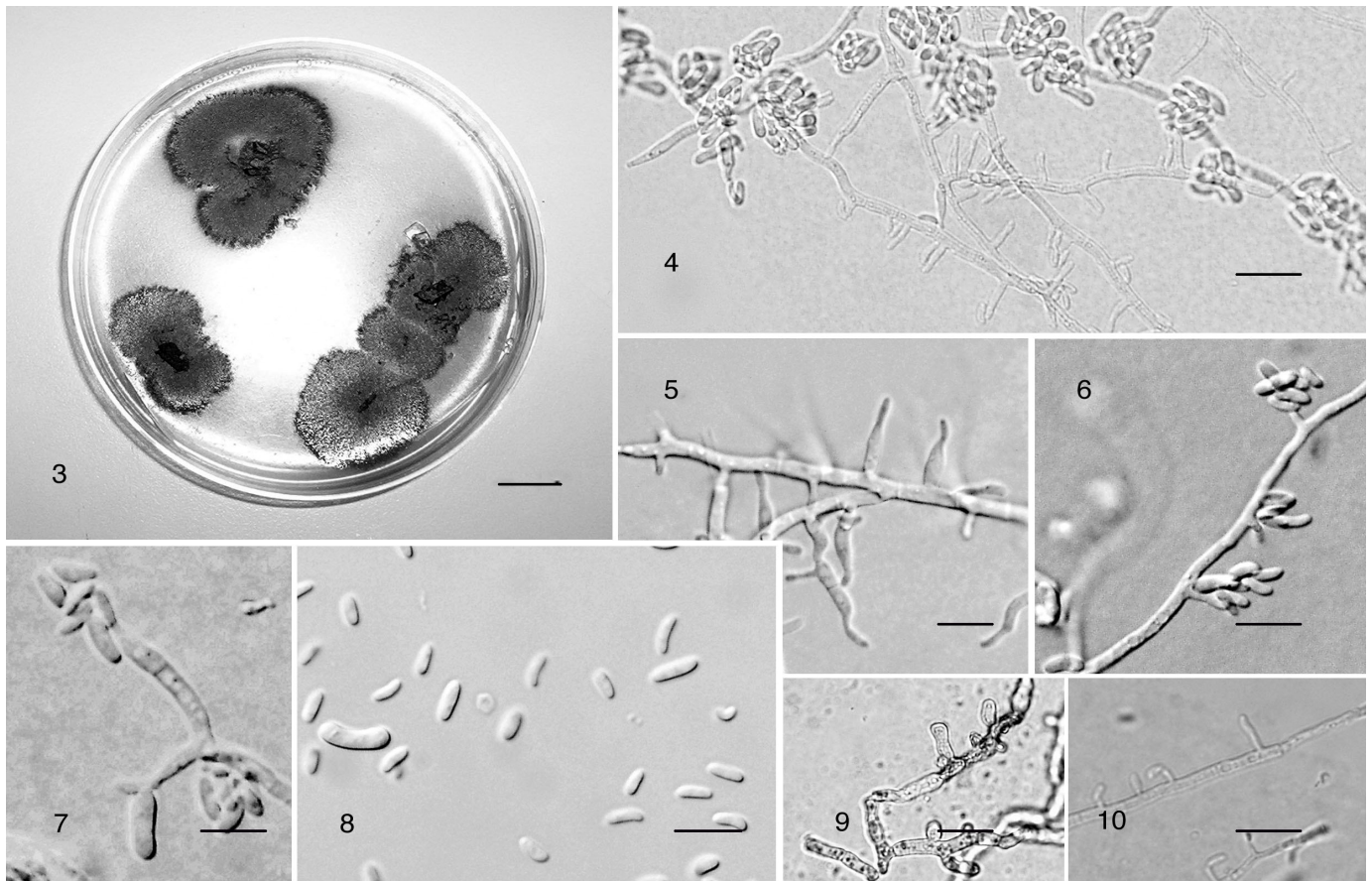
Cultural characteristics: Colony surface on MEA yellowish white (4A2), in reverse yellowish grey (4B2). Minimum temperature for growth 15 °C, optimum 30 °C, maximum above 40 °C. Colonies attaining a diameter of 30–32 mm at 20 °C after 8 d in the dark. Appearance of fruiting bodies at the border of the colony was observed after 30–45 d of incubation at 25 °C on the laboratory bench.

Substrate: Degrading wood on the forest floor.

Geographical distribution: Thailand, U.S.A.

Type specimens: **Thailand**, Chiang Mai Province, Mae Taeng, Mokfa, Degrading wood on the forest floor, 6 Jul. 2003, D. Vijaykrishna, **holotype** of *Pleurostomophora ootheca* CMU (Herbarium) 23858; ex-type culture HKUCC 10126 = CBS 115329; **isotypes** HKU(M) 17485, PDD 78745, BBH 8625 (BIOTEC Bangkok Herbarium, Thailand), MRC 1 (Mushroom Research Centre, Thailand). **U.S.A.**, South Carolina, *Quercus*, coll. M.A. Curtis, **isotype** of *Pleurostoma ootheca*, UPS.

Notes: The morphology of the collection of *Pl. ootheca* from Thailand corresponds well with that of the holotype originally collected in the U.S.A. *Ascospores* are allantoid, strongly curved, 2–2.5 × 0.5–1 µm, being quite distinct from those of *Pl. candollei*, which are allantoid, 3–4 × 0.5 µm (Barr 1985). The ascomatal venter of the Thailand specimen also falls within the range of *Pleurostoma ootheca* (220–385 µm diam). Both of these features are used to distinguish *Pl. ootheca* from *Pl. candollei*. *Pleurostoma ootheca* formed perithecia in pure culture from single-ascospore isolates. This indicates that this fungus has a homothallic mating system. Romero (1988) showed with fluorescence microscopy that the asci of *Pl. ootheca* have a thickened, upper lateral region of the wall that makes the ascus asymmetrical (Fig. 15, 16).



Figs 3–10. *Pleurostomophora* anamorph of *Pleurostroma ootheca*. 3. Four-week-old colony growing on PDA medium, showing dark coloured fruiting bodies along the margin. 4–7. Conidiogenous cells and conidia submerged in agar. 8. Conidia. 9. Older mycelium turning brown and hyphae becoming slightly thickened. 10. Young hyphae with short conidiophores and conidia. Scale bars: 3 = 1.1 cm, 4, 10 = 5 µm, 5–9 = 10 µm.

Detached asci had a thread-like structure (Fig. 16) at the base, a remnant from their attachment to the ascogenous hypha.

Pleurostomophora repens (R.W. Davidson) L. Mostert, W. Gams & Crous, **comb. nov.**
Mycobank MB500097.

Basionym: *Cadophora repens* R.W. Davidson, J. Agric. Res. 50: 803. 1935.

≡ *Phialophora repens* (R.W. Davidson) Conant, Mycologia 29: 598. 1937.

Description: See Schol-Schwarz (1970) and De Hoog *et al.* (2000).

Cultural characteristics: Colony surface on MEA brownish orange (5C3) at the centre, becoming orange-white (5A2) towards the margin, the same in reverse. Minimum temperature for growth below 10 °C, optimum 30 °C and maximum above 40 °C. Colonies attaining a diameter of 59–61 mm at 20 °C after 8 d in the dark.

Type specimens examined: United States, Florida, Caryville, pine lumber, Jun. 1939, R.W. Davidson, **holotype**

herb. CBS 7594, ex-type culture of *Cadophora repens* CBS 294.39.

Notes: *Pleurostomophora repens* has longer, penicillately branched conidiophores in comparison with the shorter, simple conidiophores of *P. ootheca*. *Pleurostomophora repens* also produces only one type of conidium, being cylindrical to allantoid, 3–6 × 1.2–2.2 µm, and thus smaller than those of *P. ootheca*. Colonies of *P. repens* grow twice as fast as those of *P. ootheca*.

Pleurostomophora richardsiae (Nannf. apud Melin & Nannf.) L. Mostert, W. Gams & Crous, **comb. nov.** Mycobank MB500098.

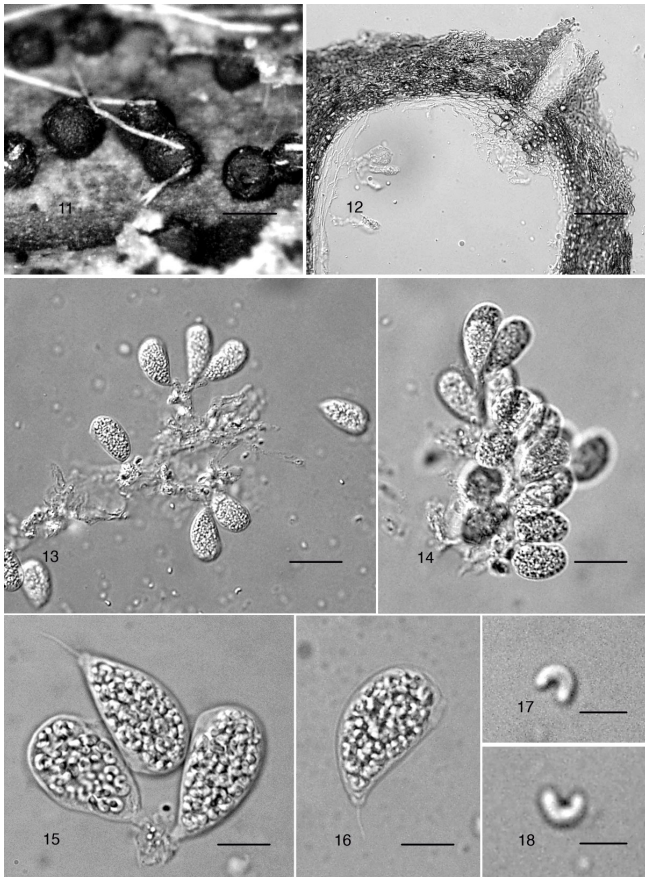
Basionym: *Cadophora richardsiae* Nannf. apud Melin & Nannf., Svenska Skogsvårdsfören. Tidskr. 32: 421. 1934.

≡ *Phialophora richardsiae* (Nannf.) Conant, Mycologia 29: 598. 1937

= *Cadophora brunnescens* R.W. Davidson, J. Agric. Res. 50: 803. 1935.

≡ *Phialophora brunnescens* (R.W. Davidson) Conant, Mycologia 29: 598. 1937.

= *Phialophora caliciformis* G. Smith, Trans. Br. Mycol. Soc. 45: 391. 1962.



Figs 11–18. *Pleurostoma ootheca*. 11. Fruiting-bodies on host tissue. 12. Vertical section through an ascoma. 13, 14. Asci on ascogenous hyphae. 15, 16. Polysporous asci with apical thickening. 17, 18. Ascospores. Scale bars: 11 = 350 μ m, 12 = 40 μ m, 13, 14 = 30 μ m, 15, 16 = 10 μ m, 17, 18 = 2.5 μ m.

Description: See Schol-Schwarz (1970), Domsch *et al.* (1980), De Hoog *et al.* (2000).

Cultural characteristics: Colony surface on MEA olive-brown (4E3), in reverse a darker shade of olive-brown (4F3). Minimum temperature for growth below 10 °C, optimum 30 °C and maximum 35 °C. Colonies attaining a diameter of 18–19 mm at 20 °C after 8 d in the dark.

Type specimens examined: Sweden, Jul. 1933, J.A. Nannfeldt, **isotypes** herb. CBS 7595 and CBS 7596, culture ex-type CBS 270.33.

Notes: *Pleurostomophora richardsiae* produces two very distinct types of conidia: brown, (sub)globose conidia, besides hyaline, allantoid to cylindrical conidia, which are clearly distinct from those of *P. ootheca*. Phialides of *P. richardsiae* are 10–35 μ m long with prominently flaring collarettes, particularly those producing the pigmented conidia, while those of *P. ootheca* are 6–19 μ m long, with inconspicuous collarettes. The brown colonies of *P. richardsiae* easily distinguish it from *P. repens* and *P. ootheca*. Morphologically, *P. richardsiae* is quite distinct from

the other two species of *Pleurostomophora*, and also clusters basal to the type of the genus, *P. ootheca* (Figs 1, 2). It is probable, therefore, that the collection of further species in this complex may eventually show *P. richardsiae* to belong to a closely related, but as yet undescribed genus. For the present this species is best accommodated in *Pleurostomophora*, as it is phylogenetically quite far removed from *Phialophora* (Fig. 1).

DISCUSSION

In the present study a new anamorph genus, *Pleurostomophora*, is introduced to accommodate the anamorph of *Pleurostoma ootheca*, and two related species, namely *P. repens* (CBS 294.39) and *P. richardsiae* (CBS 270.33). The latter two species could be linked to *Pleurostoma* based on their SSU phylogeny, suggesting that they might also have *Pleurostoma* teleomorphs. Tulasne & Tulasne (1863) reported an anamorph associated with the type, *Pl. candollei*, which had branched conidiophores with ‘innumerable agglutinated’, slender, curved conidia approximately 3 μ m long. Unfortunately, however, no cultures were available for study, and the status of the anamorph of *Pl. candollei* can only be resolved once fresh collections have been obtained.

A revision of the *Calosphaeriales* is needed to confirm the placement of the known genera within this order. To resolve the structure of the *Calosphaeriales*, cultures of its type species, *Calosphaeria pulchella* (Pers.) Schröter, are needed. The only culture of *Calosphaeria pulchella*, lodged as *Calosphaeria princeps* Tul. & C. Tul. at CBS (CBS 115.57), proved to be a misidentification or a contamination (but see Réblová *et al.*, this volume).

A morphological feature that Barr (1985) considered important of the *Calosphaeriales* is the presence of paraphyses in the centrum. Barr (1990) noted that paraphyses were absent in genera that had a spicate arrangement of asci. This is true for *Pleurostoma*, while *Togninia* has both paraphyses, as well as a spicate arrangement of asci. Members of these two genera appear to be phylogenetically closely related but there is sufficient evidence based on the 18S rDNA analyses that they represent two monophyletic lineages within the *Calosphaeriales* and are distinct genera (Fig. 1). Morphologically speaking, these two genera can also be differentiated in perithecial anatomy, asci, ascospores, the presence or absence of paraphyses, as well as in their anamorphs.

Gams (2000) resurrected the genus *Cadophora* Lagerb. & Melin for anamorphs of the *Dermateaceae* (*Helotiales*) with more or less pigmented vegetative hyphae, pale and hyaline phialides and collarettes. He also reported that this group of fungi had discomycete affinities according to 18S and 28S rDNA phylogenies

published by Paulin & Harrington (2000). In a further study, Harrington & McNew (2003) confirmed that *Cadophora* (type: *Cadophora fastigiata* Lagerb. & Melin) belonged to the *Helotiales* (discomycetes). Furthermore "*Phialophora richardsiae*" was also shown to fall within the *Sordariomycetes*, and not grouping with the true *Cadophora* species (Yan *et al.* 1995, Harrington & McNew 2003). The type species of the genus *Phialopora*, *Ph. verrucosa* Medlar, was shown to fall in the *Chaetothyriales* (Haase *et al.* 1999, Untereiner & Naveau 1999), indicating that all true *Phialophora* species should fall within this order. The *Chaetothyriales* are phylogenetically distant from the *Calosphaeriales*.

Species of the *Calosphaeriales* were usually described as being saprobic or hypersaprobic on woody plants, often being associated with stromata of other ascomycete fungi (Barr 1990) or vascular plant pathogens (Hawksworth *et al.* 1976). The ecological niche of the *Calosphaeriales*, however, does not only include plants and plant debris, but also humans. Several species of the genus *Phaeoacremonium* are also opportunistic pathogens on humans, and can cause subcutaneous phaeohyphomycotic cysts (De Hoog *et al.* 2000, Mostert *et al.* 2005). *Pleurostomophora repens* has been isolated from wood of *Pinus sylvestris* in the U.S.A. (Schol-Schwarz 1970), and has also been associated with subcutaneous infections with granulomatous nodules in humans (Meyer *et al.* 1975, Hironaga *et al.* 1989). *Pleurostomophora richardsiae*, on the other hand, has been isolated from wood, ground wood pulp, sewage and soil in North America, Europe, Africa and Asia (Schol-Schwarz 1970). *Pleurostomophora richardsiae* can also infect humans, usually through traumatic implantation, and also cause subcutaneous phaeohyphomycotic cysts (De Hoog *et al.* 2000). Both *P. ootheca* and *P. repens* could grow at 40 °C.

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