THE GENUS *PHIALOCEPHALA*: A TAXONOMIC STUDY

BY

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Preface

The classification of hyphomycetes by Hughes, based on conidial development ultimately led to the establishment of the three genera within the *Leptographium* complex. *Verticicladiella* Hughes accommodated species with sympodial conidial development and *Leptographium*, species with precurrent proliferation of conidiogenous cells. The genus *Phialocephala* Kendrick was established to accommodate those species with phialidic conidial development. These fungi are further characterized by dark mononematous conidiophores that branch occasionally at their apices. Hyaline conidia thus accumulate in slimy masses around the sporogenous heads and this facilitates dispersal by insects. Some species are characterized by solitary phialides that form direct on the mycelium.

In the first chapter of this thesis a taxonomic re-evaluation of *Phialocephala* was undertaken. The evaluation is based on the growth patterns of species, their tolerance to cycloheximide and other morphological characters. These characters form the basis on which I compiled a dichotomous and synoptic key for easy identification of species of *Phialocephala*.

In the second chapter the taxonomic placement of *Phialocephala humicola* is considered. Conidiogenesis in this species was examined using scanning, transmission and light microscopy. The relationship of *P. humicola* to other *Phialocephala* spp. as well as ophiostomatoid fungi were also determined based on molecular data.

The third chapter of this study presents an investigation using molecular data to determine the relationships between *Phialocephala* species and other related genera. This was achieved by means of sequence data obtained from the 18S and 28S subunits of the rRNA operon. The intention was to use the molecular data to determine the placement of *Phialocephala* amongst other genera of the Ascomycotina.

The overall objectives of the studies presented in this thesis were made to clarify the apparent heterogeneous nature of *Phialocephala sensu lato*. The lack of herbarium material, living cultures and the inability to induce sporulation in some species resulted in the exclusion of a limited number of species in both the re-evaluation based on morphology and molecular data. Nevertheless I would like to

believe that our understanding of this genus, its placement in the Ascomycotina and its relationships to related genera has partially been resolved.

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Dedicated to my family and Eduard

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Summary

Opsomming

Summary

Phialocephala was established to accommodate phialidic fungi in the *Leptographium* complex. They are characterized by considerable variation in morphological characters. These morphological characteristics have been used as basis to compile dichomomous and synoptic keys to known species of *Phialocephala*. These keys are supported by comprehensive descriptions accompanied by both photographs and line drawings.

The second chapter deals with the generic placement of *Phialocephala humicola*. *Phialocephala humicola* display annellidic conidiogenesis and the subsequent elongation of the conidiogenese cells. Based on the sequence data obtained from the 28S subunit of the rRNA operon, this species represent a new genus. The new genus is associated with the Ophiostomatales and might be accommodated in *Leptographium*.

The third chapter of the thesis deals with polyphyletic nature pertaining to *Phialocephala*. This study was based on sequence data obtained from the 28S and 18S regions of the rRNA operon. The results indicate that *Phialocephala* represent at least three genera associated with the Leotiales, Ophiostomatales and Microascales, respectively.

This thesis represents a comprehensive study of the morphological and molecular characteristics of the fungi accommodated in *Phialocephala sensu lato*. This genus is clearly polyphyletic. Although not all described species were included in this study, it has shed more light on the diversity and taxonomic problems pertaining to this genus.

Opsomming

Phialocephala is totstand gebring om phialidiese fungi binne die *Leptographium* kompleks te akkomodeer. Hulle word gekenmerk deur geweldige variasie in morfologiese eienskappe. Hierdie eienskappe vorm die basis vir die sleutels wat vir die identifikasie van elke spesie ingesluit is. Die sleutels word ondersteun deur volledige beskrywings, foto's en lynsketse vir elke spesie.

Die tweede hoofstuk handel oor die posisie van *P. humicola* in die Ascomycotina. *Phialocephala humicola* word gekenmerk deur annelidiese konidiogenese en die verlenging van die konidiogenese selle gedurende die vorming van elke konidium. Basispaaropeenvolgingsdata verkry vanaf die 28S subeenheid van die ribosomale RNA operon, dui daarop dat hierdie spesie 'n nuwe genus verteenwoordig. Hierdie genus word geassosieer met die Ophiostomatales, maar kan moontlik ook in *Leptographium* val.

Die derde hoofstuk handel oor die verwantskappe tussen die verskillende *Phialocephala* spesies, gebaseer op molekulêre data. Hierdie data, vanaf die 18S en 28S subeenhede van die rRNA operon dui daarop dat *Phialocephala* ten minste drie genera voorstel. Hierdie drie genera word onderskeidelik met die Leotiales, Ophiostomatales en Microascales geassosieer.

In die tesis word 'n breedvoerige studie van beide die molekulêre en morfologiese eienskappe van *Phialocephala sensu lato* saamgevat. Dit is duidelik dat *Phialocephala* meer as een genus verteenwoordig. Ten spyte van die feit dat nie alle spesies in die tesis behandel is nie, word daar egter meer lig gewerp op die komplekse taksonomiese aard van die genus.

Introduction

Phialocephala Kendrick was established in 1961 to accommodate species of fungi, in the *Leptographium* Lagerberg & Melin complex that produce conidia through phialidic development (Kendrick, 1961, 1962). These fungi are further characterized by dark mononematous conidiophores that branch occasionally at their apices (Crane, 1971). Hyaline conidia thus accumulate in slimy masses around the sporogenous heads and this facilitates dispersal (Kendrick, 1961). Some species are characterized by solitary phialides that form directly on the mycelium (Onofri & Zucconi, 1984).

Phialocephala spp. occupy a diverse range of ecological niches and include weak pathogens, saprobes and endophytes. The type species, *Phialocephala dimorphospora* Kendrick, has been isolated from a soil sample as well as decayed woody tissue (Kendrick, 1961; Barron, 1968). Other species such as *P. trigonospora* Kirschner & Oberwinkler, have been isolated from bark beetle tunnels in *Pinus* and *Picea* spp. (Kirschner & Oberwinkler, 1998), while *Phialocephala scopiformis* Kowalski & Kehr and *P. compacta* Kowalski & Kehr are endophytes of living trees (Kowalski & Kehr, 1995). *Phialocephala fortinii* Wang & Wilcox was isolated from a pseudomycorrhizal association with *Pinus* species, but also proved to be a weak pathogen on container grown conifers (Wang & Wilcox, 1985; Wilcox & Wang, 1987).

Species of *Phialocephala* are characterized by diverse morphological characteristics. This suggests that they might represent more than one genus. These characters include colony colour, presence of crystals or pigmentation in culture, rhizoids at the base of the conidiophore, sterile outgrowths on the stipes and collarette morphology to name but a few. The role these characters play in the taxonomy of these fungi has not been clearly defined, indicating the need to re-evaluate these fungi. These characteristics as well as others form the basis on which the re-evaluation of this genus was undertaken.

Taxonomic history

Phialocephala was described by Kendrick (1961) to accomodate two species, *Phialocephala dimorphospora* Kendrick and *P. bactrospora* Kendrick. The generic description was based on species characterized by dark mononematous conidiophores that are penicillately branched, with hyaline conidia accumulating in slimy masses around the sporogenous heads (Kendrick, 1961, 1962; Barron, 1968). These conidia were described as being formed by means of phialidic conidium development from deep-set collarettes. *Phialocephala* spp. are characterized by inconspicuous loosely flaring, cupulate or irregular splitted collarettes. Conidia in *Phialocephala*

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species are ovoid to pyriform in shape with a single attachment point (Wingfield, van Wyk & Wingfield, 1987) characteristic of apical wall building (Minter, Kirk & Sutton, 1982).

An amendment to the original generic description was proposed by Crane (1971) to accommodate species, such as *Phialocephala illini* Crane, where the stipes are occasionally branched and once or twice dichotomously forked. The sporogenous heads in *P. illini* are also laterally orientated on the stipe. A further amendment was proposed by Onofri and Zucconi (1984) to accommodate species such as *Phialocephala mexicana* Onofri & Zucconi, which forms solitary phialides directly on the mycelium.

Some species treated in *Phialocephala* were previously described in other genera. *Phialocephala repens* (Cooke & Ellis) Kendrick was first described as *Penicillium repens* Cooke & Ellis based on the structure of the sporogenous heads. Kendrick (1963b), however, rectified this situation by transferring *P. repens* to *Phialocephala*, based on the dematiaceous nature of the fungus and the fact that conidia are produced in slimy heads.

Phialocephala phycomyces (Auersw.) Kendrick was first described as a monotypic species in the genus *Hantzschia* Auerswald. Grosmann, however, synonymised the genera *Leptographium* and *Hantzschia*, because she had doubts about the validity of the name *Hantzschia* (Kendrick, 1964). *Hantzchia phycomyces* was then incorrectly transferred to *Graphium* Corda as *Graphium phycomyces* (Auersw.) Saccardo (Saccardo, 1932). It was later moved to *Leptographium* as *L. phycomyces* (Auersw.) Grosm. before Kendrick transferred it to *Phialocephala* (Grosmann, 1932; Shaw & Hubert, 1952). Differences in morphological characteristics such as colony colour, the presence of inconspicuous collarettes, tolerance to the antibiotic cycloheximide, together with sequence data from the 28S rRNA gene, motivated Jacobs *et al.* (2000) to describe this species as the type species of a new monotypic genus, *Kendrickiella* K. Jacobs & M. J. Wingf.

Phialocephala illini was transferred to *Chaetopsina* Rambelli by Wingfield (1987) based on the yellow colour of the conidiophores in 100% lactic acid (Samuels, 1985) as well as the large conidia and the setose conidiophores. These characteristics are typical of *Chaetopsina* but not of *Phialocephala* (Samuels, 1985; Wingfield, 1987). Some species of *Phialocephala* have been transferred to *Sporendocladia* Arnaud, Nag Raj & Kendrick based on differences in conidiogenesis. *Sporendocladia bactrospora* (Ell. & Ev.) Wingfield, the type species of *Sporendocladia*, is characterized by conidia with two attachment points. These conidia are produced in 'true chains' and are held together by wall material (Minter, Kirk & Sutton, 1983a; Mouton & Wingfield, 1992). No periclinal thickening is observed in the conidiogenous cells but secretory vesicles in and around the wall of the conidiogenous cell form a wall building ring that gives origin to new conidia (Mouton & Wingfield, 1992). Species such as *P. fumosa* (Ell. & Ev.) Sutton and *P. follicola* Kirk are also characterized by ring wall building and thus reside in *Sporendocladia*.

Teleomorph connections

No teleomorphs are known for species of *Phialocephala*. *Ophiostoma francke-grosmanniae* Davidson was thought to be the only species in *Ophiostoma* with a *Phialocephala* state. This species had been characterized by conidiogenous cells with small collarettes and periclinal thickening. However, scanning and transmission electron micrographs indicated that the conidia have only one attachment point and distinct but tightly packed annellations that give the false appearance of periclinal thickening in collarettes, when viewed with the light microscopy (Mouton, Wingfield & van Wyk, 1992). Thus, the anamorph of *O. francke-grosmanniae* was shown to be a species of *Leptographium* and not to reside in *Phialocephala* (Mouton *et al.*, 1992).

Other possible teleomorph connections of *Phialocephala* spp. had been proposed. These include reports of the ascomycete genus, *Anavirga* Fragoso, belonging to the Leotiales, with an unnamed *Phialocephala* state. Other genera such as *Tricladium* Iqbal, *Casaresia* Gonzolez, *Anguillospora* Ingold and *Lilliputia* Boud. & Pat. were also been reported to have *Phialocephala* conidial states (Descals & Sutton, 1976). A further report proposed a connection between *P. fortinii* and Leotiales genera based on the morphology of sterile apothecium-like structures with cells resembling immature asci, formed in a culture. The connection between *P. fortinii* and *P. dimorphospora* to genera in the Leotiales was recently comfirmed using rDNA ITS sequence data (Rogers, McKemy & Wang, 1999).

Conidiogenesis

Hughes (1953) first proposed the circumscription of genera based on their mode of conidiogenesis. As a direct result of the Hughes study, Kendrick (1961) established *Phialocephala*. However, since then, various aspects have been used to justify the transfer of a number of species from *Phialocephala* to more appropriate genera.

Phialocephala virens Siegfried & Siefert and *P. fusca* Kendrick, are characterized by apical wall building during conidium development (Mouton and Wingfield, 1992; Wingfield, 1985). This is characterized by the absence of elongation of conidiogenous cells (Minter *et al.*, 1983b). Periclinical thickening (Minter *et al.*, 1983a), however, forms as a result of the outer walls of the conidiogenous cells remaining in the collarette after the formation of each conidium (Hughes, 1953; Minter *et al.*, 1983a). The phialides are characterized by fixed an endogenous meristem (Minter *et al.*, 1983b). Each conidium is consequently formed by a succession of enteroblastic proliferation, holoblastic ontogeny, conidial delimitation and secession (Mouton *et al.*, 1992). The accumulation of the proliferating cell walls may result in the total blockage of the phialide and end further production of conidia by phialides (Caroll & Caroll, 1974).

The mode of conidiogenesis in the type species, *P. dimorphospora* has never been observed in any other phialidic fungus (Mouton, Wingfield & van Wyk, 1993). The process in this fungus is characterized by a conidiogenous cell in which the outer wall remnants accumulate during proliferation of the newly formed conidium, similar to typical periclinal thickening. However, the production of a new wall building apex proceeds before secession of the last conidium. The delay in conidial secession results in wall remnants that are loosely aggregated at the base of the collarette. These have been referred to as 'spent' wall thickenings because the wall layers are lost after conidial secession. In *P. dimorphospora* the first conidium is formed holoblastically, while the remaining conidia are formed by enteroblastic proliferation, holoblastic ontogeny, conidial delimitation and delayed secession (Mouton *et al.*, 1993). This process is in contrast to the accumulation of proliferation cell layers seen in other phialidic fungi such as *Fusarium crookwellense* Burgess, Nelson & Toussoun and most other species of *Phialocephala* (van Wyk, Wingfield & Marassas, 1988).

Conidiogenesis in *P. humicola* Jong & Davis is also phialidic (Onofri, Pagano & Zucconi, 1994). The conidia in this fungus revealed replacement wall building development with a particular disposition of conidia at the apex of the conidiogenous cell. A typical periclinal thickening is formed and the conidia posses only one attachment point and are not produced in chains. After the production of the first conidium, the next conidium pushes the first to the side of the conidiogenous cell. The conidia, however, have a tendency to stay attached to the conidiogenous cell, forming a conidial 'crown' (Onofri *et al.*, 1994).

Ecology

Phialocephala species are known from various habitats. *Phialocephala fortinii, P. dimorphospora, P. compacta* and *P. scopiformis* are readily isolated from roots of plants grown in cool or cold environments, such as those encountered in the alpine, sub-alpine and boreal regions (Hambleton & Currah, 1997; Stoyke & Currah, 1990). Most species are not known to cause disease, but *P. virens* was isolated from root butt rot of *Tsuga* and *Picea* spp. (Siegfried, Siefert & Bilmar, 1992).

Phialocephala fortinii and *P. dimorphospora* form part of the *mycelium radicis atrovirens* (MRA) Melin (1922) complex (Wang & Wilcox, 1985). Species belonging to this complex are isolated in the boreal forests from the surface of mycorrhizae and soil particles (Wang & Wilcox, 1985; Currah, Tsuneda & Murakami, 1993; Fernando & Currah, 1996). The *mycelium radicis atrovirens* complex, is characterized by dark sterile mycelia and consists of four taxa,

Leptodontidium orchidicola Sigler & Currah, *Phialophora finlandia* Wang & Wilcox, *Chloridium paucisporum* Wang & Wilcox, *Phialocephala fortinii* and *Phialocephala dimorphospora* (Wang & Wilcox, 1985; Fernado & Currah, 1995). However, identification of the fungi belonging to this complex is difficult because they rarely sporulate in culture (Richard & Fortin, 1973; Harvey, Rogers, & Wang, 1997; Fernando and Currah, 1996).

As part of the MRA complex, *P. fortinii* has a complex ecological role ranging from pathogenic to asymptomatic effects, depending on the host, the strain of fungus and environmental conditions (Wang & Wilcox, 1985, Wilcox & Wang, 1987; Currah *et al.*, 1993; Fernando & Currah, 1996). The variation in strains of *P. fortinii* was confirmed by Jumpponen (1999), showing that genets of *P. fortinii* isolated one year at one site, could not be found at the same site the following year. This author found that the same genet could be isolated from two different plant species up to 1,5 m apart. The allelopathic (positive and negative interactions) nature of *P. fortinii* is evident in the reports of this species being pathogenic to container-grown conifers (Wilcox & Wang, 1987; Currah *et al.*, 1993).

The mycorrhizal association of *P. fortinii* and its gymnosperm host is characterized by the formation of ectomycorrhizal structures, where the fungus colonises the vascular tissue of the plant (Currah *et al.*, 1993). The Hartig net generally does not penetrate deeper than the outer tangential walls, immediately beneath the epidermal layer of the plant cell (Fernando & Currah, 1996). This lack of deeper penetration indicates that *P. fortinii* is not as aggressive as another member of the MRA complex, *Leptodontidum orchidicola* (Fernando & Currah, 1995, 1996). The benefits of this mycorrhizal symbiosis between *P. fortinii* and its gymnosperm host, lies not only in the nutrient exchange between plant and fungus, but is also linked to reduced grazing of microfauna on the plant, giving it an advantage over competing plants species (Currah *et al.*, 1993). The disadvantages of this symbiosis for the plant are linked to the lysis of plant cells by the fungus, while colonising the vascular tissues. This is evident in very young seedlings, 3-5-day-old, where hyphal growth over the root tips causes surface cells to become contorted and to inhibit differentiation and elongation (Currah *et al.*, 1993).

Most *Phialocephala* spp. are associated with woody tissue (Kendrick, 1961, 1963; Siegfried *et al.*, 1992; Kowalski & Kehr, 1995). Some species, including the type species, *P. dimorphospora*, originate from soil (Kendrick, 1961; Barron, 1968). Other species isolated from soil samples are *P. humicola*, *P. queenslandica* Matsushima and *P. xalapensis* Persiani & Maggi (Matsushima, 1980; Sivasithamparam, 1975; Jong & Davis, 1972; Maggi & Persiani, 1984). *Phialocephala fluminis* Shearer, Crane & Miller has been isolated from water (Shearer, Crane & Miller, 1976).

The only *Phialocephala* sp. found to be associated with insects is *P. trigonospora* (Kirschner and Oberwinkler, 1998). This species has been isolated from beetle tunnels in Scots pine (*Pinus sylvestris* L.) and Norway spruce *(Picea abies* L. Korst). The occurrence, however, was shown to be constricted and the insect vector has been collected without its fungal partner in other regions.

Geographical Distribution

Phialocephala spp. have a cosmopolitan distribution that includes Scandinavian and European countries (Finland, Germany) (Siegfried *et al.*, 1992; Wang & Wilcox, 1985; Kowalski & Kehr, 1995; Kirschner & Oberwinkler, 1998) as well as some regions of Alaska (Siegfried *et al.*, 1992), the United States of America (Kendrick, 1963a; Shearer *et al.*, 1976), Canada (Kendrick, 1961; Siegfried *et al.*, 1992), Japan (Jong & Davis, 1975), Mexico (Onofri & Zucconi, 1984) and India (Rao & de Hoog, 1986). In the Southern Hemisphere, *Phialocephala* spp. have been documented from Sierra Leone (Kendrick, 1963a) and Australia (Sivasithamparam, 1975) although very little is known about them in these regions.

Materials and Methods

Isolates and colony descriptions

Isolates for all the species described in this study were obtained from various culture collections (Appendix A). Isolates were grown on malt extract agar (MEA) (20 g malt extract, 15 g NT Merck agar in 1000 ml water) and colony descriptions made on this medium. Where available isolates of each species were studied and measurements of the structures were compared with those recorded in the original descriptions. Colony colour was assigned using the colour charts of Rayner (1970).

All available herbarium specimens reflecting types of each species, as well as other collections, were studied. The lack of type specimens and the deterioration of material led to the exclusion of *P. mexicana* Onofri & Zucconi and *P. queenslandica*. Where the material was too deteriorated to use in the comparisons the original description was used and this is indicated as such in the text.

Light microscopy

Fungal structures were mounted in lactophenol on glass slides. The herbarium material was examined after placing a drop of 1% KOH on dried fungal structures. After 5 min relevant structures were removed and mounted in lactophenol on glass slides and examined. Fifty measurements of all the relevant fungal structures were recorded to compute the ranges and averages. Standard deviations for all the measurements were calculated and these are indicated in brackets.

Cycloheximide tolerance and temperature requirements

Cycloheximide tolerance of isolates was determined by tranferring 6 mm discs of each isolate on four 2% malt extract agar plates, amended with cycloheximide at concentrations of 0, 0.05, 0.1, 0.5, 1, 2.5 g/l. These plates were incubated at 25°C in the dark and the colony diameters measured for each plate. The growth was computed as an average of eight readings after eleven days. Similar procedures were followed to determine the optimal temperature for growth of each species using a temperature range between 5°C and 30°C, with 5°C intervals.

Identification of species

The species of *Phialocephala* can be divided into different groups based on cultural and morphological characteristics such as colony colour, collarette morphology, presence of rhizoids or sterile outgrowths from the stipe and the formation of second and subsequent conidia that are

different in morphology to the primary conidia. Most species are similar to the type species, *P. dimorphospora*, with respect to colony colour. Most of the species included in this study are characterized by dark colonies (Kendrick, 1961, 1963a/b; Kowalski & Kehr, 1995; Jong & Davis, 1972; Wang & Wilcox, 1985). The only exceptions are *P. virens*, with brilliant green colonies (Siegfried *et al.*, 1992) and *P. trigonospora*, with a cream colony colour (Kirschner, & Oberwinkler, 1998).

The morphology of the collarette formed, after the formation of the first conidium, also divides the *Phialocephala* spp. considered in this study, into two groups. Although the collarettes vary in morphology they can be loosely grouped into those that are conspicuous and those that are inconspicuous. *Phialocephala humicola* and *Phialocephala virens* are the only two species with inconspicuous collarettes (Jong & Davis, 1972; Siegfried *et al.*, 1992). The species with conspicuous collarettes can, however, be further subdivided. *Phialocephala dimorphospora, P. xalapensis, P. fortinii* and *P. scopiformis*, have tubular to cylindrical to sub-cylindrical collarettes (Kendrick, 1961; Maggi & Persiani, 1984; Wang & Wilcox, 1985; Kowalski & Kehr, 1995). *Phialocephala compacta* is characterized by flask to barrel-shaped collarettes, while the collarettes of *P. fluminis, P. canadensis, P. repens, P. fusca* and *P. trigonospora* are flared or irregularly torn (Kowalski & Kehr, 1995; Shearer *et al.*, 1976; Kendrick, 1962, 1963; Kirschner & Oberwinkler, 1998).

Phialocephala spp. are divided into two groups based on the formation of different types of conidia. The formation of second and subsequent conidia, different in shape to the first conidium, are reported in *P. dimorphospora, P. fortinii, P. compacta* and *P. scopiformis* (Kendrick, 1961; Wang & Wilcox, 1985; Kowalski & Kehr, 1995). Only one form of conidiu is observed in *Phialocephala fusca, P. humicola, P. xalapensis, P. canadensis, P. fluminis, P. trigonospora, P. repens* and *P. virens* (Kendrick, 1961, 1963a, 1963b; Jong & Davis, 1972; Persiani & Maggi, 1984; Shearer *et al.*, 1976; Kirschner & Oberwinkler, 1998; Siegfried *et al.*, 1992).

The presence of rhizoids and sterile outgrowths also serve to differentiate between *Phialocephala* spp. *Phialocephala fusca*, is characterized by rhizoids (Kendrick, 1963), while rhizoids are absent in all the other species. *Phialocephala canadensis* is the only species in which sterile outgrowths occur on both sides of the median septum of the conidiophore stipes (Kendrick, 1963). Although *P. fusca* and *P. fluminis* also produce sterile outgrowths, those on their stipes are not prominent (Kendrick, 1963; Shearer *et al.*, 1976).

Dichotomous key to species based on morphology

1 Phialides with typical well-defined collarettes, either flaring or cylindrcal.. 2

1'Phialides without well-defined collarettes.. 7

2 Sterile outgrowths present.. 3

2'Sterile outgrowths absent... 4

3 Conidia with lateral scar... *P. fluminis*

3'Conidia with basal scar.. *P. canadensis*

4 Conidiophore verrucose.. *P. fortinii*

4'Conidiophore not verrucose... 5

5 Conidiophore up to 700 µm long.. *P. xalapensis*

5'Conidiophore always less than 700 µm long.. 6

6 Collarette cylindrical.. 8

6'Collarette flaring 9

7 Green colony colour.. *P. virens*

7'Dark grey colony colour.. *P. humicola*

8 Formation of crystals in colony 11

8' No crystals form in colony.. *P. dimorphospora*

9 Conidia triangular in shape.. *P. trigonospora*

9'Conidia globose to ellipsoid in shape.. 10

10 Rhizoids absent.. *P. repens*

10'Rhizoids present... *P. fusca*

11 Synnematal formation.. *P. scopiformis*

11'No synnematal formation.. *P. compacta*

It should be noted that living material of some *Phialocephala* species are not available and that in the case of herbarium material, the material are in some cases so poor that structures could not be found. In these cases, some morphological data may be lacking from the synoptic key.

Synoptic key to the species

Morphological characteristics

Stipe length
A. less than 100 µm: 1;3;5;6;8;11
B. 100-200 µm: 3;4;5;6
C. 200-300 µm: 3;6
D. more than 300 µm: 7;12

Stipe smooth: 11
Stipe constricted at septa: 1;2;3;4;5;6;7;8;9;10;12

Conidiogenous apparatus length
A. 10-30 µm: 1;3;4;6;8;9;10;11
B. 30-50 µm: 1;3;4;6;8;9;10;11;12
C. 50-80 µm: 2;3;6;8;9;11;12
D. 80-100 µm: 6;7;9
E. more than 100 µm: 5;7;9

Lateral outgrowths
A. Present: 1;4;6
B. Absent: 2;3;5;7;8;9;10;11;12

Primary branch length
A. Less than 10 µm: 1;2;3;4;6;7;8;9;11;12
B. 10-15 µm: 1;3;6;8;9;10;11;12
C. 15-20 µm: 3;6;9;10;11;12
D. more than 20 µm: 9;10

Secondary branch length

A. Less than 10 μm: 1;2;3;6;7;9;10;11;12

B. 10-15 μm: 6;9;10;11

C. 15-20 μm: 9

D. more than 20 μm: 9

Phialide length

A. less than 10 μm: 2;4;6;7;8;10;11;12

B. 10-15 μm: 1;2;3;4;5;6;7;8;10;11;12

C. 15-20 μm: 3;4;6;8;9;11

D. more than 20 μm: 4;6

Collarette morphology:

A. Loosely flaring and irregularly torn: 1;4;6;8;10;11

B. Cylindrical: 2;3;9;11;12

C. Inconspicious: 7;11

Primary and secondary conidia present: 2;3;5;9

One type of conidia present: 1;4;6;7;8;10;11;12

Conidial morphology:

A. Globose: 2;5;8

B. Subglobose: 5;9

C. Transversely ellipsoid: 1

D. Triangular: 10

E. Ovoid: 2;3;6;9

F. Obovoid: 5;11

G. Spherical: 3

H. Obovate: 4

I. Ellipsoid: 6;7;9;11

J. Oblong: 11;12

Conidial length (secondary conidia)

A. 1-3 µm: 1;2;4;5;7;8;9;11;12

B. 3-5 µm: 1;3;6;7;11;12

C. 5-10 µm: 3

List of species:

1. *Phialocephala canadensis*

2. *Phialocephala compacta*

3. *Phialocephala dimorphospora*

4. *Phialocephala fluminis*

5. *Phialocephala fortinii*

6. *Phialocephala fusca*

7. *Phialocephala humicola*

8. *Phialocephala repens*

9. *Phialocephala scopiformis*

10. *Phialocephala trigonospora*

11. *Phialocephala virens*

12. *Phialocephala xalapensis*

Generic description

Phialocephala Kendrick, *Canadian Journal of Botany* **39**, 1079. 1961.

Etymology: (Phialocéphala). The name refers to the flask-shaped conidiogenous cells in the type species.

Distribution: Finland, Germany (Siegfried *et al.,* 1992; Wang & Wilcox, 1985; Kowalski & Kehr, 1995; Kirschner & Oberwinkler, 1998); Alaska (Siegfried *et al.,* 1992); the U.S.A. (Kendrick, 1963); Canada (Kendrick, 1962a); Japan (Jong & Davis, 1975); Mexico (Maggi & Persiani, 1984); India (Rao & de Hoog, 1986); Ivory Coast, Sierra Leone (Kendrick, 1963); Australia (Sivasithamparam, 1975; Jong & Davis, 1975)

Colonies fuscous black (13''''m) to olive-brown (15'''k) to cream (19'f) to green (35b). Fuscous black (13''''m) to olive green(15'''k) to eye blue (43''b), dark blue (47m) and dark green (35'm) in colour on reverse of colony. Aerial mycelium cottony to funiculose, lustrous to only at middle of colony to totally absent. Concentric rings of pigmentation present or absent. **Conidiophores**, complex, mononematous or inconspicuous, single or in groups, smooth, multiseptate numerous towards center of colony, at margin only produced from fibrils or absent. **Stipe**, mononematous, macronematous, aseptate to multiseptate, 5-700 µm long, 3-11 µm wide at base and 4-6 µm just below sporogenous apparatus, deep brown to yellowish in colour becoming lighter near the apex. **Sporogenous apparatus**, wedge-shaped, 10-125 x 45-150 µm. Comprised of 1 to 15 series of branches, distal series bearing phialides. **Primary branches,** number 2 to 5, 5-25 µm x 1-8 µm, **secondary branches,** 3-25 µm x 2-5 µm. **Tertiary branches**, 5-28 x 5-10 µm and **quartenary branches,** 15-25 x 2-8 µm. **Phialides** and solitary phialides 4-25 x 1-8 µm, numerous, subparallel, lageniform or fan-shaped to flask-shaped. **Collarettes**, variable in morphology, inconspicuous to conspicuous, flask-shaped, barrel-shaped or loosely flaring and irregularly torn to cylindrical and deep-set. **Conidia,** slimy, globose, subglobose, transversely ellipsoid, triangular, ovoid, obovoid, spherical, obovate, ellipsoid, oblong, very dilute brown to hyaline in colour, 1-15 x 1-5µm.

Hosts and substrates *Acer* (Kendrick, 1963a), *Pinus* (Kendrick, 1963a; Wang & Wilcox, 1985), *Picea* (Kowalski & Kehr, 1995; Kendrick, 1963a), *Tsuga* (Kendrick, 1963a; Siegfried *et al.*, 1992), *Boungainvilea* (Kendrick, 1963a), *Alnus* (Kowalski & Kehr, 1995) and *Triticum* (Sivasithamparam, 1975), decayed wood, water and soil (Kendrick, 1961; Jong & Davis, 1972; Shearer *et al.*, 1976). Bark beetle tunnels in *Picea abies* and *Pinus sylvestris* (Kirschner & Oberwinkler, 1998)

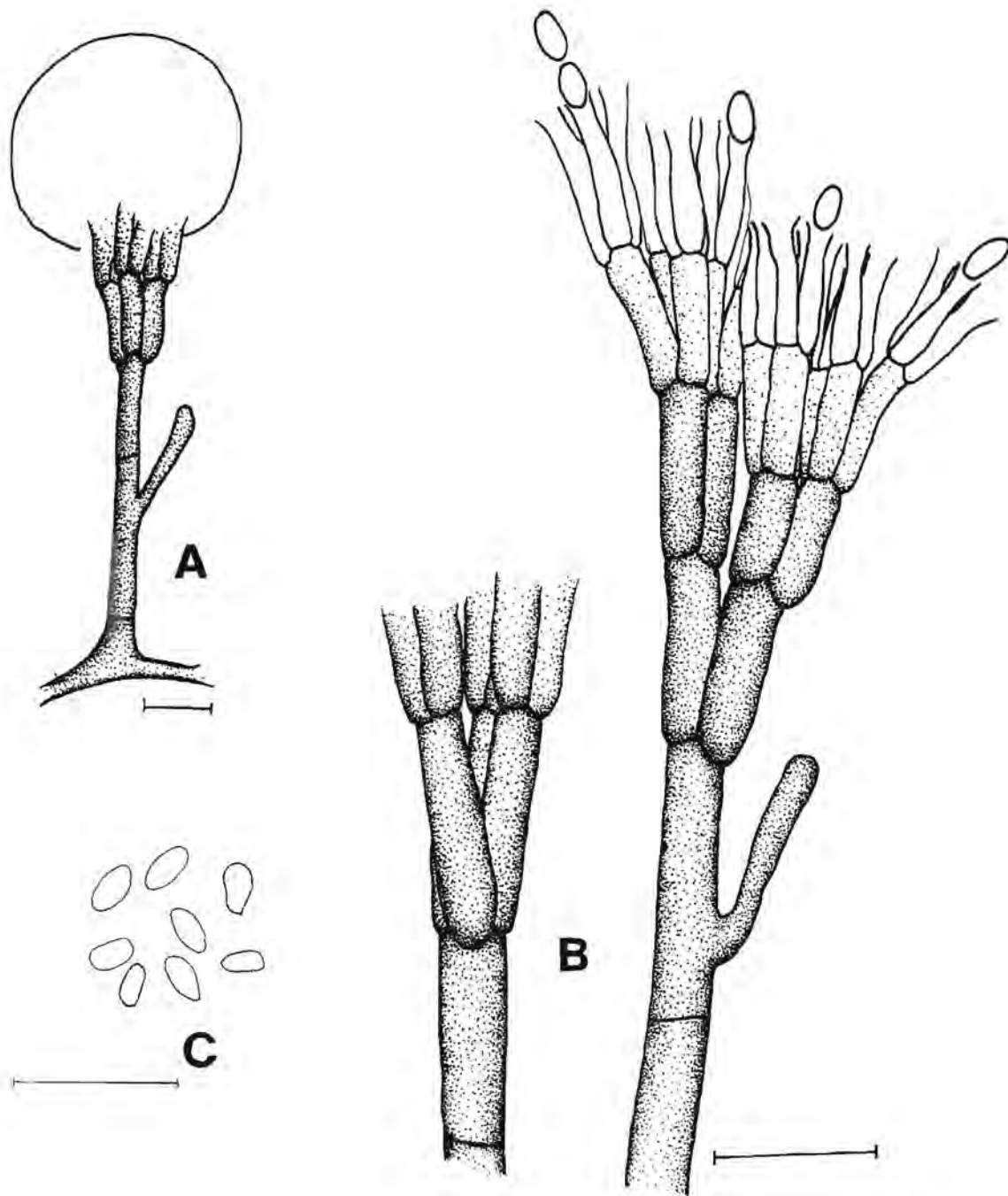**Type species:** *Phialocephala dimorphospora* DAOM (71465c).

Fig. 1. Conidiophores and conidia of *P. canadensis* (DAOM 71971). **A.** Habit sketch (Bar = 10 μm). **B.** Conidiogenous apparatus (Bar = 10 μm). **C.** Conidia (Bar = 10 μm).

Species description

Phialocephala canadensis Kendrick, *Canadian Journal of Botany* **41**, 1018. 1963. (**Fig. 1**)

Etymology: (canadénsis). The specific epithet refers to the origin of this species in Quebec, Canada.

Distribution: Canada (Kendrick, 1963)

Hosts: *Acer* (Kendrick, 1963)

Colonies fuscous black (13''''m). Reverse of colony, fuscous black (13''''m). Immersed mycelium composed of dark, septate, hyphae aggregated in radiating fibrille, dendritic toward the margin. Aerial mycelium is usually absent. **Conidiophores,** complex, numerous towards center of colony, toward margin only produced from immersed fibrils, 15-73(-85) µm. **Stipe** mononematous, 5-36(-47) µm long; 8 septate, 4.5 µm wide at base and 4 µm just below sporogenous apparatus, Brussels brown (15m) in colour becoming lighter near the apex. Two sterile outgrowths generally produced from opposite sides of stipe, immediately below penultimate septum. One or two further outgrowths develop adjacent to lower septa. Other outgrowths observed on lower parts and alongside primary branches. These outgrowths are straight, non-septate, 26 µm x 4 µm, concolourous with stipe becoming hyaline at rounded apex. **Sporogenous apparatus,** concolourous with stipe, 10-38(-42) µm in length. One to 3 series of branches. **Primary branches,** number 2 to 4, 6-13 µm x 1-4 µm. **Secondary branches,** 4-8 x 2-4 µm. **Phialides,** 15 x 1-2(-4) µm, numerous, subparallel. **Collarettes,** variable but conspicuous, loosely flaring and irregularly torn but sometimes cupulate. **Conidia,** slimy, transversely ellipsoid, sometimes slightly curved, very dilute brown, 1.5-2.0 x 2-4 µm (Kendrick, 1963).

Specimens examined: Holotype: Gatineau Park, Quebec, Canada, November 1960, collected: S.J. Hughes, DAOM 71971.

Notes

Phialocephala canadensis can be distinguished based on the presence of two sterile outgrowths produced from opposite sides of stipe, immediately below the penultimate septum and conidial shape. Although sterile outgrowths are occasionally produced in *P. fusca*, they are, however, not conspicuous. Nothing is known regarding the ecology and pathogenicity of this species.

Phialocephala compacta Kowalski & Kehr, *Canadian Journal of Botany* **73**, 27. 1995. (**Fig. 2**)

Etymology: (compácta). The specific epithet refers to the compact conidiogenous heads observed in older cultures.

Distribution: Braunschweig, Germany (Kowalski & Kehr, 1995)

Hosts: *Alnus glutinosa* and *Picea* species (Kowalski & Kehr, 1995)

Colonies olive black (27''''m) to black (1*), cottony aerial mycelium, only at middle. Black (1*) in colour on reverse of colony. Concentic rings of pigmentation present. **Hyphae,** dark olive (21'''m) in colour, 2.5-5 µm wide. Crystals form. **Conidiophores,** mononematous, arising vertically or laterally from mycelium, smooth, multiseptate, dark olive brown (15'''k) in colour. **Sporogenous apparatus,** 50-70 x 50-67 µm. Two to five series of branches. Branches concolorous with stipe, later becoming dark olive (21'''m) to olive black (27''''m). **Primary branches,** 2 to 3 in number, 8 x 5-7.5 µm. **Secondary branches,** 3-10 x 3-5 µm, **tertiary branches,** 8-28 x 5-10 µm, **quaternary branches,** 15-25 x 2.5-7.5 µm. **Phialides,** lighter in colour than branches, flask-shaped to slightly curved, 8-12.5 x 4 µm. **Collarettes,** cylindrical to barrel-shaped. **Primary conidia,** 4 x 2-3 µm, hyaline to subhyaline, ovoid in shape. **Secondary conidia,** globose, hyaline to subhyaline, 2-3 µm in length (Kowalski & Kehr, 1995).

Specimens examined: Holotype: Braunschweig, Germany, August 1990, collected: T. Kowalski, IMI 363203. **Cultures:** CBS 507.94 (same as CMW 4946 and ATCC 96754), Braunschweig, Germany, August 1990, collected: T. Kowalski.

Notes

Colonies sporulate readily on MEA after several weeks at room temperature. Storing cultures in the dark at 4°C for several months can also induce abundant sporulation (Kowalski & Kehr, 1995). An important characteristic of *P. compacta* is that there is an increase in pigmentation and sclerotization of the conidiogenous apparatus with age. No branches are recognizable at the end of the process. Furthermore, *P. compacta* is the only *Phialocephala* sp. in which the formation of crystals is recorded in culture. *Phialocephala compacta* is further characterized by two types of conidia. The first conidia produced by the conidiogenous cells are ovoid, similar to *P. dimorphospora*, while the remaining conidia are globose in shape. This is in contrast to the second and subsequent conidia of *P. dimorphospora* that are spherical. *Phialocephala compacta*, and *P. scopiformis* are endophytes of *Pinus* and *Picea* spp. (Kowalski & Kehr, 1995).
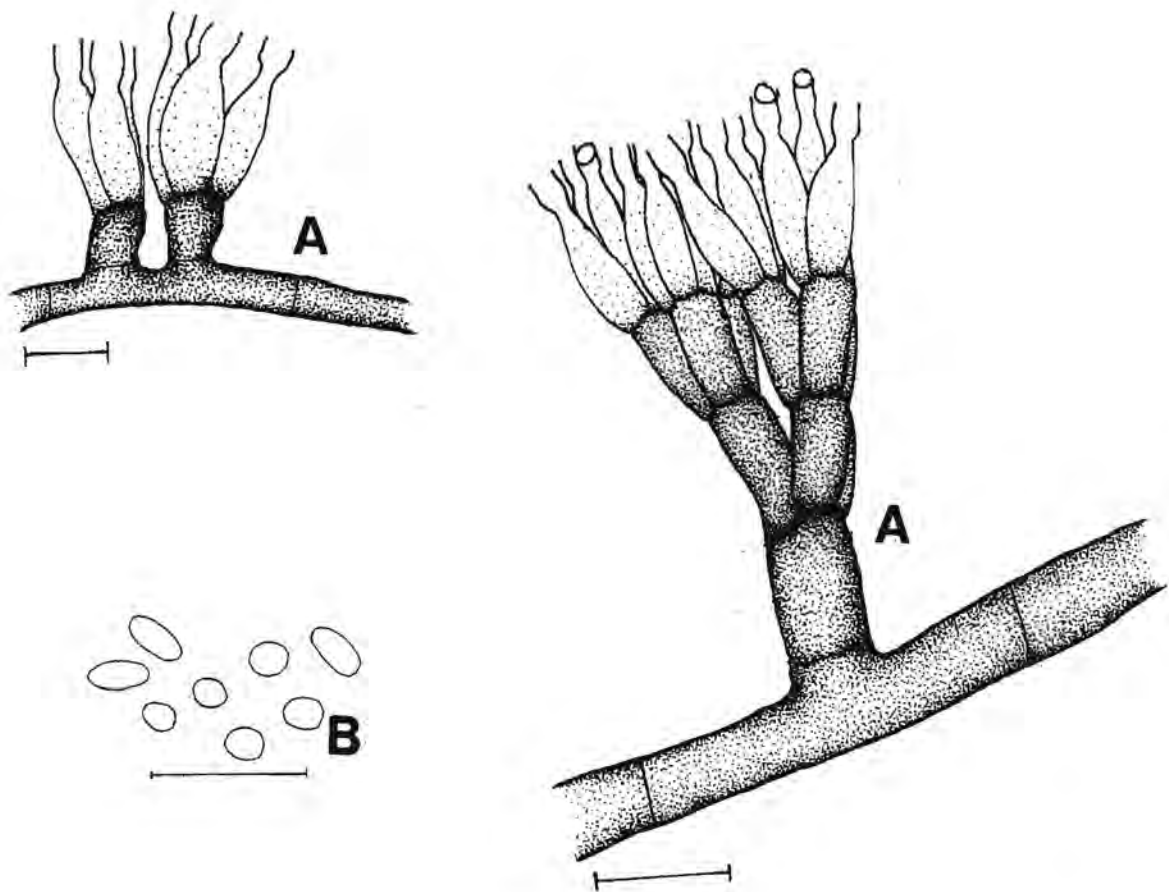
Fig. 2. Conidiophores and conidia of *P. compacta* (IMI 363203). **A.** Conidiogenous apparatus (Bar = 10 μm). **B.** Conidia (Bar = 10 μm).

Phialocephala dimorphospora Kendrick, *Canadian Journal of Botany* **39**, 1080. 1961. (**Figs. 3-4**)

Etymology: (dimorphospóra). From the Greek adjetive διζ: twice, and the Greek noun μορφη: form and the Greek noun σπορα: spore or seed. This specific epithet refers to the two forms of conidia produced by this species, namely ovoid first conidia and spherical second and subsequent conidia.

Distribution: Gatineau province of Quebec, Canada, Maryland and Alabama, U.S.A. (Kendrick, 1961).

Substrate: Decayed wood (Kendrick, 1961).

Colonies with optimal growth at 30°C on 2% MEA, reaching 8 mm in diam. in 11 days. No growth below 5°C or above 35°C. Able to withstand high concentrations of cycloheximide with a 68 % reduction in growth on 0.5 g/l cycloheximide after 11 days at 25 °C in the dark. Thick turf of deep mouse gray (15'''''i) to dark mouse gray (15'''''k) aerial mycelium. Fuscous black (13''''m) in colour on reverse of colony. **Hyphae,** sepia (17''m), irregularly, smooth to rough-walled, 3-5 μm wide. Margin diffuse and finely striate. **Conidiophores,** 30-132 (-265) μm, inconspicious in floccose mycelium. **Sporogenous apparatus,** 15-46 (-70) μm. Two to four series of branches. **Primary branches** 2 to 3 in number, (5-)7-12.5(-18) x 3-5 μm. **Secondary branches,** 3-9(-10) x 3-5 μm, **tertiary branches,** 8-25(-28) x 5-9(-10) μm and **quaternary branches,** (15-)18-25 x 3-6(-8) μm. **Phialides,** 10-16(-18) x 3-4(-8) μm. **Collarettes,** tubular, well-developed, deeply-set. **Primary conidia,** ovoid, 5-6 x 2-3 μm. **Secondary conidia,** spherical, 2-3 x 2-3 μm.

Specimens examined: Holotype: Ontario, Canada, November 1960, collected: S.J. Hughes, DAOM 71465(c). **Cultures:** ATCC 24087 (same as CMW 508) Alabama, U.S.A., 1987, collected: G. Morgan-Jones. CBS 300.62 (same as DAOM 87232 and CMW 665), New Brunswick, U.S.A., 1961. DAOM 165556A (same as CMW 505), Gatineau Park, Quebec, Canada, collected: S.J. Hughes. CMW 168, Maryland, U.S.A., 1986, collected: C.A. Shearer.

Notes

The most characteristic feature of *P. dimorphospora* is the presence of long tubular collarettes formed after the production of the first conidium. *Phialocephala dimorphospora* is also characterized by the formation of two conidial shapes. This is similar to *P. fortinii, P. compacta,* and *P. scopiformis*. The first conidia of *P. dimorphospora* are ovoid in shape, similar to those of *P. compacta.* This is in contrast to *P. fortinii* where the first conidia are obovoid. The second and subsequent conidia vary in shape from spherical in *P. dimorphospora* to globose in *P. compacta.*

The second and subsequent conidia in *P. fortinii* are globose to sub-globose. Hyphal loops such as those described in *P. fortinii* have been observed in cultures of *P. dimorphospora*.

Phialocephala dimorphospora forms part of the *Mycelium radicis atrovirens* (MRA) Melin (1922) complex (Richard & Fortin, 1973). Fungi in this complex can be readily isolated in the boreal forests from the surface of mycorrhizae and especially from pseudomychorrhizae on conifers and soil particles (Wang & Wilcox, 1985).

Fig. 3. Light micrographs of conidiophores and conidia of *Phialocephala dimorphospora* (CMW 168). **A.** Conidiophore (Bar = 3 μm). **B.** Conidiogenous apparatus (Bar = 5 μm). **C.** Conidiogenous cell (Bar = 3 μm). **D.** Conidia (Bar = 5 μm). **E.** Subsequent conidia (Bar = 5 μm).
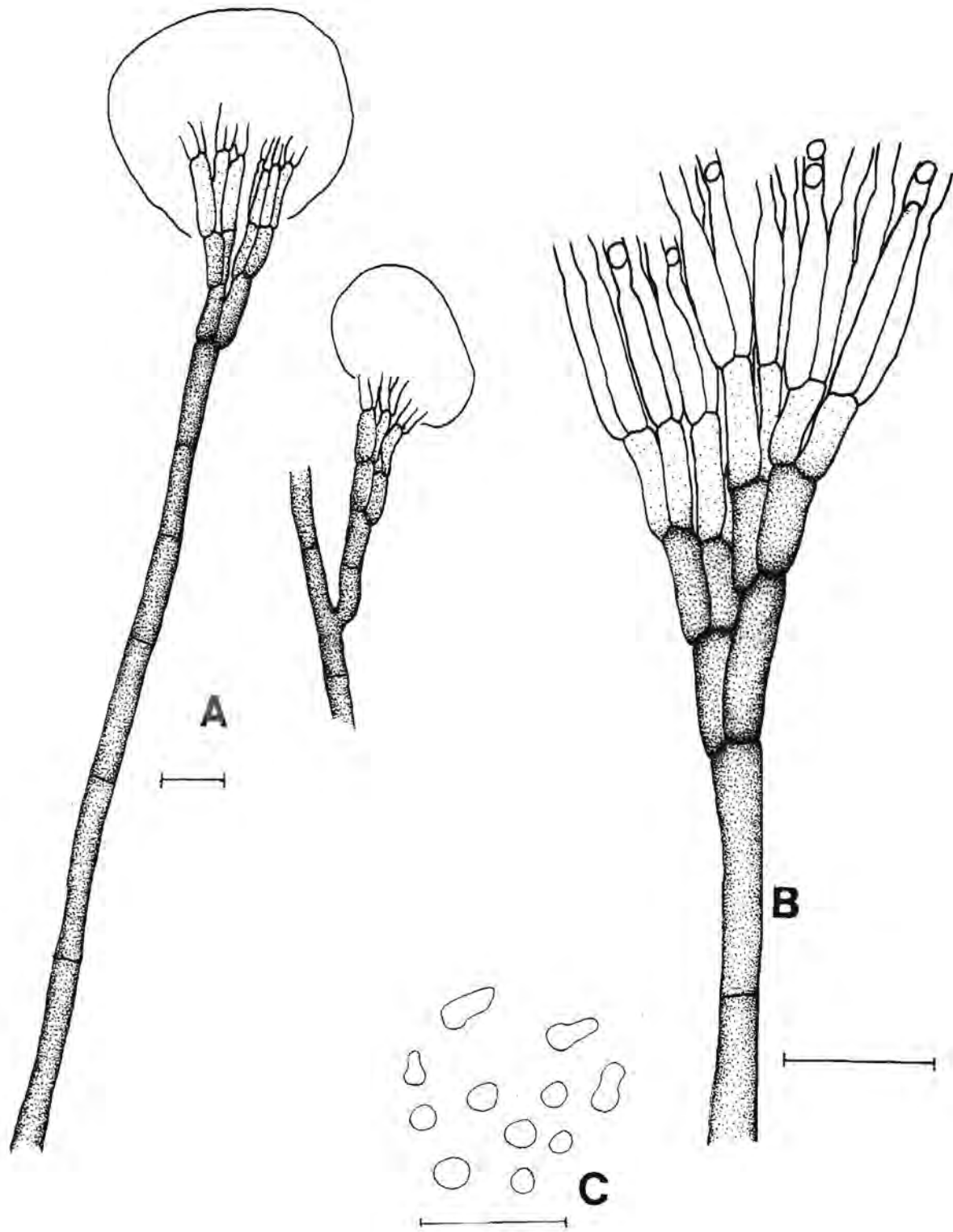
Fig. 4. Conidiophores and conidia of *P. dimorphospora* (DAOM 71456c). **A.** Habit sketch (Bar = 10 μm). **B.** Conidiogenous apparatus (Bar = 10 μm). **C.** Conidia (Bar = 10 μm).

Phialocephala fluminis Shearer, Crane &Miller *Mycologia* **68**, 186. 1976. (**Figs. 5-6**)

Etymology: (flumínis). The specific epithet refers to the fact that the fungus was first isolated from water.

Distribution: Macon County, Illinois, U.S.A. (Shearer *et al.*, 1963).

Substrate: Water

Colonies immersed mycelium composed of branched, dark, septate, subhyaline to fawn (13''') hyphae. Concentric rings of pigmentation absent. **Conidiophores** singly or in groups, 124-202(-214) μm, terminal or lateral on hyphae. **Stipe** mononematous, stout, septia (17''m), 115-196(-199) μm long, septate, 5.5 μm wide at base and 5 μm just below sporogenous apparatus. One to three sterile outgrowths produced laterally, singly on stipe, below septum present or absent. One or two further outgrowths develop adjacent to lower septa. Other sterile outgrowths observed on lower parts and alongside primary branches. **Sporogenous apparatus,** concolourous with stipe, 13-34(-40) μm in length. One to three series of branches. **Primary branches,** subhyaline to fawn (13'''), 4-6(-7) μm x 1-2(-4) μm. **Phialides,** hyaline to subhyaline, flask-shaped, 7-25 x 1-2(-4) μm, arising from stipe or directly from mycelium. **Collarettes,** flaring. **Conidia,** obovate, hyaline, 1.5-2 x 0.5-1.5(-2) μm (Shearer *et al.*, 1976)

Specimens examined: Holotype: Sagamon River, Decatur, Macon Couty, Illinois, Apr 1975, ILLS 36160.

Notes

Phialocephala fluminis resembles *P. canadensis* and *P. fusca* because of the presence of flaring collarettes on the phialides as well as sterile outgrowths on the conidiophores. *Phialocephala fluminis*, however, differs from *P. canadensis* in that it has conidial scars that are perpendicular in contrast to the basal scars of *P. canadensis*. *Phialocephala fluminis* is similar to *P. virens* in the formation of solitary phialides directly on the mycelium. Nothing is known regarding the ecology of this species, other than the fact that it occurs in water.
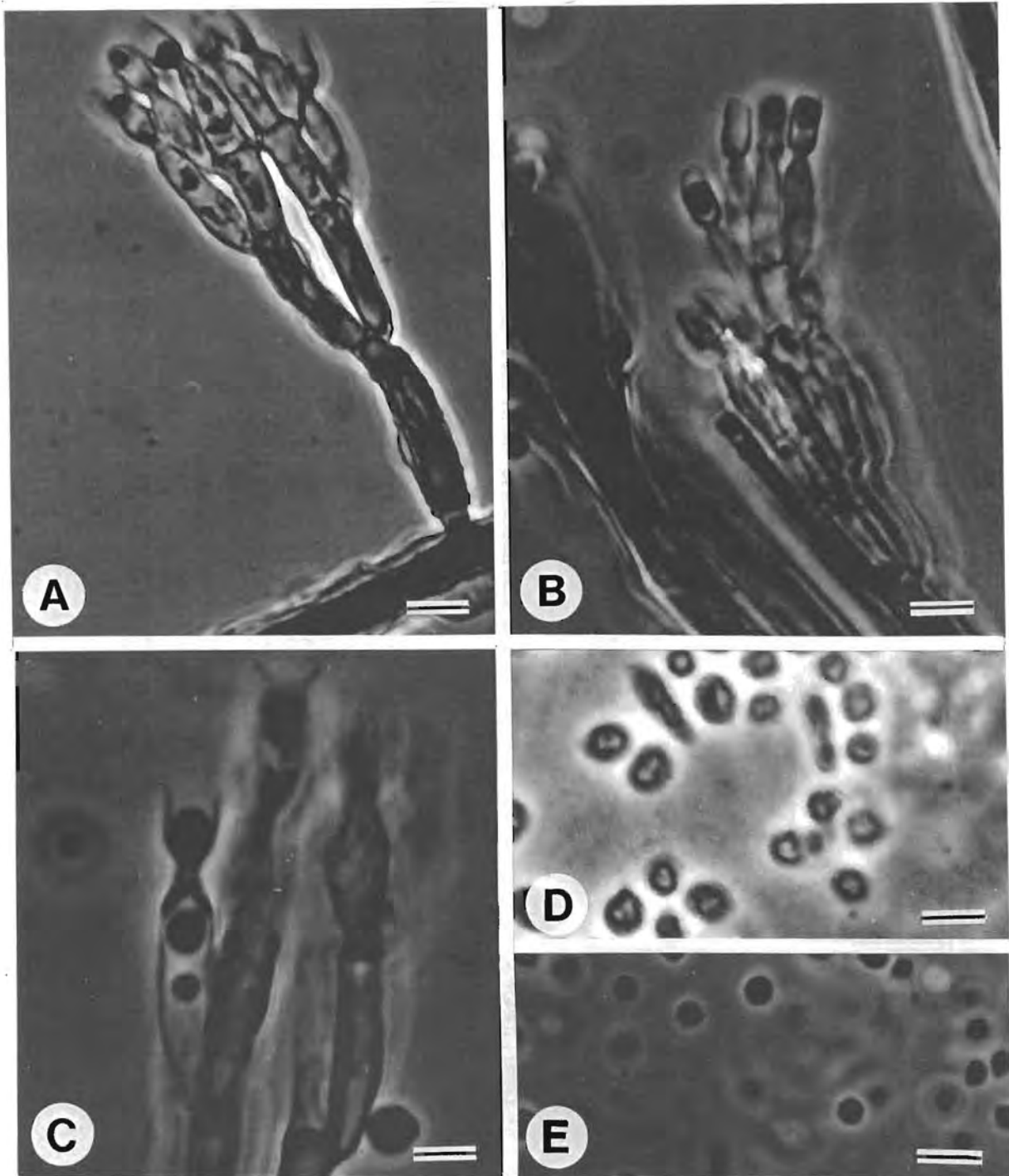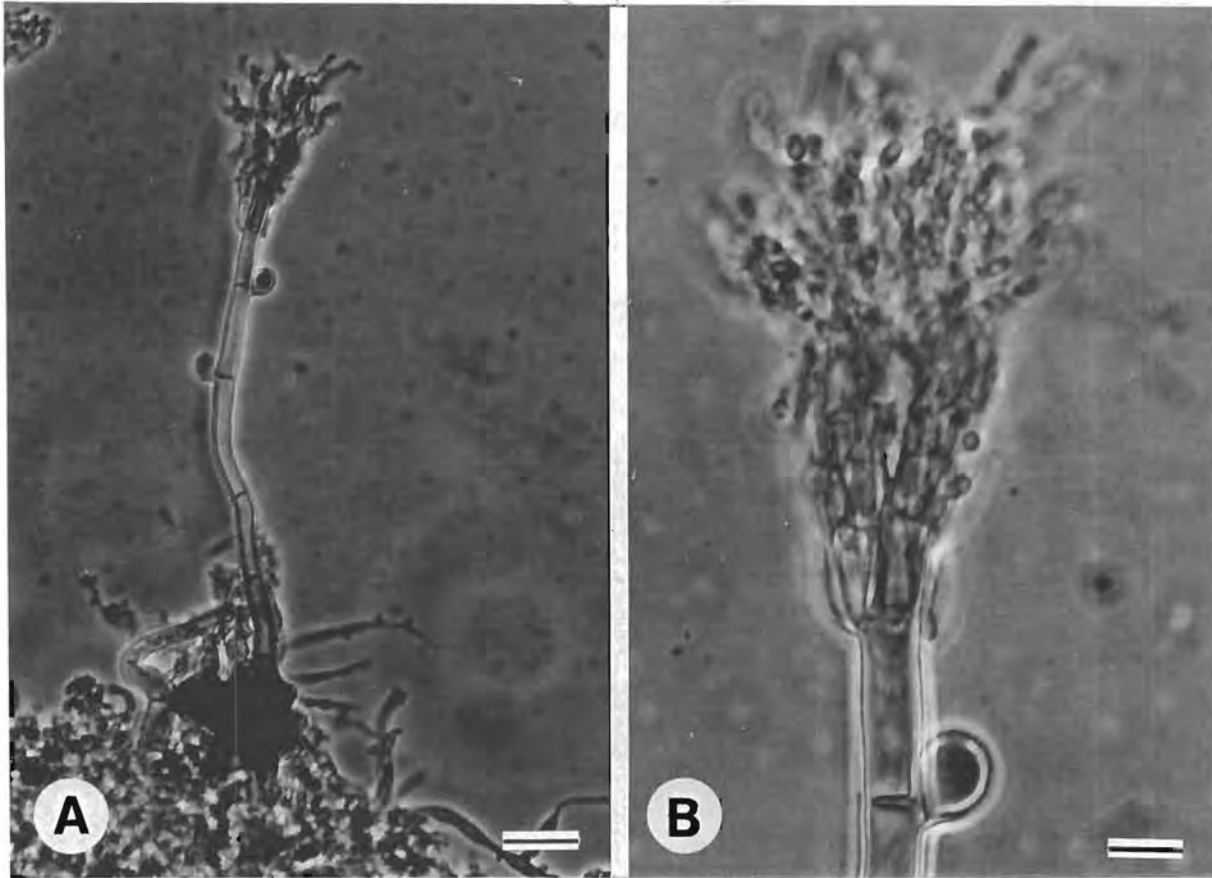
Fig. 5. Light micrographs of conidiophores and conidia of *Phialocephala fluminis* (ILLS 36160). **A.** Conidiophore (Bar = 19 µm). **B.** Conidiogenous apparatus (Bar = 4 µm).
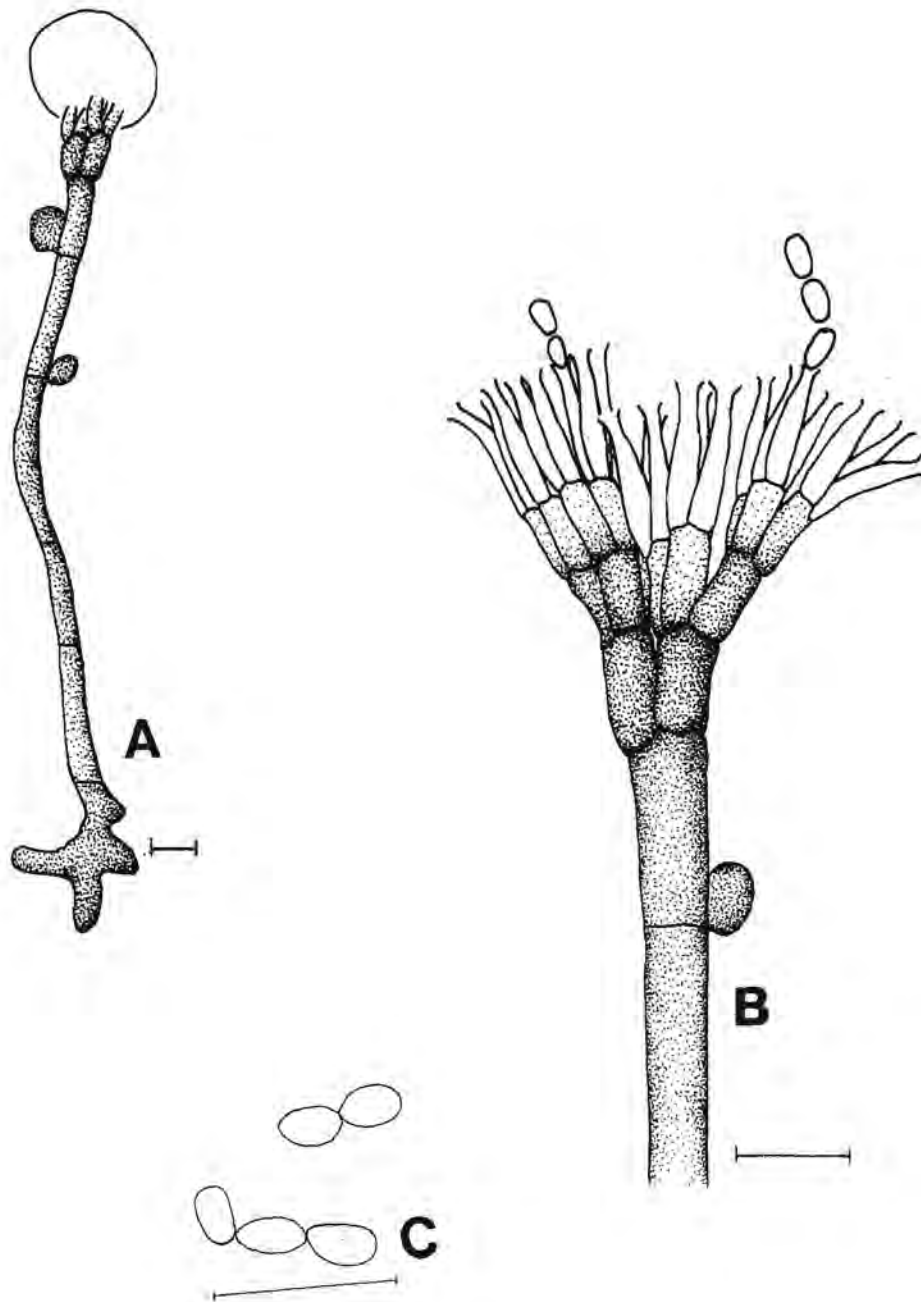
Fig. 6. Conidiophores and conidia of *P. fluminis* (ILLS 36160). **A.** Habit sketch (Bar = 10 μm). **B.** Conidiogenous apparatus (Bar = 10 μm). **C.** Conidia (Bar = 10 μm)

Phialocephala fortinii Wang & Wilcox, *Mycologia* 77, 955. 1985. (**Fig. 7**)

Etymology: (fortínii). The Latin genetive of Fortin. This specific epithet indicate that this fungus was named after Fortin.

Distribution: Finland (Wang & Wilcox, 1985).

Hosts: *Pinus sylvestris* (Wang & Wilcox, 1985).

Colonies fast-growing, 15mm in 11 days on 2% malt extract agar. No growth below 5°C or above 30°C. Able to withstand high concentrations of cycloheximide with a 55 % reduction in growth on 0.5 g/l cycloheximide after 11 days at 25 °C in the dark. Greenish black (31'''''k) to fuscous (13''''k), floccose, aerial mycelium in older cultures. Fuscous black (13''''m) in colour on reverse of colony. Margin submerged. Hyphae, septate, Brussels brown (15m), 10 μm wide. Hyphal loops occasionally present. **Sporogenous apparatus,** complex, wedge-shaped; 110 μm x 140 μm. Three to seven series of branches. **Stipe,** sepia (17''m), verrucose, 10-120 x 3-4 μm, excluding conidiogenous apparatus. **Phialides,** 15 x 2-3.5 μm and fawn (13''') in colour. Collarettes, light brown, conspicuous. **Primary conidia,** obovoid in shape, 3 x 1-1.5 μm. **Secondary conidia,** globose to subglobose in shape, 1.5-2 μm. All conidia hyaline in colour (Wang & Wilcox, 1985).

Specimens examined: Holotype: Suonenjoki experiment Station for Forest Regeration, Research Institute, Suonenjoki, Finland, July 1975, collected: H.E.Wilcox, FAP 7. **Cultures:** CBS 443.86 (same as CMW 5590), 1975, collected: J. Wang.

Notes

Isolates of *P. fortinii* differ from other *Phialocephala* spp. in that no slimy heads are observed and they sporulate sparsely in culture. Two types of conidia are formed, which is similar to *P. dimorphospora* and *P. compacta*. The first conidia are obovoid in shape, in contrast to the ovoid first condia of *P. dimorphospora* and *P. compacta*. The second and subsequent conidia are globose to sub-globose. These are similar to those of *P. compacta*, that are globose, but different to the spherical second and subsequent conidia formed by *P. dimorphospora*. Similar to *P. compacta*, storage of *P. fortinii* at 4°C for an extensive period of time is necessary for sporulation (Wang & Wilcox, 1985).

Phialocephala fortinii also forms part of the MRA (*Mycelium radicis atrovirens*) complex and is a common endophyte of the roots of ericaceous plants, conifers and terrestrial orchids in boreal forests (Currah *et al.*, 1993; Fernando & Currah, 1995, 1996). Isolates of *P. fortinii* have also been reported as weak pathogens of container-grown conifers (Wilcox & Wang, 1987).
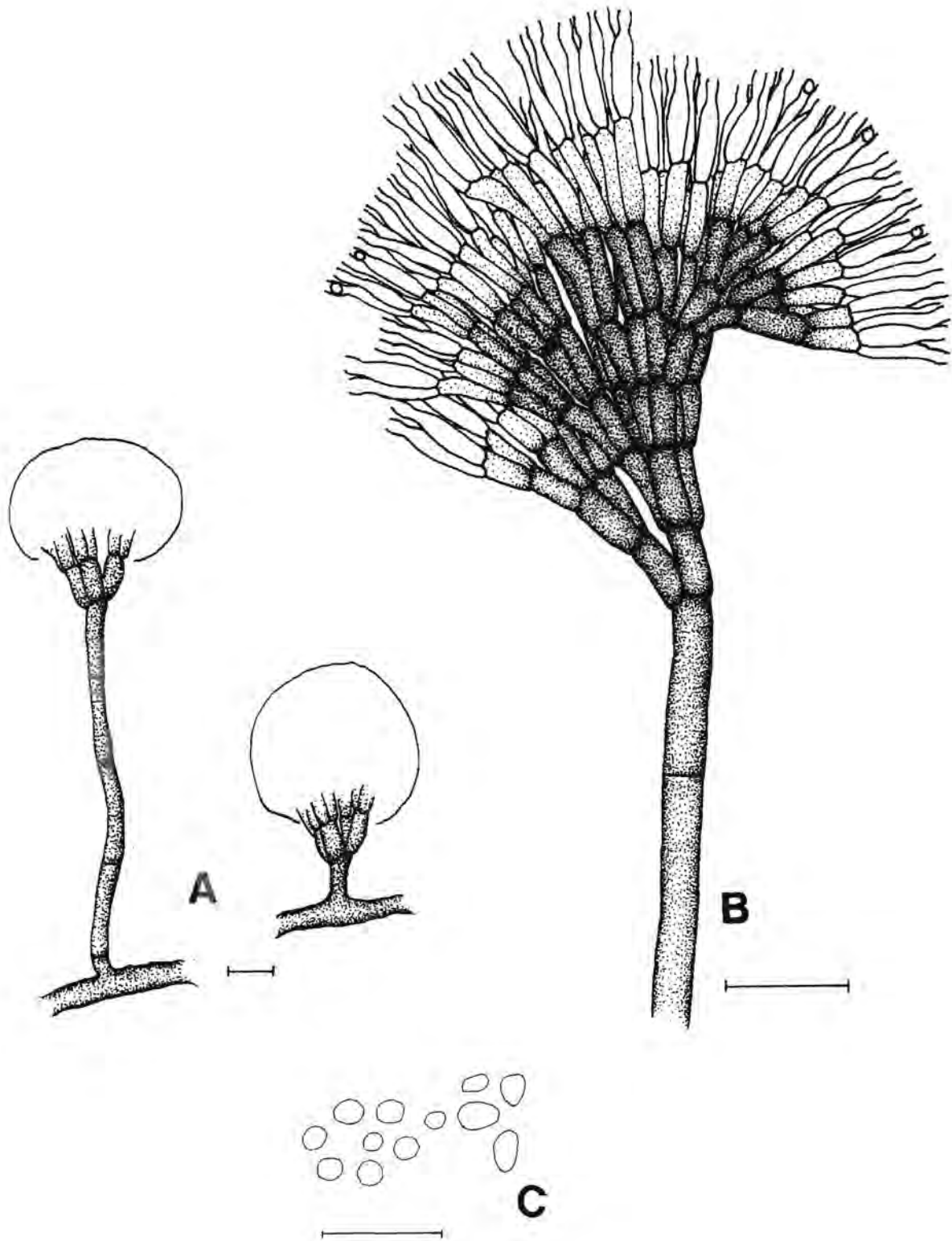
Fig. 7. Conidiophores and conidia of *P. fortinii* (FAP 7). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidia (Bar = 10 µm).

Phialocephala fusca Kendrick, *Canadian Journal of Botany* **41**, 1015. 1963. (**Figs. 8-9**)

Etymology: (fúsca). From the Latin noun <u>fuscus</u>: dark. This specific epithet refers to the dark colour of the colonies.

Distribution: Canada, Sweden and Sierra Leone.

Hosts: *Picea glauca, Pinus* spp., *Tsuga heterophylla, Acer, Bougainvillea spectabilis* (Kendrick, 1963).

Colonies with optimal growth at 25 °C on 2 % MEA, reaching 18 mm in diameter in 11 days. No growth below 15°C or above 30°C. Not able to withstand high concentrations of cycloheximide with a total cessation of growth on 0.5 g/l cycloheximide after 11 days at 25°C in the dark. Colonies, olive–brown (17'''k) in colour. Reverse of colony, fuscous black (13''''m). Concentric, zones of alternately lightly and heavily pigmented immersed hyphae. Margins very finely fibrillose, slightly diffuse. Immersed hyphae, sepia (17''m) in colour, 3 µm wide, sometimes exhibiting lateral outgrowths. Aerial mycelium, few or absent. **Conidiophores,** complex, numerous, absent towards margin, 27.5-257(-338) µm. Rhizoidal hyphae sometimes present. **Stipe,** mononematous 7.5-85(-300) µm in length, 3-7.5 µm wide at the base and 2.5-5 µm just below sporogenous apparatus, up to 7 septate, Brussels brown (15m) in colour becoming lighter colour near the apex. **Sporogenous apparatus,** fawn (13'''), 12.5-57(-100) µm in length. One to three series of branches. **Primary branches,** 2 in number, 5-12(-18) x 3-5 µm. **Secondary branches,** 5-11(-15) x 3-4 µm. **Phialides,** 5-19(-25) x 1-3(-3.5) µm, subparallel. **Collarettes,** very variable but conspicuous, cupulate with margin somewhat involute, but more usually loosely flaring and irregularly split. Occasionally peripheral phialides develop lateral outgrowths and produce one or more secondary phialides. **Conidia,** ellipsoid to ovoid, sometimes slightly curved, subhyaline to dilute fuscous, 3-5 x 3-4 µm.

Specimens examined: Holotype: Petawawa Forest Station, Chalk River, Ontario, Canada, October, 1959, collected: K. Shields, DAOM 75852. **Cultures:** DAOM 67995 (same as CBS 301.85 and CMW 172), Ontario, Canada, 1959, collected: W.B. Kendrick. DAOM 74598 (same as CBS 300.85, ATCC 60839 and CMW 520), Gatineau park, Canada, collected: W.B. Kendrick. CBS 238.74 (same as CMW 658), Stockholm, Sweden, 1971, collected: T. Nilsen.

Notes

Phialocephala fusca can be distinguished from all other *Phialocephala* spp. by its characteristic flaring collarettes, rhizodial hyphae and the formation of secondary phialides. *Phialocephala fusca* is similar to *P. canadensis* in that both form sterile outgrowths on the stipe, but in *P. fusca* these are

not as obvious as those in *P. canadensis*. Cultures of this species may deteriorate with long storage and the conidiogenous heads may be replaced by solitary phialides. Nothing is known regarding the ecology of this fungus.

Fig. 8. Light micrographs of conidiophores and conidia of *Phialocephala fusca* (CMW 172).
A. Conidiophore (Bar = 6 μm). **B.** Conidiogenous apparatus (Bar = 7 μm). **C.** Conidiogenous cell (Bar = 7 μm) (collarette indicated by arrow). **D.** Conidia (Bar = 7 μm).
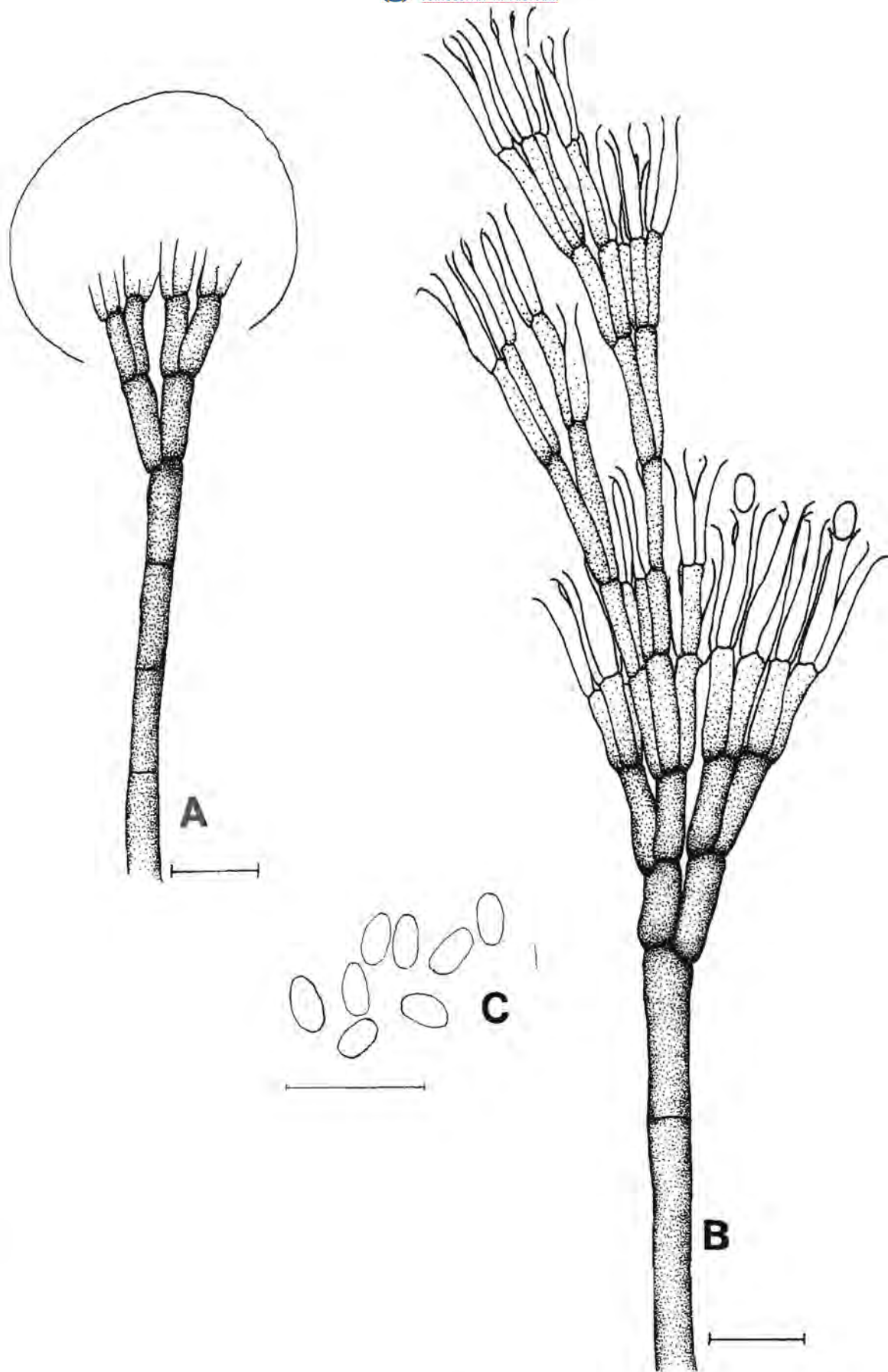
Fig. 9. Conidiophores and conidia of *P. fusca* (DAOM 75852). **A.** Habit sketch (Bar = 10 μm). **B.** Conidiogenous apparatus (Bar = 10 μm). **C.** Conidia (Bar = 10 μm).

Phialocephala humicola Jong & Davis *Mycologia,* **64**, 1353. 1972. **(Figs. 10-11)**

≡*Phialocephala gabalongii* Sivasithamparam *Transactions British Mycological Society* **64**. 336. 1975.

Etymology: (humicóla). From the Latin noun <u>humus</u>: soil and the Latin verb <u>solere</u>: to inhabit. This specific epithet refers to the origin of this species from soil.

Distribution: New Jersey, U.S.A. and Gabalong, western Australia (Jong & Davis, 1972; Sivasithamparam, 1975).

Substrate: Soil.

Hosts: *Triticum* (Sivasithamparam, 1975).

Colonies with optimal growth at 25 °C on 2 % MEA, reaching 18 mm in diam. in 11 days. No growth below 15°C or above 30°C. Able to withstand high concentrations of cycloheximide with a 60% reduction of growth, on 0.5 g/l cycloheximide, after 11 days at 25°C, in the dark. Colonies, deep mouse gray (15'''''i) to dark mouse gray (15'''''k) aerial mycelium. Reverse of colony fuscous black (13''''m) in colour. **Hyphae,** irregularly, 3-5 µm wide. **Conidiophores,** macronematous, arising singly or in small groups from hyphae, 92-340(-450) µm. **Stipe,** cylindrical, 5-12 septate, 125-196(-200) µm x 8 µm. Sepia (17''m) at base becoming paler towards the apex. One to three series of branches, distal series bearing phialides. **Primary branches,** fawn (13'''), 2 to 3 in number, 3-9.5(-10) x 2.5-3-5 µm. **Secondary branches,** 5-7 x 2.0-2.5µm, **tertiary branches,** 5-7 x 2.0-2.5µm. **Phialides,** hyaline, 4-8(-10) x 1-1.5µm and with inconspicuous openings. **Conidia,** ellipsoid, hyaline, (1.8-)3-4 x 1.5-2µm.

Specimens examined: Holotype: *P. gabalongii*, Australia, July 1973, collected: K. Sivasithamparam, IMI 177255. *Phialocephala humicola*, Cape May, New Jersey, U.S.A., 1971, ATCC 22801. **Cultures:** ATCC 38881 (same as CBS 302.85 and CMW 510), Alabama, U.S.A., 1987, collected: Morgan-Jones. ATCC 22801 (same as CBS 420.73, IMI 173641 and CMW 659), New Jersey, U.S.A., 1971.

Notes

There are some similarities in conidial shape between *P. humicola* and *P. virens*. Both these species have ellipsoid conidia. *Phialocephala humicola* and *P. virens* differ from all other *Phialocephala* spp. in the inconspicuous nature of their collarettes. *Phialocephala humicola* and *P. gabalongii* both occur in the soil environment and are generally recognized as synonyms.

Phialocephala humicola, P. gabalongii and *P. dimorphospora*, are the only species isolated from soil, all other species were isolated from woody tissue.

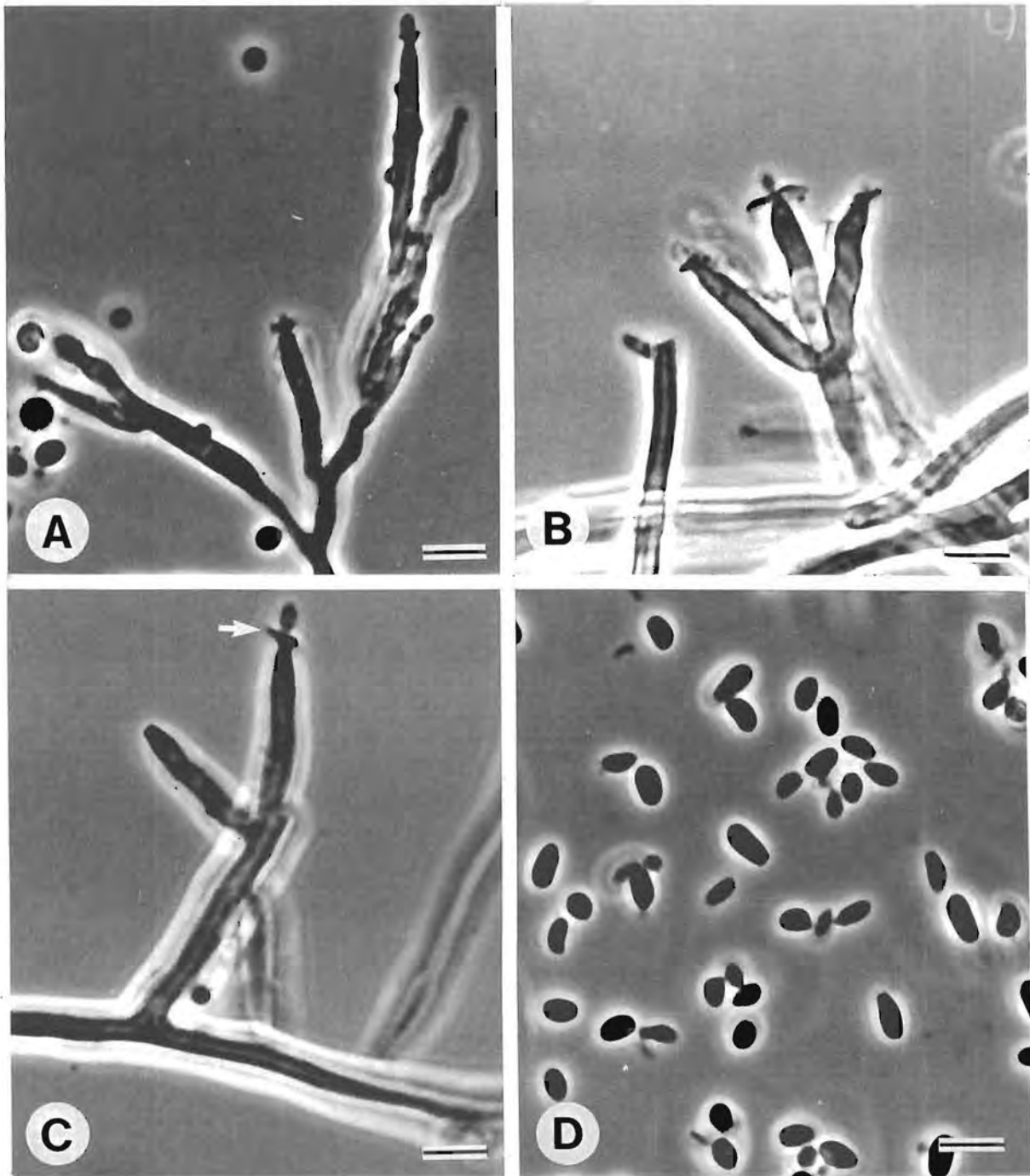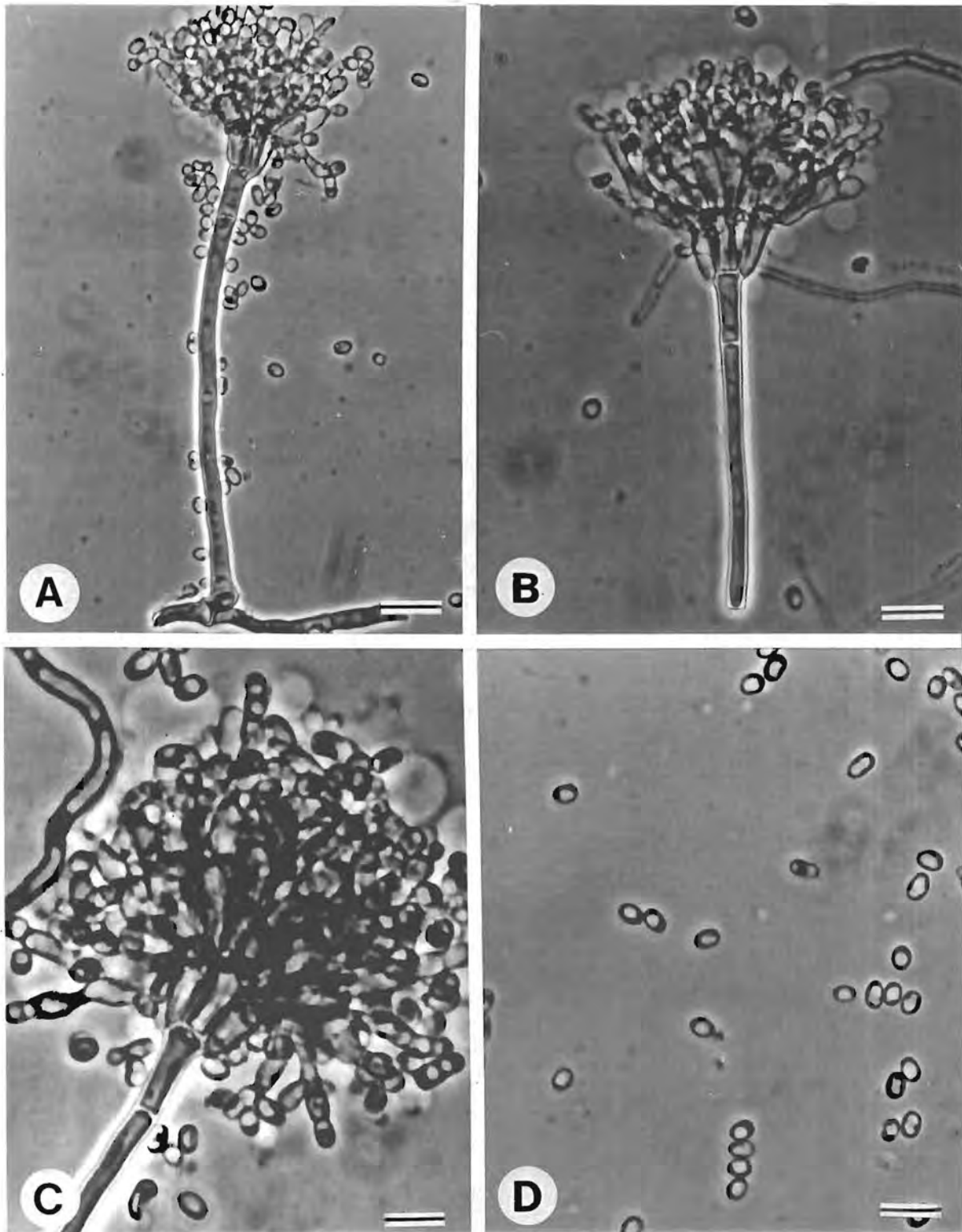**Fig. 10**. Light micrographs of conidiophores and conidia of *Phialocephala humicola* (CMW 170). **A.** Conidiophore (Bar =14 µm). **B.** Conidiogenous apparatus (Bar = 7 µm). **C.** Conidiogenous cell (Bar = 7 µm). **D.** Conidia (Bar = 4 µm).
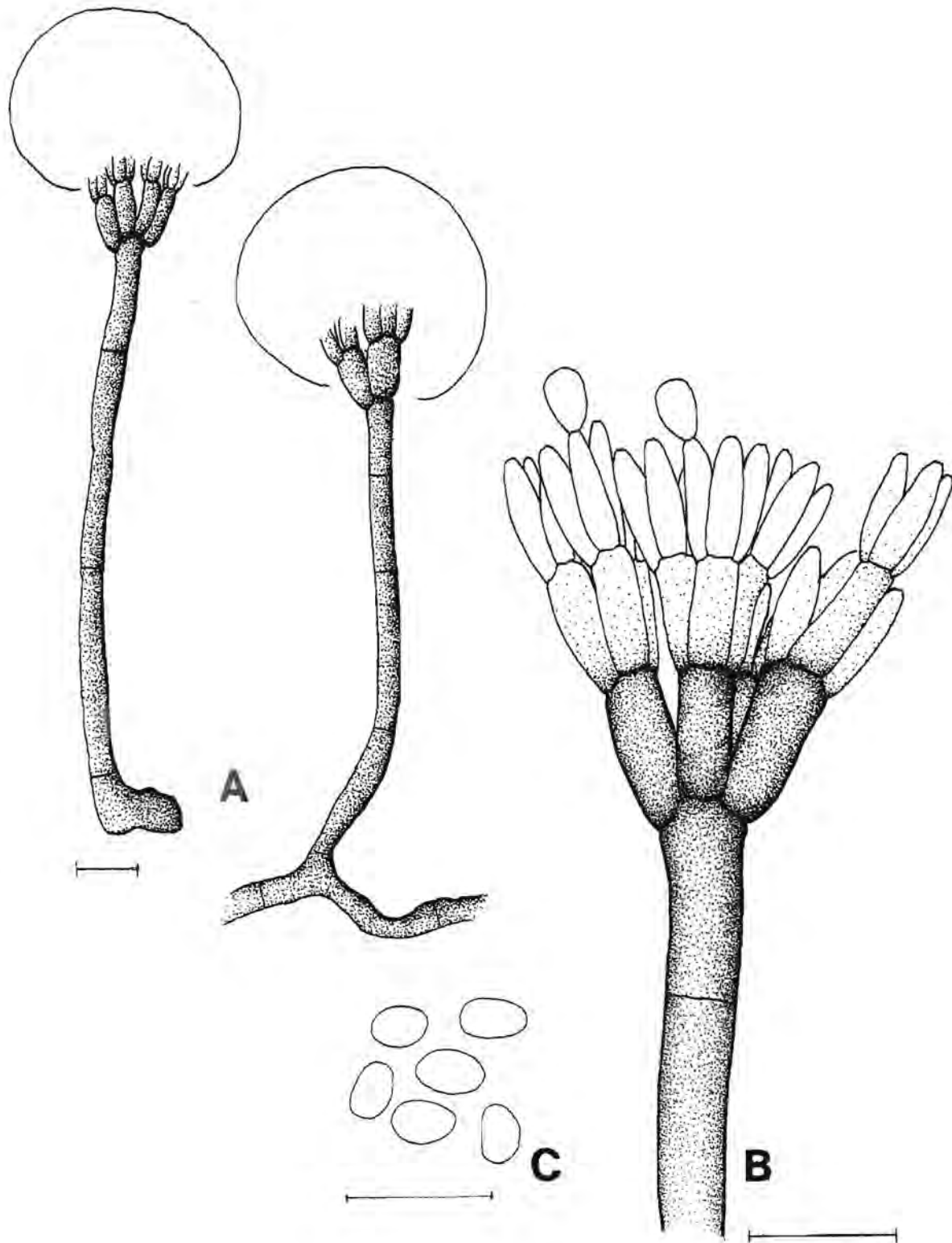
Fig. 11. Conidiophores and conidia of *P. humicola* (ATCC 22801). **A.** Habit sketch (Bar = 10 μm). **B.** Conidiogenous apparatus (Bar = 10 μm). **C.** Conidia (Bar = 10 μm).

Phialocephala repens (Cooke and Ellis) Kendrick, *Canadian Journal of Botany* **41**, 574. 1963.
(Figs. 12)

≡*Penicillium repens* Cooke and Ellis *Grevillea* 7, 6. 1878.

Etymology (repéns). The specific epithet indicates the creeping, prostrate nature of this species in culture.

Distribution New Jersey, U.S.A. (Kendrick, 1963).

Hosts *Magnolia* and *Populus* (Kendrick, 1963).

Colonies with optimal growth at 25°C on 2% MEA, reaching 12 mm in diameter in 11 days. No growth below 5°C or above 30°C. Not able to withstand high concentrations of cycloheximide with total accesion of growth on 0.5 g/l cycloheximide, after 11 days at 25°C, in the dark. Colonies, thick floccose mycelium, chaetura drab (17''''m) aerial mycelium, except margins. Aerial mycelium, lustrous, sepia (17''m) hyphae, 2.6-5.4 µm wide, regularly septate. Reverse of colony, fuscous black (13''''m). Hyphae produce side branches, growing at right angles to main stipe, further branching also observed. **Conidiophores**, 25-92(-100) µm, scattered throughout aerial mycelium developing as short, fertile, side branches on aerial hyphae. **Stipe**, mononematous, sepia (17''m), relatively thin, 10-62(-70) µm long, 2.2-4.4 µm thick at base and 2-4 µm thick near apex. One septa in shorter conidiophores, longer conidiophores develop extra septa. **Sporogenous apparatus**, 15-45(-54) µm long, consisting of 2-3 series of branches ultimately bearing a cluster of phialides. **Primary branches**, 2-3 in number, 7-13(-15) x 2-6(-8) µm. **Secondary and tertiary branches,** shorter and lighter pigmented than primary branches. **Phialides**, fan-shaped in shape. Main stipe axis recognized by successive series of branches. **Collarettes**, short flaring, 8-15 x 1-4 µm. **Conidia** hyaline, globose, 1.5-2 µm.

Specimens examined: Holotype: Ontario, October 1960, collected: G.L. Hennebert, DAOM 74357. **Cultures:** DAOM 74357 (same as MUCL 1849 and CMW 5539), 1960, collected: G.L. Hennebert.

Notes

Phialocephala repens is most similar to *P. fusca* in collarette morphology and in the formation of only one type of conidium. It differs from *P. dimorphospora* in the absence of a long collarette needed to accommodate the elongated conidium, typically formed by *P. dimorphospora*. Colonies of *P. repens* are characterized by a thick weft of aerial mycelia, which is absent in colonies of *P. dimorphospora*. *Phialocephala repens* is similar to *P. canadensis*, *P. fluminis* and *P. fusca* in the formation of only one type of conidium. These conidia are globose in shape, in contrast to those of

P. canadensis that are transversely ellipsoid and those of *P. fluminis* that are obovate. The conidia of *P. fusca* are ellipsoid in shape. Nothing is known about the ecology of *P. repens*.
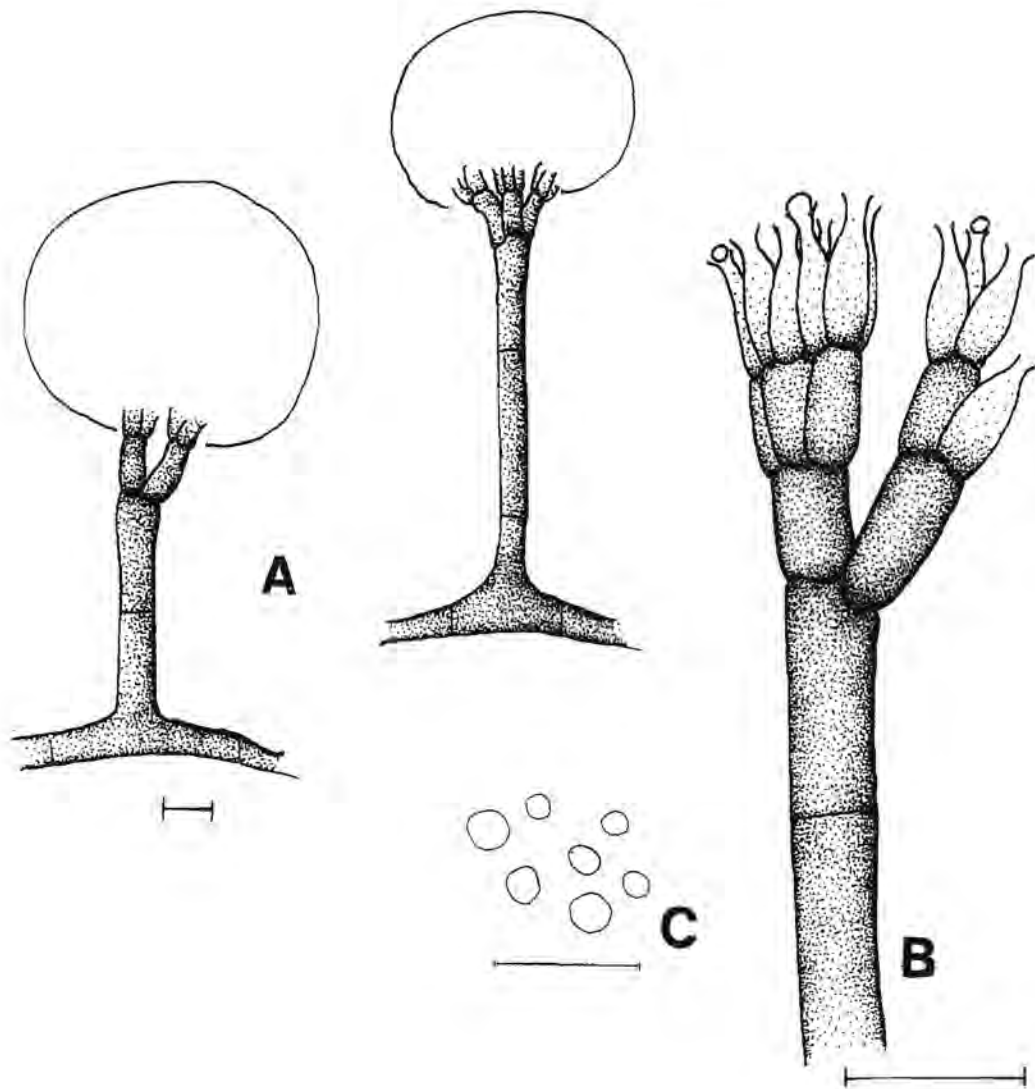
Fig. 12. Conidiophores and conidia of *P. repens* (DAOM 74357). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidia (Bar = 10 µm).

Phialocephala scopiformis Kowalski & Kehr, *Canadian Journal of Botany* **73**, 27. 1995. **(Fig. 13**)

Etymology (scopifórmis). The specific epithet refers to the 'brush-like' appearance of the culture.

Distribution Regenberg, Germany (Kowalski & Kehr, 1995).

Hosts *Larix*, *Abies* and *Picea* species (Kowalski & Kehr, 1995).

Colonies woolly aerial mycelium, near colony center, composed of olive-brown (17'''k) hyphae, 2.5- 4.5 μm wide, smooth to rough or ornamented. Black (1*) in colour on reverse of colony. Aerial mycelium near center forming hyphal fascicles, sparse towards colony margin. Colony surface with a slight metallic gleam. Yellow crystalline concentric rings on colony surface. **Conidiophores** macronematous, mononematous, concolorous with aerial mycelium, multiseptate, 20-280 x 2.5-3.0 μm. **Sporogenous apparatus,** 45-150 x 25-125 μm. One to fifteen series of branches. **Primary branches** concolorous with conidiophore becoming lighter ending in hyaline phialides. **Primary and secondary branches**, 7.5-25 x 2.5-3 μm. Branches of first few series, divergent, forming separate conidiogenous heads. **Phialides**, 17-21 x 3 μm. **Collarettes** cilindrical, conspicuous, hyaline. **First conidia** hyaline, one or two celled, ovoid to ellipsoid, 5-8 x 2-2.5 μm. **Subsequent conidia**, present in older cultures, subglobose in shape, 2-2.5 x 2-2.5 μm, with droplet (Kowalski & Kehr, 1995).

Specimens examined: Holotype: Regensburg, Germany, September 1990, IMI 363204. **Cultures:** CBS 468.94 (same as CMW 4947, IMI 363204 and ATCC 96753), Regensberg, Germany, September 1990.

Notes

Phialocephala scopiformis sporulates at room temperature, but storage at 4°C in the dark, enhances sporulation (Kowalski & Kehr, 1995). This species is characterized by exceptionally complex conidiogenous heads. Two-celled conidia are observed only in older cultures. Two types of conidia are present, similar to those of *P. dimorphospora* and *P. fortinii*. The first conidia of *P. scopiformis* are obovoid to ellipsoid and the second and subsequent conidia are sub-globose in shape. This is in contrast to the obovoid first and sub-globose to globose second and subsequent conidia of *P. fortinii*. This species can be distinguished from *P. dimorphospora* with its ovoid first and spherical subsequent conidia.

Fig. 13. Conidiophores and conidia of *P. scopiformis* (IMI 363204). **A.** Habit sketch (Bar = 10 μm). **B.** Conidiogenous apparatus (Bar = 10 μm). **C.** Conidia (Bar = 10 μm).

Phialocephala trigonospora Kirschner & Oberwinkler *Sydowia* **50**, 207. 1998. (**Fig. 14**)

Etymology (trigónospora) From the Greek adjetives τριγονοζ : triangular and σπορα: spore. This specific epithet refers to the triangular shape of the conidia.

Distribution Germany (Kirschner & Oberwinkler, 1998)

Hosts *Pinus sylvestris* and *Picea abies* (Kirschner & Oberwinkler, 1998)

Colonies cream (19'f) in colour. Aerial mycelium poorly developed. Immersed hyphae septate, hyaline, 1 μm wide. **Conidiophores** macronematous, single, erect, including condiogenous apparatus 70-131(-185) μm long. **Stipe,** 5-9 μm wide at base, 3-5 μm thick below conidiogenous apparatus, 2-6 septate; sepia (17''m) in colour. **Sporogenous apparatus,** 12.9-3(-37) μm complex, sepia (17''m) to hyaline in colour. Two to three series of branches, distal series bearing phialides. **Primary branches,** 10-21x2-4 μm. **Secondary branches,** 8-12 μm long. **Tertiary branches,** 7-9x1.5-2 μm. **Phialides,** hyaline, with deep collarettes, 8-15 x 1-1.5 μm. **Conidia,** triangular, smooth, hyaline (Oberwinkler & Kirschner, 1998)

Specimens examined: Holotype: Odenwald, Rohrbach, Germany, September 1994, collected: R. Kirschner. CBS (CBS 100161). **Cultures:** CMW 5591, Germany, September 1994, collected: R. Kirschner.

Notes

Phialocephala trigonospora differ from all species in that the conidia are triangular in shape. It is also the only *Phialocephala* sp. that has been isolated from bark-beetle tunnels and occur on *Pinus sylvestris* and *Picea abies*. Furthermore, *P. trigonospora*, is the only species with cream coloured colonies, all the other *Phialocephala* spp. have dark colonies.
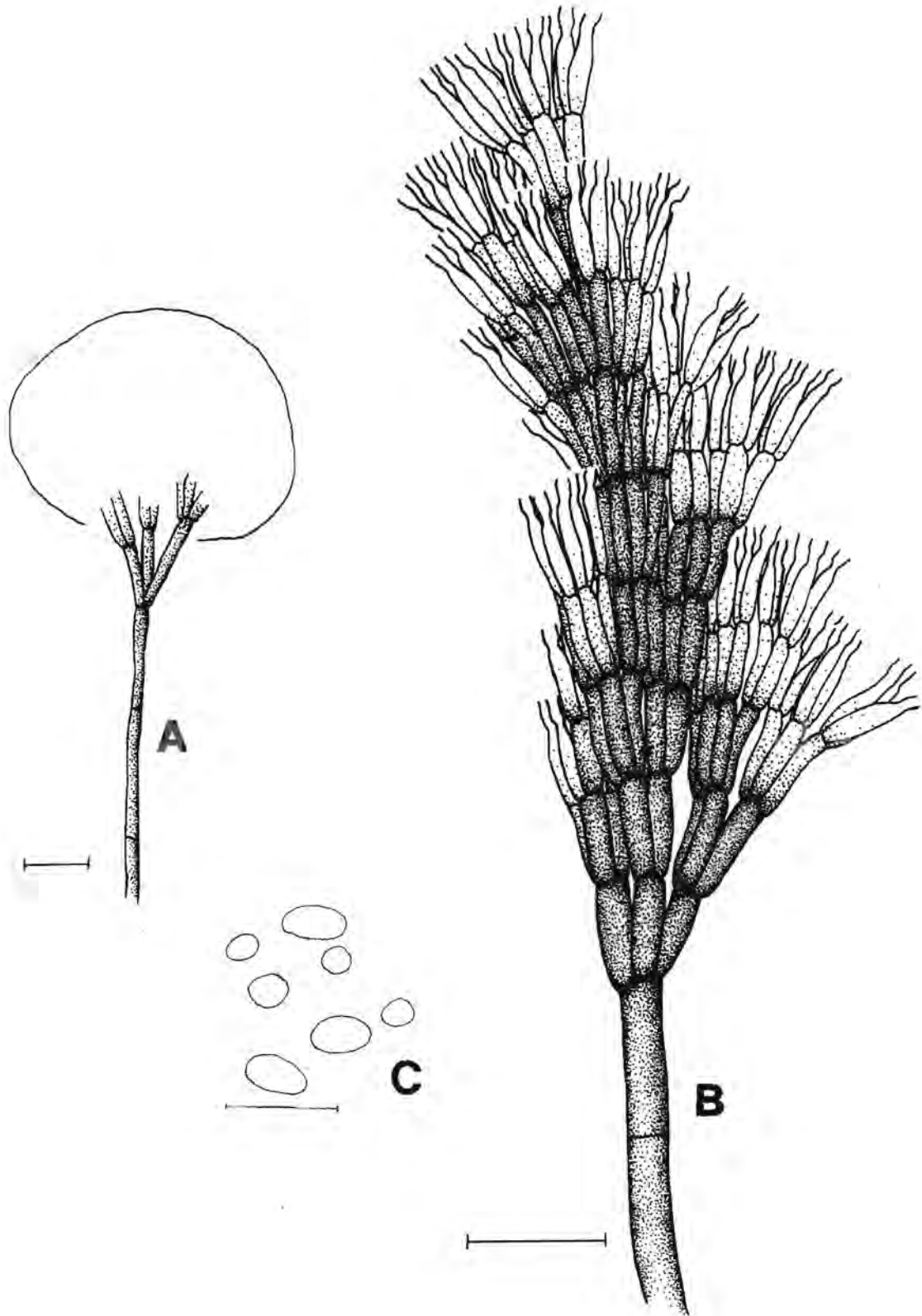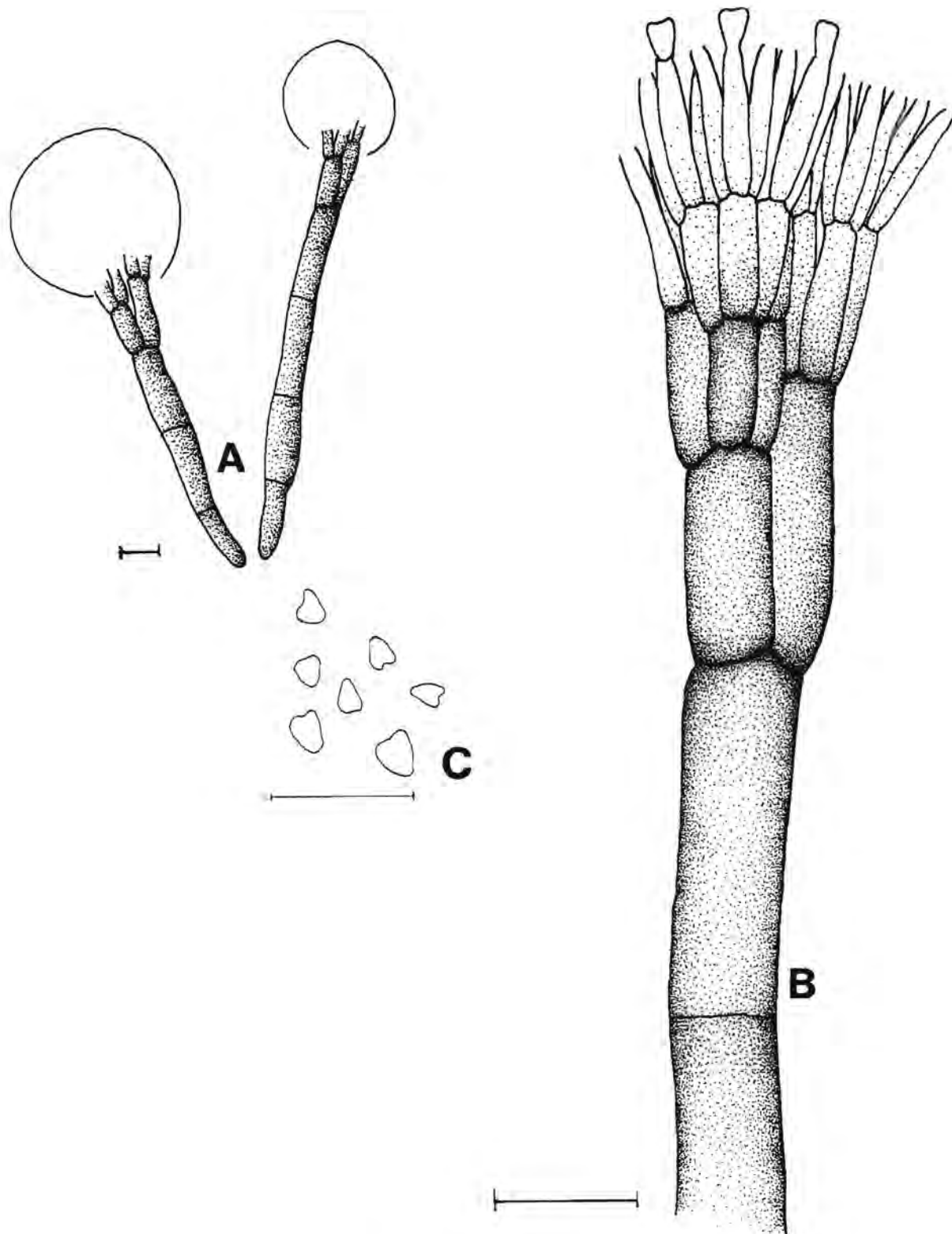
Fig. 14. Conidiophores and conidia of *P. trigonospora* (CBS 100161). **A.** Habit sketch (Bar = 10μm). **B.** Conidiogenous apparatus (Bar = 10 μm). **C.** Conidia (Bar = 10 μm).

Phialocephala virens Siegfried & Seifert *Canadian Journal of Botany* 70, 2485. 1992. (**Fig. 15-16**)

Etymology (virén̅s) The specific epithet refers to the green colour of the fungus and its ability to cause green stain in the sapwood of a variety of sapwood species.

Distribution Finland, Alaska and Canada (Siegfried *et al.*, 1992)

Hosts Isolated from *Tsuga heterophylla* and *Picea* sp (Siegfried *et al.*, 1992).

Colonies on 2 % MEA, reaching 14 mm in diameter in 14 days. Green (35b) colour, appearing greyish green (21'''') in centre, surrounded by mouse grey(15'''''), greyish blue-green (37'''') and eye blue (43''b) concentric zones. Margin greyish violet (51''b) in colour. Aerial mycelium, green (35b), sparsely cottony to funiculose. Submerged green in colour. Sparse drops of a dark exudate present in older cultures. Reverse of colony eye blue (43''b) centre, dark blue (47m) and dark green (35'm) margin. **Conidiophores**, complex, near margin directly from surface, towards centre from aerial mycelium or funicles, singly or in groups. Two to three conidiophores form rudimentary synnemata. Mycelium, smooth, irregular, septate and subhyaline. **Hyphae,** submerged, 1-4 μm in diameter. **Stipe**, stout, mononematous, subhyliane, generally aseptate, 9-30 x 3-5 μm. **Sporogenous apparatus**, 10-75 x 8-50μm. Conidiophores, monoverticillate. Conidiophore branching bi- to quarterverticillate, sometimes monoverticillate, 3-5.5 μm wide. **Primary branches**, hyaline, 2 to 4 in number, 3-8 x 1.5-3.5 μm. **Subsequent branches**, 6.5-11.5 x 1.5-3μm. **Phialides**, 5-11 x 1.5-3 μm, hyaline, slightly flask-shaped to subulate. Intercalary phialides with apical, lateral conidiogenous extensions, 5 μm long. **Collarettes**, inconspicious or cylindrical to slightly flaring, hyaline. Solitary phialides on mycelium, 10-14 x 2-2.5 μm. **Conidia,** 1.5-3 x 1.6-2 μm, ellipsoidal to oblong-ellipsoid or obovoid in shape, hyaline and smooth (Siegfried *et al.*, 1992).

Specimens examined: Holotype: U.S.A., Alaska, Kenai Peninsula, 1988, collected: K. Reynolds, DAOM 214746. **Cultures:** CBS 504.61 (same as CMW 5591), Italy, March 1961, collected: W. Gams. CBS 365.76 (same as CMW 5592), Finland, July 1976, collected: W. Gams.

Notes

Phialocephala virens differs from other *Phialocephala* spp. in colony colour. Some colonies of *P. virens* is greyish green to blue-green compared to most other species which are greyish-black in colour. The conidiophores, phialides and conidia of *P. virens* are shorter and more compact than in *P. dimorphospora*. This species is also the only *Phialocephala* sp. where phialides occur on primary, secondary and tertiary branches on a single conidiophore. *P. virens* is characterized by solitary phialides forming directly on the mycelium as well as the formation of synnemata. Little is

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known about the ecology of this species except that *P. virens* causes green stain in the sapwood of timber.

Fig. 15. Light micrographs of conidiophores and conidia of *Phialocephala virens* (CMW 5591). **A.** Conidiophore (Bar = 6 μm). **B.** Conidiogenous apparatus (Bar = 7 μm). **C.** Conidiogenous cell (Bar = 7 μm) (collarette indicated by arrow). **D.** Conidia (Bar = 4 μm).

Fig. 16. Conidiophores and conidia of *P. virens* (DAOM 214746). **A.** Habit sketch (Bar = 10 μm). **B.** Conidiogenous apparatus (Bar = 10 μm). **C.** Conidia (Bar = 10 μm).

Phialocephala xalapensis Persiani & Maggi, *Mycotaxon* **20**, 253. 1984. (**Fig. 17-18**)

Etymology: The specific epithet indicates the region in Mexico, where the species were first isolated, namely Xalapa-Veracrux.

Distribution: Xalapa-Veracrux, Mexico (Maggi & Persiani, 1984)

Hosts: Isolated from *Coffeae arabinae* L. (Maggi & Persiani, 1984)

Colonies with optimal growth at 25 °C on 2 % MEA, reaching 16 mm in diameter in 11 days. No growth below 5 °C or above 35 °C. Able to withstand high concentrations of cycloheximide with a 97% reduction of growth on 0.5 g/l cycloheximide, after 11 days at 25°C, in the dark. Colonies, effuse Brussels-brown (15m) to fuscous black (13''''m) in colour, Brussels-brown (15m) in colour on reverse of colony. Hyphae branched, septate, subhyaline, smooth, 1.8-2.7µm wide. **Conidiophores** macronematous, mononematous, solitary, erect, 5-39(-48) µm. **Stipe** straight, 700 µm long, 5-5.5 µm wide, 4-7 septate; Brussels-brown (15m) in colour becoming paler near apex. Lobed at the base or with a true foot cell. **Sporogenous apparatus** complex, obconical; 12-22(-26) µm in length. Two to three series of branches, distal series bearing phialides. Occasionally stipe bears lateral branch on which branches and phialides in apical apex of the conidiogenous head are inserted. **Primary branches,** 2 to 4 in number, concolorous with stipe, 4-13(-18) µm x 1-2 µm. **Secondary branches**, 2-4 in number, 7.2-9 x 2.5-3 µm, **tertiary branches**, 2-4 in number, 6-7.5 x 2.5-3 µm. **Phialides** conspicuous, subhyaline, sub-cylindrcal, 5-13(-15) x 1-2 µm. **Collarettes** defined, short. **Conidia** oblong with rounded apices, fawn (13'''), 2-3(-4) µm x 2 µm.

Specimens examined: Holotype: Could not be obtained from herbarium. **Cultures:** CBS 218.86 (same as CMW 5594), India, 1985, collected: V. Rao. CBS 390.71(same as CMW 5595), Sweden, 1971, collected: T. Nilsson.

Notes

Phialocephala xalapensis forms only one type of conidium. This is similar to *P. canadensis, P. fluminis, P. repens* and *P. fusca.* These conidia, oblong in shape, differ from the transversely ellipsoid, obovate, globose, and ellipsoid conidia of *P. canadensis, P. fluminis, P. repens* and *P. fusca*, respectively. No sterile outgrowths have been observed in *P. xalapensis* as in the case of *P. fusca, P. canadensis* and *P. fluminis.* Furthermore, the length of the stipe and the presence of a true foot cell separate *P. xalapensis* of all other *Phialocephala* species. Nothing is known about the ecology or pathogenicity of this fungus.
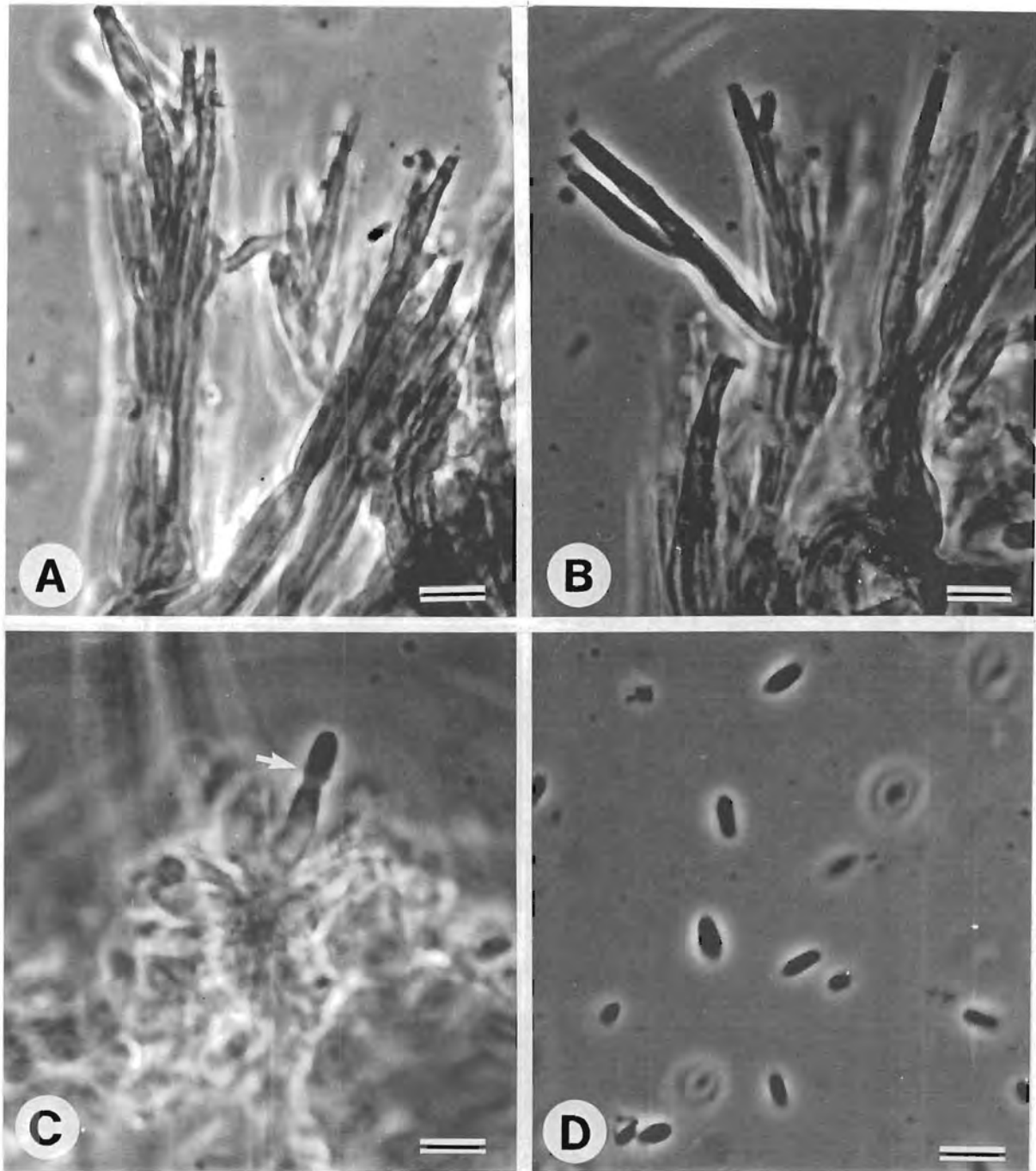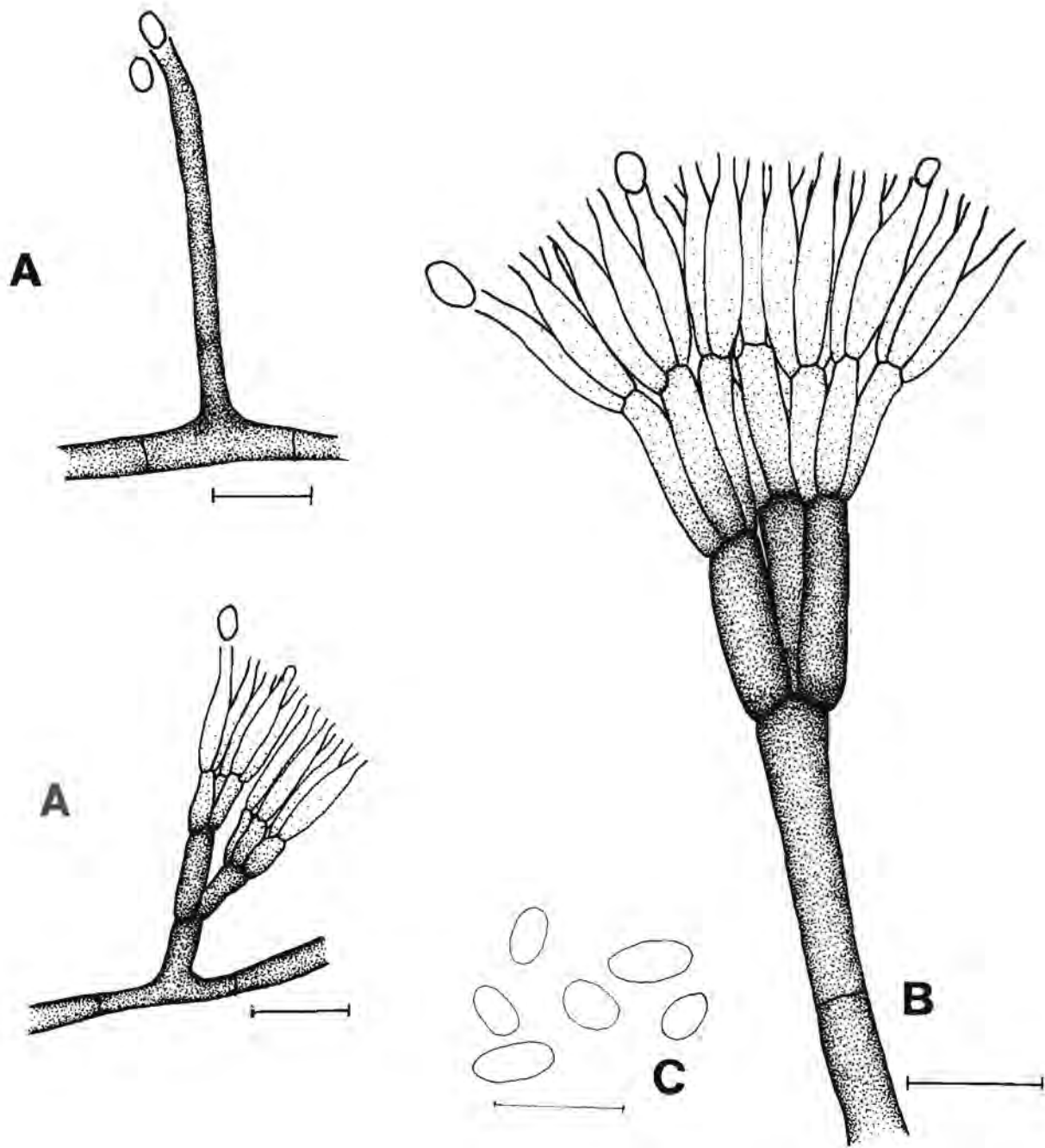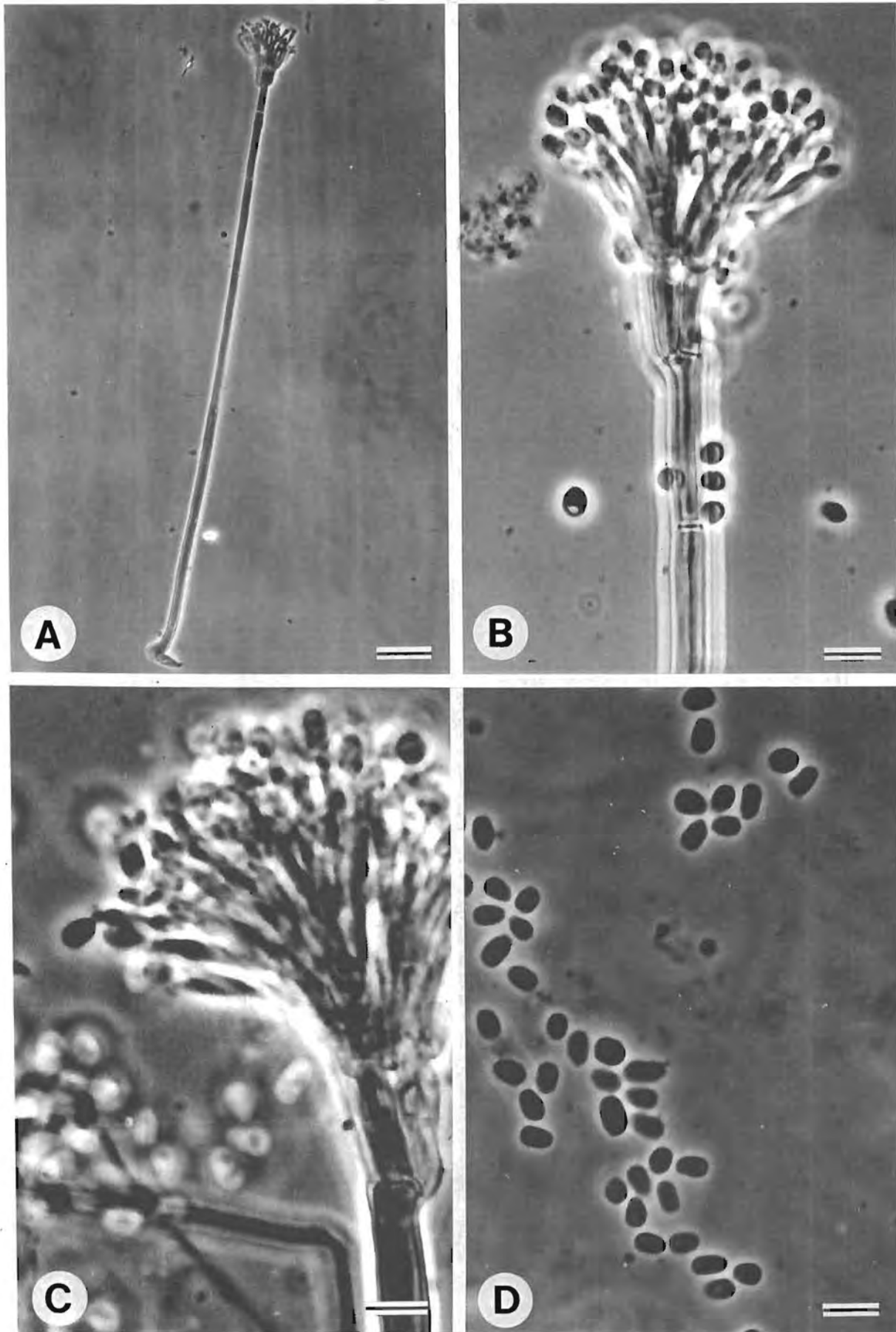
Fig. 17. Light micrographs of conidiophores and conidia of *Phialocephala xalapensis* (CMW 5594). **A.** Conidiophore (Bar = 35 μm). **B.** Conidiogenous apparatus (Bar = 10 μm). **C.** Conidiogenous cell (Bar = 6 μm). **D.** Conidia (Bar = 6 μm).
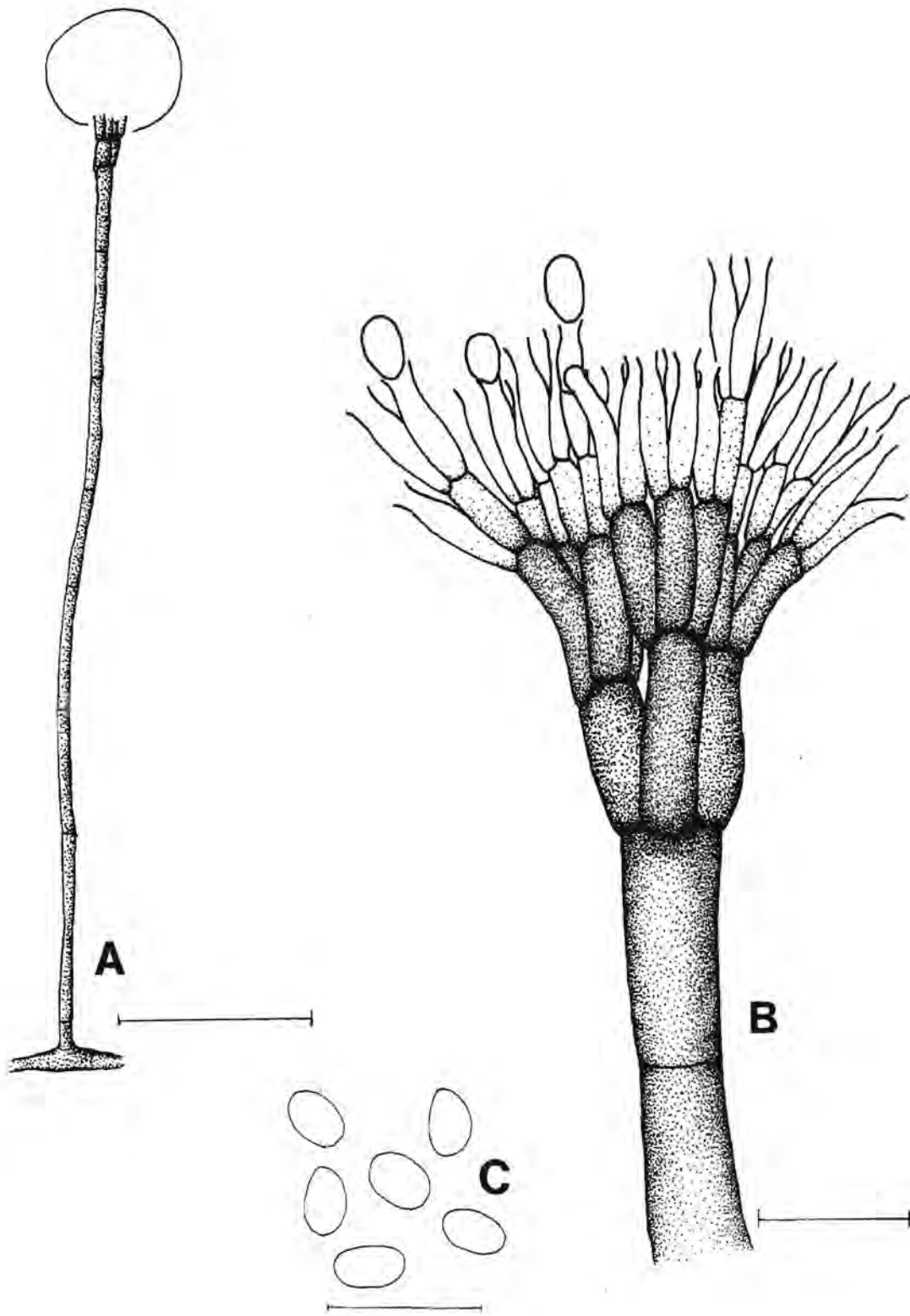
Fig. 18. Conidiophores and conidia of *P. xalapensis* (CBS 468.94). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidia (Bar = 10 µm).

Excluded species

The following species are excluded, due to the lack of sufficient material for examination.

Phialocephala mexicana Onofri & Zucconi *Mycotaxon* **20, 185-195, 1984**

Phialocephala mexicana was isolated from *Coffea arabica* (Onofri & Zucconi). It is similar to *P. virens* in forming solitary phialides and only primary conidia. The conidia of *P. mexicana* are subacerose often slightly curved or sigmoid, (Onofri & Zucconi, 1984), while those of *P. virens* are ellipsoidal to oblong-ellipsoid or obovoid. Herbarium material of this species was lost.

Phialocephala queenslandica Matsushima *Mycological Memoirs* **1, 112, 1980**

Phialocephala queenslandica was isolated from a soil sample, originating from Queensland, Australia. This species is characterized by obolong-ellipsoid conidia, similar to *P. mexicana* (Matsushima, 1980). Herbarium material of this species was lost.

Phialocephala victorinii Vijanovic and St.-Arnaud *Mycologia* **92, 571-576, 2000**

Phialocephala victorinii is an endophyte isolated from living roots of the terrestrial orchid, *Cypripedium parviflorum*. Sterile outgrowths are present on the hyphae and not on the conidiophores, as is the case with other *Phialocephala* species. Two kinds of conidia are observed, obovoid and allantoid in shape (Vujanvic *et al.*, 2000). This species was described after the completion of the current work and could not be included.

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Appendix A

CMW refers to the culture collection of the Tree Pathology Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

IFO refers to the culture collection of the Institute for Fermentation, Osaka, Japan.

CBS refers to the culture collection of the Centraalbureau voor Schimmelcultures, Baarn & Delft, The Netherlands.

ATCC refers to the culture collection of the American Type Culture Collection, U.S.A.

MUCL refers to the culture collection of the Belgian Co-ordinated Collections of Micro-organisms, Brussel, Belgium.

IMI refers to the herbarium of the International Mycological Institute, CABI Bioscience U.K Centre, Egham, U.K.

DAOM refers to the National Mycological Herbarium, Ottawa, Canada.

HBR refers to the Herbario "Barbosa Rodrigues", Santa Catarina, Brasil.

Taxonomic re-evaluation of *Phialocephala humicola*

Phialocephala was established for species belonging to the *Leptographium* complex that produce their conidia in phialides at the apices of dark mononematous conidiophores. *Leptographium* spp. are characterised by producing mitospores via percurrent proliferation of conidiogenous cells. *Phialocephala* species are found in a wide range of ecological niches including soil and decayed wood. In contrast *Leptographium* species are best known as associates of insects that infest conifers. *Phialocephala humicola* was first isolated from a soil sample collected in Alabama, U.S.A.. In this study, the taxonomy of *P. humicola* is reconsidered based on morphological charateristics, molecular data and physiology. *Phialocephala humicola* was found to have distinct percurrent proliferation of conidiogenous cells which is typical of *Leptographium* spp. However, comparison based on the fungus' tolerance to high concentrations of cycloheximide, indicated no relationship between this species and *Leptographium* spp. Analysis of the sequence data from the 28S rRNA operon also showed that this fungus is related to the Ophiostomatales. The fungus would best be accommodated in *Leptographium* or a new genus closely related to it.

Keywords: *Leptographium reconditum, Phialocephala humicola*, phylogeny, morphology

Phialocephala Kendrick together with *Leptographium* Lundberg & Melin, forms part of the *Leptographium*-complex, which originally also included *Verticicladiella* Hughes. *Leptographium* species and species previously accommodated in *Verticicladiella* are indistinguishable and the genera were synonymised (Wingfield, 1985). *Phialocephala* is distinguished from *Leptographium* and *Verticicladiella* based on differences in conidium development (Kendrick, 1961, 1962). *Phialocephala* is broadly characterised by conidia that develop in phialidic conidiogenous cells. This is in contrast to the annellidic development of conidia in *Leptographium* (Kendrick, 1961; 1962). *Phialocephala* spp. are characterised by having dark mononematous conidiophores that branch occasionally at their apices (Kendrick, 1961). Some species are characterised by the solitary phialides that can form directly on the mycelium (Onofri & Zucconi, 1984). Hyaline ameroconidia accumulate in slimy masses around the sporogenous heads (Kendrick, 1961; 1962).

Leptographium and *Phialocephala* spp. can be distinguished based on their tolerance to the antibiotic, cycloheximide. *Phialocephala* spp. are sensitive to low concentrations of cycloheximide, while *Leptographium* spp. are able to tolerate high concentrations of this antibiotic (Harrington, 1988). No teleomorphs have been reported for *Phialocephala* spp. although a connection to Leotiales has been proposed (Currah, Tsuneda & Murakami, 1993). This is in contrast to *Leptographium* spp. that are known anamorphs of *Ophiostoma* (Wingfield, 1993; Harrington, 1987).

Phialocephala spp. occupy a diverse range of ecological niches. They include weak pathogens as well as saprotrophs and endophytes of trees (Wang & Wilcox, 1985; Currah *et al.*, 1993; Kowalski & Kehr, 1995). This distinguishes them from *Leptographium* spp. that are mainly associated with insects that infest conifers (Harrington, 1988). No *Phialocephala* sp. has been associated with disease, although *P. virens* Siegfried & Siefert has been isolated from root rot symptoms (Siegfried, Siefert & Bilmar, 1992). This is in contrast to *Leptographium* spp. that are well-known agents of blue-stain of timber (Siefert, Webber & Wingfield, 1993) while others are important pathogens (Harrington & Cobb, 1987).

Phialocephala humicola Jong & Davis (1972) is one of several *Phialocephala* spp. that has been isolated from soil. Others include the type species, *P. dimorphospora* Kendrick and *P. queenslandica* Matsushima (Kendrick, 1963; Barron, 1968; Matsushima, 1980). Based on morphological characters such as colony colour, conidiophore shape, number of primary branches, conidial diameters and inconspicuous collarettes, Jong & Davis (1975) synonymised *P. humicola* with *P. gabalongii* Sivasithamparam. The latter species was isolated from the roots of *Triticum* collected from Gabalong, Western Australia (Sivasithamparam, 1975). Onofri, Pagano and Zucconi

(1994), showed through light and scanning electron microscopy studies that *P. humicola* has phialidic conidiogenesis. They also found that *P. humicola* displays the formation of a conidial 'crown', which is formed when the conidia from the same conidiogenous cell remain attached around its apex.

Leptographium reconditum was described by Jooste (1978) as the only *Leptographium* sp. isolated from the roots of *Triticum*. This is an unusual habitat for *Leptographium* spp. that are usually associated with conifers and living woody tissue (Lagerberg, Lundberg & Melin, 1927; Harrington, 1988). The association of *L. reconditum* with roots of wheat makes the fungus similar to *P. gabalongii*, also known from this niche.

Previous studies have shown that *Phialocephala* is taxonomically heterogenous (Wingfield, van Wyk & Wingfield, 1987). Some species such as *P. bactrospora* with ring wall building conidial development (Minter, Kirk & Sutton, 1983) have already been transferred to *Sporendocladia* Arnaud: Nag Raj & Kendr. (Wingfield *et al.*, 1987), but remaining species display diverse morphological characters and ecological habitats. The aim of this study was to re-evaluate the taxonomic placement of *P. humicola* based on a variety of characteristics including DNA sequence comparisons.

MATERIALS AND METHODS
Morphology and growth in culture

Cultures: Material for examination included the herbarium type specimens of *P. dimorphospora* Kendrick (ATCC 38881), *L. lundbergii* Lagerberg & Melin (PREM 50548), *L. reconditum* Jooste (PREM 45016), *P. humicola* Jong & Davis (ATCC 22801) and *P. gabalongii* Sivasithamparam (ATCC 22801). Also included were living isolates of *L. reconditum* (CMW 15), *P. humicola* (CMW170, 1618), *L. lundbergii*, (CMW 30) and *P. dimorphospora*, (CMW 168, 508). These isolates were used in all the morphological comparisons, growth in culture and cycloheximide tolerance studies.

Light microscopy: Fungal structures produced on 2% Malt extract agar (MEA, 20 g NT Merck malt extract, 20 g Biolab agar and 1000 ml distilled water) were used for the morphological comparison. For light microscopy, relevant structures from the cultures, as well as herbarium

specimens, were mounted in lactophenol on glass slides. Fifty measurements of each relevant morphological structure were made and ranges and averages computed.

Growth in culture: The optimal temperatures of growth for *P. humicola, L. reconditum, L. lundbergii* and *P. dimorphospora* were determined by inoculating four MEA plates with agar disks taken from the actively growing margins of 2-wk-old isolates. Plates were incubated at temperatures ranging from 5 to 30 °C at 5 °C intervals. Cycloheximide tolerance was determined for the species previously mentioned as well as *O. piliferum* (Fr.: Fr.) Sydow (CMW2481). This was done by inoculating 4 MEA plates amended with increasing concentrations (0, 0.05, 0.1, 0.5, 1.0 g/l) of cycloheximide and incubating them at 25°C. Colony diameters were measured after 11 d and growth was computed as an average of eight readings.

Electron microscopy: For scanning electron microscopy (SEM), small blocks of agar cut from sporulating colonies were fixed in 3% glutaraldehyde and 0.5% osmium tetroxide in a 0.1 M phosphate buffer, dehydrated in a graded acetone series and critical-point dried. Specimens were mounted and coated with gold palladium alloy and examined using a JSM 6400 Scanning Electron microscope.

For ultrastructural examination, the isolate of *P. humicola* was grown on 2% malt extract agar in Petri dishes at 25°C. Small blocks of agar were cut form the colony and fixed in the same manner as described for scanning electron microscopy. The material was then embedded in Spurr epoxy resin (Spurr, 1969) and ultrathin sections (60 nm) were cut with a diamond knife, using an LKB Ultratome III. Sections were stained for 10 min with 4% uranyl acetate and 2 min in Reynolds' lead citrate and examined with a Philips CM100 Transmission Electron Microscope.

Molecular comparisons

Fungal isolates: The isolates used for the molecular comparisons included *P. dimorphospora, P. humicola, L. lundbergii,* and *L. reconditum* (Table 1). Other species for which sequence data have previously been published and that were included for comparison are summarised in Table 2.

DNA extraction: Isolates were grown in liquid malt extract (ME) (2% w/v, NT Merck) at 25°C in the dark for 14 d. DNA was isolated using a modified version of DNA extraction as described by

Raeder and Broda (1985). Mycelium was ground to a fine powder in liquid nitrogen, to which 1ml extraction buffer (200 mM Tris-HCl, pH 8; 25 mM EDTA; 250 mM NaCl; 0.5% SDS) was added. This was followed by further homogenization and incubation, for 1 h at 60°C. Cell debris were precipitated by centrifugation (ca. 15700 x g, 30 min) and a series of phenol:chloroform (0.5 v/v) extractions were performed until the interface was clear. The nucleic acid was precipitated from the aqueous phase by adding cold 100% ethanol (2:1 v/v) and incubated for two days at -20°C. The mixture was subsequently centrifuged at ca. 15700 x g for 30 min, the pellet was washed in 70% ethanol and resuspended in 300 µl sterile water.

Polymerase chain reaction (PCR): Extracted DNA was used as template to amplify a region of the nuclear large (28S) subunit (LSU) of the ribosomal RNA operon using PCR. The ITS 2 region and a portion of the LSU gene was amplified using the primer set ITS 3 (5'-GCATCGATGAAGAACGCAGC-3') (Visser *et al.*, 1995) and LR 3 (5'-CCGTGTTTCAAGACGGG-3') (White *et al.*, 1990). The PCR reaction mixture included 25mM MgCl$_2$, Expand HF buffer without MgCl$_2$, dNTPs (1.25 mM), 25 pmol of each primer in the primer set, 250 ng template DNA and 1.75U Expand^{TM} High Fidelity PCR System (Roche Pharmaceuticals, Germany). The PCR reaction conditions, for the amplification of the large subunit, was an initial denaturation at 94°C for 2 min, followed by annealing at 48°C for 1 min, ramping at 5°C/s to 72°C for 2 min. This was repeated for 40 cycles and a final elongation step was done at 72°C for 8 min. The resulting PCR products were purified using a QIAquick PCR Purification kit (QIAGEN, Germany).

DNA sequencing: DNA sequences were determined using an ABI 377 automated sequencer. The ABI PRISM ^{TM} Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, (Applied Biosystems, UK) was used in the sequence reactions. Both strands were sequenced using primer sets ITS3 and LR3. For the purpose of sequencing, an internal primer was used, namely 404X (5'- GTGAAATTGTTGAAAGGG -3') (Jacobs, 1999).

Sequence analysis: DNA sequences were manually aligned by inserting gaps. These gaps were treated as missing data. Analysis was done using the heuristic search option of PAUP 4.0* (Phylogenetic Analysis Using Parsimony* and Other Methods version 4.30b2a, Swofford, 1993). The branch-swapping and tree bisection-reconnection algorithm (TBR) was used to find the tree with most parsimony. Bootstrap analysis was performed to determine branch confidence (1000 replicates). *Filobasidiella neoformans* Kwon-Chung was used as outgroup.

RESULTS

Morphological comparison

Culture characteristics: Comparison of the isolates of *P. humicola* (CMW 170, 1618) with the herbarium type specimens (ATCC 22801) and the complete description provided by Jong & Davis (1972) confirmed their identity. The synonymy of *P. humicola* and *P. gabalongii* could not be re-evaluated because the herbarium material of *P. gabalongii* has deteriorated and no structures were available for examination. Cultures of the isolates, as well as the herbarium material of *P. humicola* are 'deep mouse gray' (15'''''i) to 'dark mouse gray' (15'''''k) (Rayner, 1970) in colour. The reverse of the colonies are 'fuscous black' (13''''m) in colour. *Phialocephala humicola* can be distinguished from other *Phialocephala* spp. based on conidial shape and the presence of inconspicuous collarettes at the apices of the conidiogenous cells.

Microscopy: *Phialocephala humicola* differs from *L. reconditum* based on a number of morphological characteristics. *Leptographium reconditum* has oblong conidia with truncate ends and rounded apices, while those of *P. humicola* are ellipsoidal (Fig. 1). The number of primary branches ranges from one to three in *P. humicola*, while *L. reconditum* has two to five primary branches. The presence of rhizoids in *L. reconditum* also distinguishes it from *P. humicola*, in which rhizoids are absent (Fig. 1).

Examination of *P. humicola* using scanning as well as transmission electron microscopy showed that the conidiogenous cells of *P. humicola* are distinctly annellidic with conspicuous signs of percurrent proliferation (Fig.2). The conidia have a tendency to remain attached to the conidiogenous cells giving them the appearance of having developed sympodially (Fig. 2).

Growth in culture: Differences in physiology also distinguish *L. reconditum* and *P. humicola* from each other. *Leptographium reconditum* has an optimum growth rate at 20°C and attained a diameter of 36 mm in 8 d. *Phialocephala humicola* grows optimally at 30°C and attains an average diameter of 18 mm in 11 d. Furthermore, *P. humicola* was unable to tolerate high concentrations of cycloheximide and ceased to grow at a cycloheximide concentration of 0.5 g/l. This sensitivity is typical of other species of *Phialocephala* included in the study (Table 3). The isolate of *L. reconditum* was found to be able to tolerate high concentrations of cycloheximide (Table 3).

Molecular comparison

The total number of characters used in the comparison of species based on the LSU rRNA gene was 337 of which 246 were parsimony uninformative and 91 parsimony informative. Two trees were obtained and one representative tree is presented here (Fig. 3). The shortest tree length was 230 with a consistency index (CI) of 0.557 and a retention index (RI) of 0.690.

In the phylogenetic tree constructed based on the 28S rDNA gene sequence *P. humicola* isolates grouped together. They were, furthermore, positioned within a selected group of fungi representing the Ophiostomatales. Isolates of *P. dimorphospora* were clearly unrelated to *P. humicola*.

DISCUSSION

Results of this study have shown that *P. humicola* is unrelated to *P. dimorphospora*, on which *Phialocephala* is based. This is perhaps not surprising given the fact the two fungi originate from very different ecological niches. They also have very distinct morphological features, including markedly different conidiogenous cells, which represents a feature that has in the past been considered taxonomically informative (Hughes, 1953; Kendrick, 1961; Wingfield *et al.*, 1987; Wingfield, 1985).

Our results have shown that *P. humicola* has conidia that develop through percurrent proliferation of the condiogenous cells. The reason why the fungus was thought to have phialidic conidium development is most probably due to the fact that annellations in *P. humicola* are tightly packed. Using light microscopy, these conidiogenous cells can be mistaken for phialides, which has previously been shown in *Ophiostoma francke-grosmanniae* Davidson (Mouton, Wingfield & van Wyk, 1992).

Annellidic conidium development in *P. humicola* makes the fungus morphologically suited for accommodation in *Leptographium*. One of the interesting features of most *Leptographium* spp. and their *Ophiostoma* teleomorphs is that they are able to tolerate high levels of cycloheximide in culture (Harrington, 1988; Marais, 1993). The fact that *P. humicola* is sensitive to low concentrations of cycloheximide might suggest that it is not closely related to *Ophiostoma* and its anamorphs. There are, however, other species of *Leptographium* such as *L. wageneri* var. *pseudotsugae* Harrington and Cobb that are sensitive to cycloheximide (Marais, 1993) and the type species of *Graphium*, *G. penicilloides* is able to tolerate relative high levels of the antibiotic, but is not related to *Ophiostoma* (Okada *et al.*, 2000).

DNA sequence data presented in this study show that *P. humicola* is unrelated *to P. dimorphospora*. The latter species appears to reside in the Leotiales as suggested by Currah *et al.* (1993) after they observed apothecium-like structures with cells resembling immature asci in culture. Based on sequence comparisons, *P. humicola* is firmly placed in the Ophiostomatales. This is consistent with its conidium development, which suggests that it could be accommodated in *Leptographium*.

Phialocephala humicola has been found in the soil and in the rhizosphere of wheat (Jong & Davis, 1972; Sivasithamparam, 1975). In this respect, the fungus is similar to *L. reconditum* which also originates from the rhizosphere of wheat (Jooste, 1978). The two fungi are distinctly different based on morphology and physiology, although they are clearly related. Further studies, based on alternative gene sequences are required to determine whether these two anamorphic fungi are more closely related to each other than to other species of *Leptographium*. Although there is some justification for providing a name in *Leptographium* to accommodate *P. humicola*, we have elected not to do so. The fungus is clearly misplaced in *Phialocephala* but we prefer to await a more complete study of additional species of *Phialocephala* before generic names are provided for these mitosporic fungi.

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Table 1. List of fungi studied.

Culture number[a]	Alternative designation[b]	Name	Origin	Collector
CMW508	ATCC 24087	*P. dimorphospora*	Maryland, U.S.A.	C.A. Shearer
CMW168	-	*P. dimorphospora*	Maryland, U.S.A.	C.A. Shearer
CMW 30	-	*L. lundbergii*	New Zealand	M. Dick
CMW 170	ATCC 38881	*P. humicola*	Alabama, U.S.A.	G. Morgan-Jones
CMW1618	IFO 30007	*P. humicola*	Japan	K. Tubaki
CMW 15	-	*L. reconditum*	South Africa	W. J. Jooste

a) CMW refers to the culture collection of the Tree Pathology Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

b) IFO refers to the culture collection of the Institute for Fermentation, Osaka, Japan. ATCC refers to the culture collection of the American Type Culture Collection, U.S.A.

Table 2. Species included in phylogenetic analyses for which sequence data were derived from GenBank.

Species	GenBank accession number
Ceratocystis albofundus de Beer, Wingfield & Morris	AF043605
Ceratocystis fimbriata Ellis & Halst	U17401
Cercophora septentrionalis Lundqvist	U47823
Chaetomium globosum Kunze: Fr.	U47825
*Chromocleista malachitea*Yaguchi & Udagawa	AB000621
Filobasidiella neoformans Kwon-Chung	AF075526
Fonsecaea pedrosoi (Brumpt) Negroni	L36997
Glomerella cingulata (Stoneman) Spaulding & von Schrenk	AF222490
Hamigera avellanea (Thom and Turesson) Stolk & Samson	D14406
Hypocrea schweinitzii (Fr.) Sacc.	L36986
Leptographium abietinum (Grosmann) Siemaszko	AF269212
Ophiostoma piceaeperdum (Rumbold) Arx	AF269215
Ophiostoma francke-grosmanniae Davidson	AF269213
Ophiostoma penicillatum (Grosmann) Siemaszko	AF269214
Ophiostoma piliferum(Fr.: Fr.) Sydow	U47837
Ophiostoma quercus (Georgev.) Nannf.	AF128931
Xylaria curta Fr.	U47840

Table 3. Tolerance of *P. humicola*, and other relevant fungi to various concentrations of cycloheximide.

Cycloheximide concentration (g/l)	Average colony diameter (mm)[a]				
	L. lundbergii	*O. piliferum*	*P. dimorphospora*	*P. humicola*	*L. reconditum*
0	40	14	9	7	22
0.05	29	11	7	3	16
0.1	30	11	6	1	18
0.5	24	9	4	0	16
1	24	10	4	0	20

a) Colony diameter represents growth after six days at 25°C in Petri dishes. Measurements represent an average of eight colony diameters.
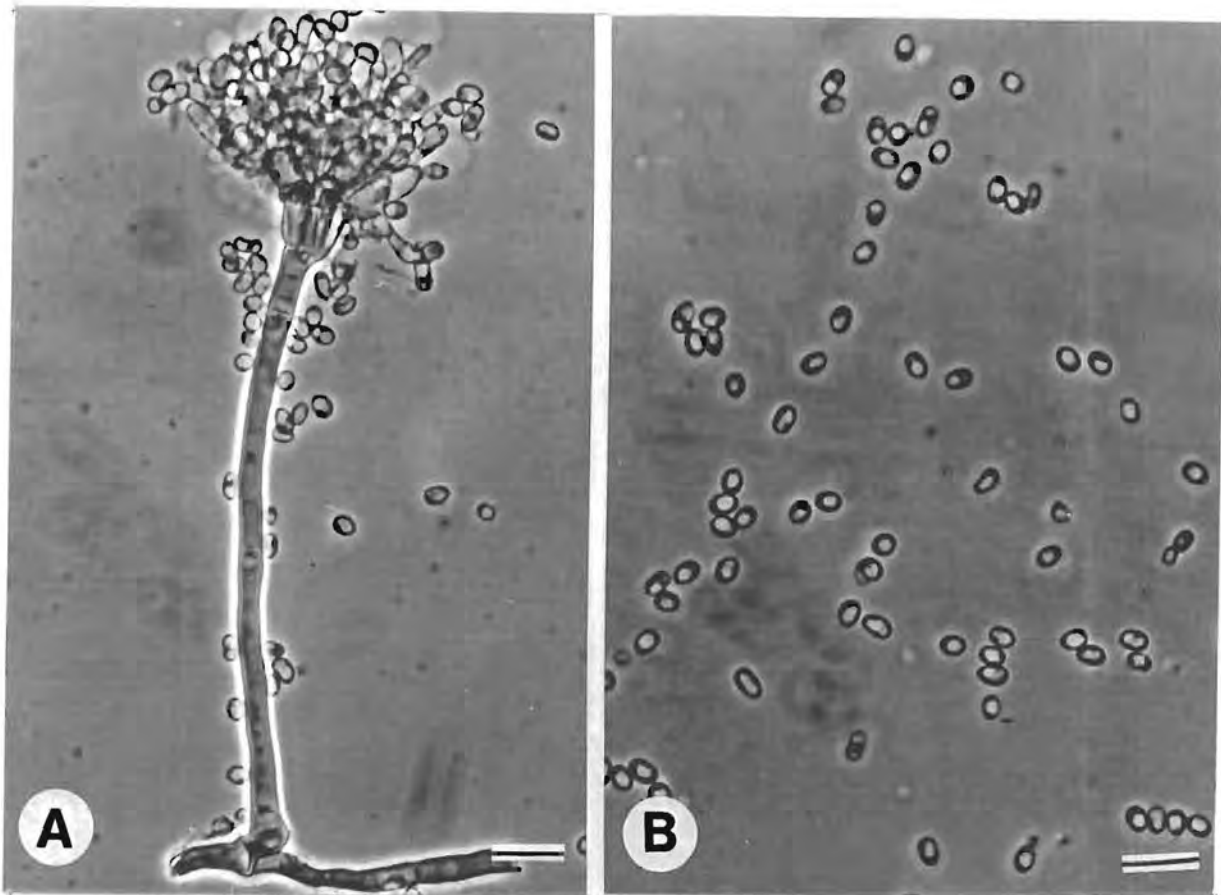
Fig. 1. Light micrographs of conidiophore and conidia of *Phialocephala humicola* (CMW 170). **A.** Conidiophore (Bar = 10 μm). **B.** Conidia (Bar = 10 μm).
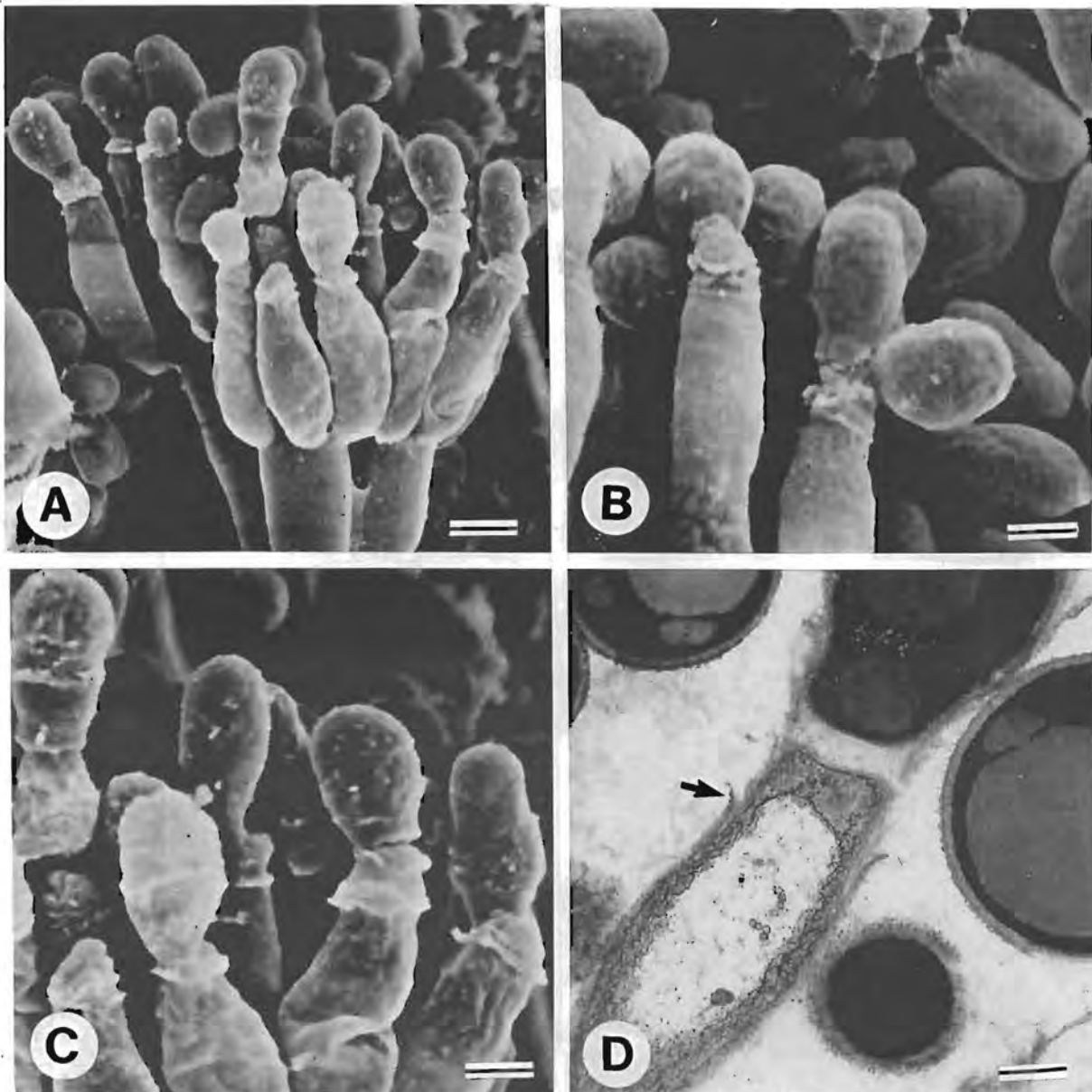
Fig. 2. Scanning and transmission micrographs of conidiophores and conidia of *Phialocephala humicola* (CMW 170). **A.** Conidiogenous apparatus (Bar = 1 μm). **B.** Conidiogenous cell with conidial crown (Bar = 1 μm). **C.** Conidiogenous cell with distinct annellides (Bar = 1 μm). **D.** Section through a conidiogenous cell. The arrow indicates annellation (Bar = 1 μm).

Figure 3. Phylogenetic tree produced by PAUP heuristic option of the LSU rDNA with *Filobasidiella neoformans* as outgroup. Bootstrap values above 50% (percentages of 1000 bootstrap replicates) are indicated in bold below the branches of the tree. Branch length values are indicated above the branches of the tree.

2 Ophiostoma quercus
7
100 1 Ophiostoma piliferum

2 LEPTOGRAPHIUM LUNDBERGII
2
3 Ophiostoma europhoides
2
1 LEPTOGRAPHIUM RECONDITUM

3
64

3
1 Leptographium abietinum
1
2 Ophiostoma penicillatum
5
3 Ophiostoma franckegrosmanniae

Ophiostomatales

15
93

16
100
1 PHIALOCEPHALA HUMICOLA170
1 PHIALOCEPHALA HUMICOLA1618

4 Xylaria curta
3
53
1 Ceratocystis fimbriata
13
100
5 Ceratocystis albofundus
5

Xylariales

Microascales

5
10 Hypocrea schweinitzii

Hypocreales

6

5
3 Cercophora septentrionalis
8
98
5 Chaetomium globosum

Sordiales

9

5 Chromocleista malachi
14
100
9 Hamigera avellanea

Eurotiales

12 Fonsecaea pedrosoi

Dothideales

30
12
100
PHIALOCEPHALA DIMORPHOSPORA168

PHIALOCEPHALA DIMORPHOSPORA508

Filobasidiella neoformans

5 changes

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Phylogenetic relationships of *Phialocephala* species based on rRNA sequence data

Phialocephala was established for species that produce their conidia from phialides at the apices of dark mononematous conidiophores in the *Leptographium* complex. Some species previously residing in *Phialocephala* were reallocated to *Sporendocladia*, due to the fact that they resembled *Chalara* in having ring wall building conidial development and conidia with two attachment points, produced in false chains. Despite this significant realignment of the genus, a great deal of morphological heterogenecity remains in *Phialocephala*. The objective of this study was to consider this heterogenecity based on comparisons of sequence data derived from the large (28S) and small (18S) subunit of the rRNA operon. *Phialocephala dimorphospora*, the type species of the genus, grouped together with *P. fortinii* and genera of the Leotiales. *Phialocephala virens* grouped with *P. dimorphospora* and *P. fortinii*. *Phialocephala xalapensis* and *P. fusca* are clearly unrelated to *Phialocephala sensu stricto* and probably represent a new genus in the Ophiostomatales. *Phialocephala compacta* resides with representatives of the Microascales and probably represents a distinct genus in this order. *Phialocephala scopiformis* and *P. repens* are not closely related to the other *Phialocephala* species. Results of this study have clearly shown that the morphological heterogenecity amongst species of *Phialocephala* is clearly reflected by phylogenetic analysis of sequence data from two conserved ribosomal RNA genes. Appropriate genera now need to be found to accommodate these fungi. In addition, cultures of *Phialocephala* species not treated in this study should be collected and included in future studies.

Keywords: *Phialocephala*, phylogeny, morphology

Phialocephala Kendrick was established to accommodate species in the *Leptographium* Lundberg & Melin complex, that produce their conidia in phialides (Kendrick, 1961). This distinguishes them from *Leptographium* spp. that are characterised by percurrent proliferation of the conidiogenous cells. *Phialocephala* spp. are further characterised by having dark mononematous conidiophores that branch occasionally at their apices (Crane, 1971). Hyaline ameroconidia accumulate in slimy masses around the sporogenous heads (Kendrick, 1961, 1963). Some species are also characterized by solitary phialides, which are formed directly on the mycelium (Onofri & Zucconi, 1984). These morphological characters suggest an adaptation to insect dispersal, although insect associations are not known for most species.

Phialocephala spp. occupy a diverse range of ecological niches (Wang & Wilcox, 1985; Kowalski & Kehr, 1995). *Phialocephala fortinii* Wang & Wilcox, *P. dimorphospora* Kendrick, *P. compacta* Kowalski & Kehr and *P. scopiformis* Kowalski & Kehr are readily isolated from plants, growing in cool or cold environments, such as those encountered in the alpine, sub-alpine and boreal regions (Wang & Wilcox, 1985; Hambleton & Currah, 1997; Stoyke & Currah, 1990). *Phialocephala trigonospora* Kirschner & Oberwinkler was isolated from bark beetle tunnels in *Pinus sylvestris* L. and *Picea abies* L. Korst., while *P. scopiformis* and *P. compacta* are endophytes of *Pinus* and *Picea* spp. (Kowalski & Kehr, 1995; Kirschner & Oberwinkler, 1998). Most species are not associated with disease, but *P. virens* Siegfried & Siefert was isolated from root rot of *Tsuga* and *Picea* spp. (Siegfried, Siefert & Bilmar, 1992), while *P. fortinii* has also been reported as a weak pathogen of container-grown conifers (Wilcox & Wang, 1987).

No known teleomorph associations have been determined for any species of *Phialocephala,* although a connection to the Leotiales has been proposed (Currah, Tsuneda & Murakami, 1993). This was based on the morphology of apothecium-like structures with cells resembling immature asci, produced in some cultures (Currah, *et al.*, 1993). Previously, the anamorph of *Ophiostoma francke-grosmanniae* Davidson was also suggested to represent a *Phialocephala* species. However, the presence of closely packed annellations, observed in an ultrastructural study showed that the anamorph of this fungus should rather reside in *Leptographium* (Mouton, Wingfield & van Wyk, 1992).

The morphologically heterogeneous nature of *Phialocephala* was emphasised when the species with inconspicuous collarettes and ring wall building conidial development were moved to *Sporendocladia* Arnaud, Nag Raj & Kendr. (Wingfield, van Wyk & Wingfield,

1987). However, based on morphological and physiological variability, the remaining *Phialocephala* spp. clearly remain a heterogeneous group. The variable morphological characteristics include a wide diversity of conidial forms and variously structured collarettes at the apices of conidiogenous cells. Furthermore, the variable presence of rhizoids at the base of conidiophores and sterile outgrowths on stipes suggest that many of these fungi are phylogenetically unrelated.

Phialocephala fusca Kendrick is the only *Phialocephala* sp. that forms rhizoids at the base of its conidiophore stipes (Kendrick, 1963). Likewise, *P. canadensis* Kendrick, *P. fluminis* Shearer, Crane & Miller and *P. fusca* are unique in having sterile outgrowths on their stipes (Kendrick, 1963; Shearer, Crane & Miller, 1976). Collarette morphology in *Phialocephala* spp. varies from being broadly flared in *P. fusca* to deeply-set in *P. dimorphospora* and inconspicuous in *P. humicola* Jong & Davis (Kendrick, 1961; 1963; Jong & Davis, 1972). Conidial shapes in *Phialocephala* spp. range from ellipsoid to globose and sub-globose. Some species have two distinct forms of conidia and one species, *P. trigonospora*, has uniquely triangular spores (Kirschner & Oberwinkler, 1998).

Phialocephala spp. vary in their tolerance to the antibiotic cycloheximide, which is a characteristic that can indicate connections to the Ophiostomatales (Harrington, 1988). Species such as *P. dimorphospora* Kendrick display 84% reduction in growth, in the presence of 0.5 g/l of cycloheximide, while *P. fusca* is unable to grow in the presence of the antibiotic (Marais, 1993).

Very limited molecular data are available from which to infer phylogenetic relationships for *Phialocephala* spp. To our knowledge, the only species that have been considered at this level are *P. fortinii* and *P. dimorphospora* (Rogers, McKemy & Wang, 1999). Based on ITS sequences comparisons, these two species appear to be closely related. This relationship is also strongly supported by their morphological and ecological characteristics.

The aim of this study was to consider phylogenetic relationships between *Phialocephala* spp. for which cultures are available. A further aim was to evaluate their placement within orders of the Ascomycotina. This was achieved by means of analysis of partial sequences of the 18S (SSU) and 28S (LSU) genes of the ribosomal RNA operon.

MATERIALS AND METHODS

Fungal isolates: Isolates were obtained from a wide variety of sources (Table 1). Comparisons were also made with sequences deposited in GenBank for taxa that are apparently closely related to *Phialocephala* species (Table 2).

DNA extraction: Isolates were grown in liquid malt extract (ME) (2% w/v, NT Merck) at 25°C in the dark for 14 d. DNA was isolated using a modified version for DNA extraction described by Raeder & Broda (1985). Mycelium was ground to a fine powder in liquid nitrogen, to which 1ml extraction buffer (200 mM Tris-HCl, pH 8; 25 mM EDTA; 250 mM NaCl; 0.5% SDS) was added. This was followed by further homogenisation and incubation for 1 h at 60°C. Cell debris was precipitated by centrifugation (ca.15700 x g, 30 min) and a series of phenol:chloroform (0.5 v/v) extractions were performed until the interface was clear. The nucleic acid was precipitated from the aqueous phase by adding cold 100% ethanol (2:1 v/v) and incubated for two days at -20°C. The mixture was subsequently centrifuged at ca. 15700 x g for 30 min, the pellet was washed in 70% ethanol and resuspended in 300 µl sterile water.

Polymerase chain reaction (PCR): Extracted DNA was used as template to amplify regions of the nuclear large (28S) (LSU) and small (18S) (SSU) subunits of the ribosomal RNA operon using PCR. The SSU gene was amplified using the primer set NS 3 (5'-GCAAGTCTGGTGCCAGCAGCC-3') and NS 8 (5'-TCCGCAGGTTCACCTACGGA-3') (White *et al.*, 1990) or primer set 2F (5'-ATCTGGTTGATCCTGCCAGTAG-3') and 1794R (5'-GATCCTTCCGCAGGTTCACC-3') (Okada, Takmatsu & Takamura, 1997). The ITS 2 region and a portion of the LSU gene was amplified using the primer set CS 3 (5'-CGAATCTTTGAACGCACATTG-3') (Visser, *et al.*, 1995) and LR 3 (5'-CCGTGTTTCAAGACGGG-3') (White *et al.*, 1990). The PCR reaction mixture included 25 mM MgCl$_2$, Expand HF buffer without MgCl$_2$, dNTPs (1.0 mM), 25 pmol of each primer in the primer set, 250 ng template DNA and 1.75 U Expand^{TM} High Fidelity PCR System (Roche Pharmaceuticals, Germany). The PCR reaction conditions, for the amplification of the LSU, was an initial denaturation at 94°C for 2 min, followed by annealing at 48°C for 1 min, ramping at 5°C/s to 72°C for 2 min. This was repeated for 40 cycles and a final elongation step was done at 72°C for 8 min. The SSU was amplified following the same

PCR reaction conditions given above but for only 25 cycles. The resulting PCR products were purified using a QIAquick PCR Purification kit (QIAGEN, Germany).

DNA sequencing: DNA sequences were determined using an ABI 377 automated sequencer. The ABI PRISM ^{TM} Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, (Applied Biosystems, UK) was used in the sequence reactions. Both strands were sequenced using primer sets CS3, LR3 and NS3, NS8 (Fig. 1). For the purpose of sequencing two internal primers were used, namely 404X (5'-CCCTTTCAACAATTTCAC-3') and 1332R (5'- AAGGTCTCGTTCGTTATCG -3') (Okada *et al.*, 1997) for the large and small subunit, respectively (Fig. 1) (Jacobs, 1999).

Sequence analysis: DNA sequences were manually aligned by inserting gaps. These gaps were treated as missing data in the analysis. Analysis was done using the heuristic search option of PAUP 4.0* (Phylogenetic Analysis Using Parsimony* and Other Methods version 3.1.1, Swofford, 1993). The branch-swapping and tree bisection-reconnection algorithm (TBR) was used to find the parsimonious tree. A bootstrap analysis was performed to determine branch confidence intervals (1000 replicates).

In the SSU and LSU phylograms *Filobasidiella neoformans* Kwon-Chung sequences, was used as the outgroup. The tree was rooted by defining the ingroup as monophyletic and the outgroup as a monophyletic sister group to the ingroup.

RESULTS
Small subunit RNA gene analysis

Two phylograms are included in the analysis of the small subunit data. The first comparison (Fig. 2) was done to achieve a comparison between the small and large subunit data sets for the *Phialocephala* species studied. The alignment was accomplished by inserting gaps, which resulted in a total of 421 characters used in the comparison of species, of which 97 characters were parsimony-informative and 324 parsimony uninformative. A total of 100 trees were obtained. The shortest tree length was 223 with a consistency index (CI) of 0.574 and a retention index (RI) of 0.665 (Fig. 2). The second phylogram (Fig. 3) based on the sequence data obtained from the 18S gene, was included for comparison between *Phialocephala* species and representatives of the Leotiales and *Phialophora finlandia*. These were not included in the first phylogram, because the published 28S data

for these specific representatives are not available. The total number of characters used in the second comparison was 421 of which 325 were parsimony uninformative and 96 parsimony informative. A total of 100 trees were obtained. The shortest tree length was 225 with a consistency index (CI) of 0.578 and a retention index (RI) of 0.683.

In the first comparison (Fig. 2) *P. xalapensis* and *P. fusca* resided in a separate group representing the Ophiostomatales. Isolates of *S. bactrospora* were firmly placed in the Microascales together with *Ceratocystis* Ellis & Halst. *Phialocephala compacta* grouped closely with representatives of the Microascales. *Phialocephala dimorphospora* and *P. fortinii* grouped together and apart from all other *Phialocephala* species. Representatives of the orders Dothideales and Eurotiales form the fourth clade.

In the phylogram based on 18S data (Fig. 3) *P. xalapensis* and *P. fusca* grouped together with representatives of the Ophiostomatales. *Sporendocladia* isolates and representatives of the Microascales grouped together. *Phialocephala compacta*, was again closely related to these fungi. *Phialocephala dimorphospora* and *P. fortinii* group firmly together in the Leotiales. *Phialophora finlandia*, another endophyte species, also resided in this clade.

Large subunit RNA gene analysis

Two phylograms are included in the analysis of the large subunit data. The first analysis (Fig. 4) was done to achieve a comparison with the data obtained from the 18S gene. The alignment was accomplished by inserting gaps, which resulted in a total of 333 characters used in the comparison of species, of which 116 characters were parsimony informative and 217 parsimony uninformative. A total of 8 trees were obtained. The shortest tree length was 400 with a consistency index (CI) of 0.489 and a retention index (RI) of 0.577. The second phylogram includes species for which only LSU data were available (Fig. 5). The total number of characters used in the comparison of species based on the LSU rRNA gene was 333 of which 212 were parsimony uninformative characters and 121 parsimony informative characters. A total of 8 trees were obtained. The shortest tree length was 456 with a consistency index (CI) of 0.463 and a retention index (RI) of 0.598 (Fig. 5).

In the phylogenetic tree constructed using the 28S rRNA gene (Fig. 4) *P. xalapensis* and *P. fusca* grouped with the genera, *Ophiostoma*, *Diaporthe* and *Leptographium*. *Phialocephala compacta* resided with the Microascalean genus, *Melanospora*.

Sporendocladia bactrospora isolates were tightly grouped with *Ceratocystis fimbriata*. The relationship of *Sporendocladia* as part of the Microascales was thus confirmed and mirrors the results observed from 18S sequence data. *Phialocephala dimorphospora* and *P. fortinii* grouped together, supporting the relationship between these two species. *Phialocephala scopiformis* and *P. repens*, are distinct from fungi in all other clades.

In the phylogenetic tree constructed using the 28S rRNA gene for species, excluded from the 18S datasets, *S. bactrospora*, *P. compacta* and representatives of the Microascalean group together. *Phialocephala fusca*, *P. xalapensis* and *P. humicola* grouped in the Ophiostomatales. *Phialocephala dimorphospora*, *P. fortinii* and another *Phialocephala* species, *P. virens*, grouped together and separate from other *Phialocephala* species. This suggested that *P. virens* is a member of *Phialocephala sensu stricto*.

DISCUSSION

Results of this study confirmed views based on morphology and ecology that species of *Phialocephala* are phylogenetically unrelated. Analyses of sequence data have thus shown that species considered in this study represent taxa in the Leotiales, Ophiostomatales and Microascales. Although this has not been an objective of the present study, various species will require new representative taxa outside *Phialocephala*.

Phialocephala dimorphospora is the type species of this genus. The fungus has very characteristic deeply set conidiogenous loci with tubular collarettes (Kendrick, 1961) that give rise to the characteristically long first conidia. Subsequently produced conidia are ovoid. *Phialocephala fortinii* has conidiogenous cells and conidia similar to those of *P. dimorphospora* and the two fungi share similar ecological niches. It is, therefore, not surprising that results of this study showed that the two fungi are phylogenetically related. Furthermore, our results confirm those of a previous study that has suggested that these fungi probably reside in the Leotiales (Rogers *et al.*, 1999; Currah *et al.*, 1993).

Another *Phialocephala* species also related to *P. dimorphospora* and *P. fortinii* is *P. virens*. Despite the relatedness based on the 28S data there are substantial morphological differences between *P. dimorphospora* and *P. virens*. These morphological differences include the presence of inconspicuous collarettes in *P.virens*, compared to the deep-set collarettes of *P. dimorphospora*. *Phialocephala virens* is also the only *Phialocephala* species associated with disease and the formation of root rot and green stain (Siegfried *et al.*, 1992). The similarity of *P. dimorphospora* and *P. virens* molecular data needs to be further investigated by including data from other conserved genes.

In many respects, *Phialocephala* spp. are morphologically similar to *Leptographium* species. Species in both genera have erect conidiophores with conidia produced in slimy masses at the apices of branched conidiogenous apices. In *Leptographium*, this morphological form is an adaptation to facilitate an association with insect vectors (Harrington, 1988). It is thus not surprising that three *Phialocephala* spp. (*P. fusca, P. humicola* and *P. xalapensis*) included in this study were found to be related to *Leptographium* in the Ophiostomatales. Our results furthermore suggest that distinct phialides with well developed collarettes should not exclude species from *Leptographium*. This is despite the fact that the latter genus has previously been reserved for species with precurrent proliferation of conidiogenous cells. Results of this study did not enable us to suggest phylogenetic relatives for *P. scopiformis* and *P. repens*. These fungi are clearly unrelated to other *Phialocephala* spp. studied, and also distinctly different from each other. Sequence data for a greater number of fungi from other taxonomic levels will be required in order to suggest an appropriate ordinal positions for these species. Although they are clearly not *Phialocephala* spp. in the strict sense, they should probably reside in this genus until further information is available to suggest more appropriate taxa for them.

In this study we included a number of isolates of *S. bactrospora*, a species that was previously accommodated in *Phialocephala* as *P. bactrospora* (Kendrick, 1961). Based on a study of conidiogenesis and the presence of ring wall building conidial development in this fungus, Wingfield *et al.* (1987) transferred it to *Sporendocladia*. The fact that conidia are produced through ring wall building makes this fungus morphologically similar to *Chalara* anamorphs of *Ceratocystis*, in which conidia are typically produced in this manner (Nag Raj & Kendrick, 1975). *Sporendocladia bactrospora* is also sensitive to the antibiotic, cycloheximide, which makes it similar to *Chalara* spp. It was thus not surprising that isolates of *S. bactrospora* included in this study grouped together with *Ceratocystis* in the Microascales.

Leptographium kitajimina Aoshima is a fungus lodged in the culture collection of the Institute of Fermentation, Osaka, Japan (IFO 6908). As far as we have been able to ascertain, this fungus was never formally described. It is also not mentioned in the thesis of Aoshima (1965) in which a number of new species were presented but not validly described (Yamaoka, Wingfield, Takahashi & Solheim, 1997). Inspection of a culture of *L. kitajimina* in this study showed that it is morphologically similar to *S. bactrospora*. Sequence data have also shown that it is the same as *S. bactrospora*. As far as we are aware, this is the first record of the fungus from Japan.

This study has enabled us to suggest appropriate phylogenetic placements of a number of *Phialocephala* spp. It has been possible to confirm previous contentions that the genus is heterogenous and that most species are unrelated. Future names in *Phialocephala* should be restricted to those species that are similar to *P. dimorphospora*, both in morphology and based on sequence data. Alternative generic names will be needed for other species.

One of the limitations of this study has been that isolates are not available for a number of *Phialocephala* spp. Where isolates are available, these are one or only a few in number. Furthermore, a few species for which cultures are available grow extremely slowly and for this, or some other reason we have not succeeded in producing sequence data for them. It is hoped that in the future, additional cultures and sequence data will become available for further study.

Table 1. List of fungi for which sequence data were generated in this study.

Culture number[a]	Alternative designation[b]	Name	Origin	Collector	GenBank accession number
CMW 4946	CBS 507.94 ATCC 96754	*P. compacta*	Braunschweig, Germany	T. Kowalski	To be released at a later date
CMW508	ATCC 24087	*P. dimorphospora*	Maryland, U.S.A.	C.A. Shearer	"
CMW168		*P. dimorphospora*	Maryland,U.S.A.	C.A. Shearer	"
CMW5590	CBS 443.86 ATCC 60614	*P. fortinii*	Suonenjoki, Finland	J. Wang	"
CMW 172	CBS 301.85 ATCC 62326	*P. fusca*	Ottawa, Canada	W.B. Kendrick	"
CMW 170	ATCC 38881	*P. humicola*	Alabama,U.S.A.	G. Morgan-Jones	"
CMW1618		*P. humicola*	Japan	K. Tubaki	"
CMW5339	MUCL 1849	*P. repens*	Regensberg, Germany	T. Kowalski	"
CMW 4947	CBS 468.94 ATCC 96754	*P. scopiformis*	Regensberg, Germany	T. Kowalski	"
CMW 5592	CBS 365.76	*P. virens*	Finland	W. Gams	"
CMW 5594	CBS 218.86	*P. xalapensis*	India	V. Rao	"
CMW 30		*L. lundbergii*	New Zealand	M. Dick	"
CMW 15		*L. reconditum*	South Africa	W. Jooste	"
CMW 664	CBS 299.62 ATCC 44606	*S. bactrospora*	Chiem, Columbia	J. Foster	"
CMW 1592	IFO 8770	*S. bactrospora*	Japan	K. Tubaki	"
CMW 1593	IFO 8852	*S. bactrospora*	Japan	M. Ichanoe	"
CMW 1636	IFO 6908	*L. kitajimana*	Japan	K. Aoshima	"

a) CMW refers to the culture collection of the Tree Pathology Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

b) IFO refers to the culture collection of the Institute for Fermentation, Osaka, Japan. CBS refers to the culture collection of the Centraalbureau voor Schimmelcultures, Baarn & Delft, The Netherlands. ATCC refers to the culture collection of the American Type Culture Collection, U.S.A.. MUCL refers to the culture collection of the Belgian Co-ordinated Collections of Micro-organisms, Brussel, Belgium.

Table 2. Species included in phylogenetic analyses for which sequence data were derived from GenBank.

Species	GenBank accession number (LSU)	GenBank accession number (SSU)
Ceratocystis fimbriata Ellis & Halst	U17401	U32418
Cercophora septentrionalis Lundqvist	U47823	U32400
Chaetomium globosum Kunze: Fr.	U47825	U20379
Chromocleista malachitea Yaguchi & Udagawa	AB000621	D88323
Cudonia confusa Bresadola	-	Z30240
Diaporthe phaseolorum (Cooke & Ellis) Sacc.	U47830	L36985
Filobasidiella neoformans Kwon-Chung	AF075526	D12804
Fonsecaea pedrosoi (Brumpt) Negroni	L36997	AF050276
Glomerella cingulata (Stoneman) Spaulding & von Schrenk	AF222490	AF222531
Hamigera avellanea (Thom & Turesson) Stolk & Samson	D14406	AB000620
Hypocrea schweinitzii (Fr.) Sacc.	L36986	U47833
Magnaporthe grisea	AB026819	AF056626
Melanospora fallax Zukal	U17404	U47842
Ophiostoma piliferum (Fr.: Fr.) Sydow	U47837	U20377
Phialocephala fortinii Wang & Wilcox	-	L76626
Phialophora finlandia Wang & Wilcox	-	AF056373
Sclerotinia sclerotiorum (Libert) de Bary	-	X69850
Spathularia flavida Fr.:Fr.	-	Z30239
Xylaria curta Fr.	U47840	U32417
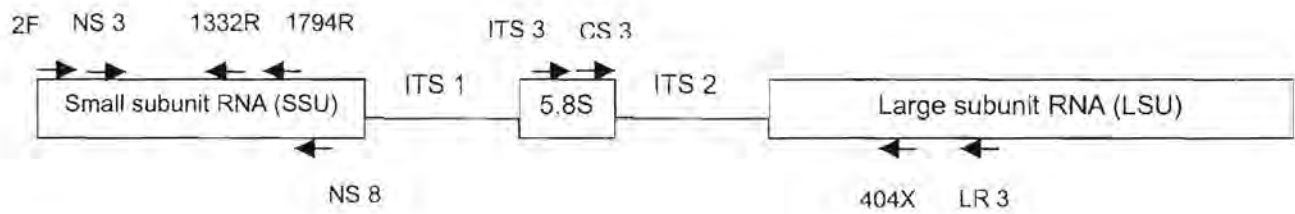
Figure 1. Schematic representation of the ribosomal RNA operon and the annealing sites of the primer sets used.

Figure 2. Phylogenetic tree produced by PAUP heuristic option of the SSU rDNA with *Filobasidiella neoformans* as outgroup. Bootstrap values above 50% (percentages of 1000 bootstrap replicates) are indicated in bold below the branches of the tree. Branch length values are indicated above the branches of the tree.

4 Diaporthe phaseolorum — Diapothales

3 **LEPTOGRAPHIUM LUNDBERGII** — Anamorphic Ophiostomatales

3 **PHIALOCEPHALA XALAPENSIS**

14
100

3 **PHIALOCEPHALA FUSCA**

6
52

1 Ophiostoma piliferum — Ophiostomatales

3 Glomerella cingulata — Phyllachorales

2
7 Ceratocystis fimbriata — Microascales

4
88 **SPORENDOCLADIA BACTROSPORA664**

7
100 **SPORENDOCLADIA BACTROSPORA1593**

2
4 Melanospora fallax — Laboulbeniales

2
1 Hypocrea schweinitzii — Hypocreales

4
73

7 **PHIALOCEPHALA COMPACTA**

3
11 Cercophora septentrionalis — Sordiales

100 2 Chaetomium globosum

1 Magnaporthe grisea

1
1

18

68 **PHIALOCEPHALA FORTINII**
1

Phialocephala fortinii genbank

5
89 **PHIALOCEPHALA DIMORPHOSPORA168**
1

3 **PHIALOCEPHALA DIMORPHOSPORA508**

12

4 Xylaria curta — Xylariales

3
11 Fonsecaea pedrosoi — Dothideales

7
71 1 Hamigera avellanea — Eurotiales

12
100 2 Chromocleista malachitea

1 **PHIALOCEPHALA SCOPIFORMIS**

27
9 **PHIALOCEPHALA REPENS**

Filobasidiella neoformans

— 5 changes

Figure 3. Phylogenetic tree produced by PAUP heuristic option of the SSU rDNA with *Filobasidiella neoformans* as outgroup and representatives of Leotiales included. Bootstrap values above 50% (percentages of 1000 bootstrap replicates) are indicated in bold below the branches of the tree. Branch length values are indicated above the branches of the tree.

Diapothales

Anamorphic
Ophiostomatales

Ophiostomatales

Phyllachorales

Laboulbeniales

Microascales

Hypocreales

Sordiales

Leotiales

Xylariales

Dothideales

Eurotiales

Figure 4. Phylogenetic tree produced by PAUP heuristic option of the LSU rDNA with *Filobasidiella neoformans* as outgroup. Bootstrap values above 50% (percentages of 1000 bootstrap replicates) are indicated in bold below the branches of the tree. Branch length values are indicated above the branches of the tree.

Phylogenetic tree:

- 12 Fonsecaea pedrosoi — Dothideales
- 8
  - 30 / **79**
    - 10 Hamigera avellanea — Eurotiales
    - 21 Xylaria curta — Xylariales
- 9
  - 11 Chromocleista malachi — Eurotiales
  - 7
    - 4
      - 5 Magnaporthe grisea
      - 10 / **86**
        - 9 Cercophora septentrionalis — Sordiales
        - 2 Chaetomium globosum
    - 6
      - 5 Diaporthe phaseolorum — Diapothales
      - 6
        - 5
          - 4 **LEPTOGRAPHIUM LUNDBERGII** — Anamorphic Ophiostomatales
          - 9 Ophiostoma piliferum — Ophiostomatales
        - 4
          - 7 / **93**
            - 3 **PHIALOCEPHALA FUSCA**
            - 8 **PHIALOCEPHALA XALAPENSIS**
    - 17
      - 3
        - 4 / **62**
          - 5
            - 8 Ceratocystis fimbriata — Microascales
            - 10
              - **SPORENDOCLADIA BACTROSPORA1636**
              - **SPORENDOCLADIA BACTROSPORA1592**
        - 6
          - 2
            - 3 Melanospora fallax — Laboulbeniales
            - 7 **PHIALOCEPHALA COMPACTA**
      - 14 Glomerella cingulata — Phyllachorales
      - 10 Hypocrea schweinitzii — Hypocreales

- 16
  - 24
    - 18
      - 77 / **100**
        - 2
          - **PHIALOCEPHALA DIMORPHOSPORA168**
          - **PHIALOCEPHALA DIMORPHOSPORA508**
        - 5 **PHIALOCEPHALA FORTINII**
    - 14 **PHIALOCEPHALA SCOPIFORMIS**
  - 40
    - 12 **PHIALOCEPHALA REPENS**
    - Filobasidiella neoformans

— 5 changes

104

Figure 5. Phylogenetic tree produced by PAUP heuristic option of the LSU rDNA with *Filobasidiella neoformans* as outgroup. Species for which only LSU sequence data were obtained are included in this phylogram. Bootstrap values above 50% (percentages of 1000 bootstrap replicates) are indicated in bold below the branches of the tree. Branch length values are indicated above the branches of the tree.
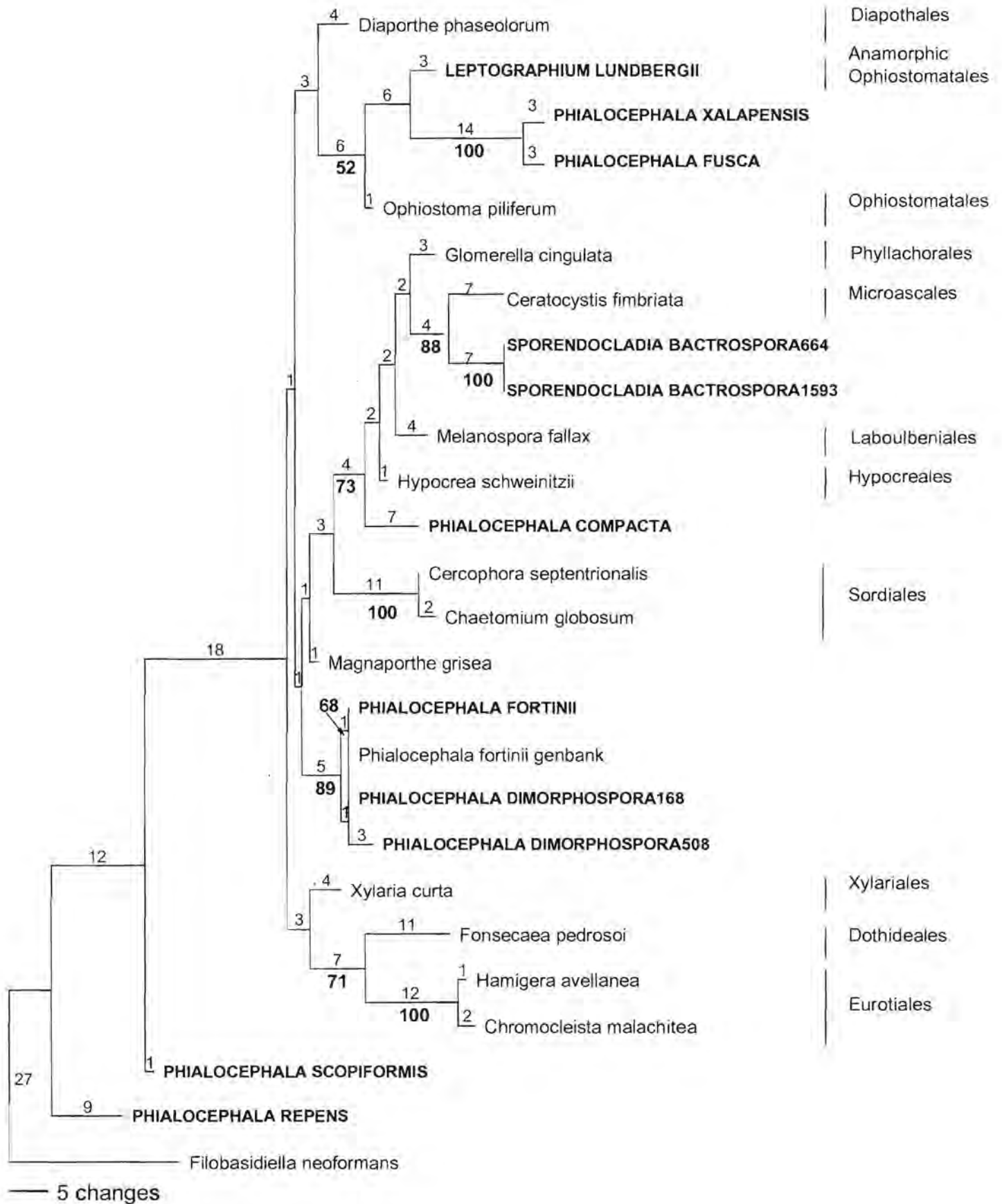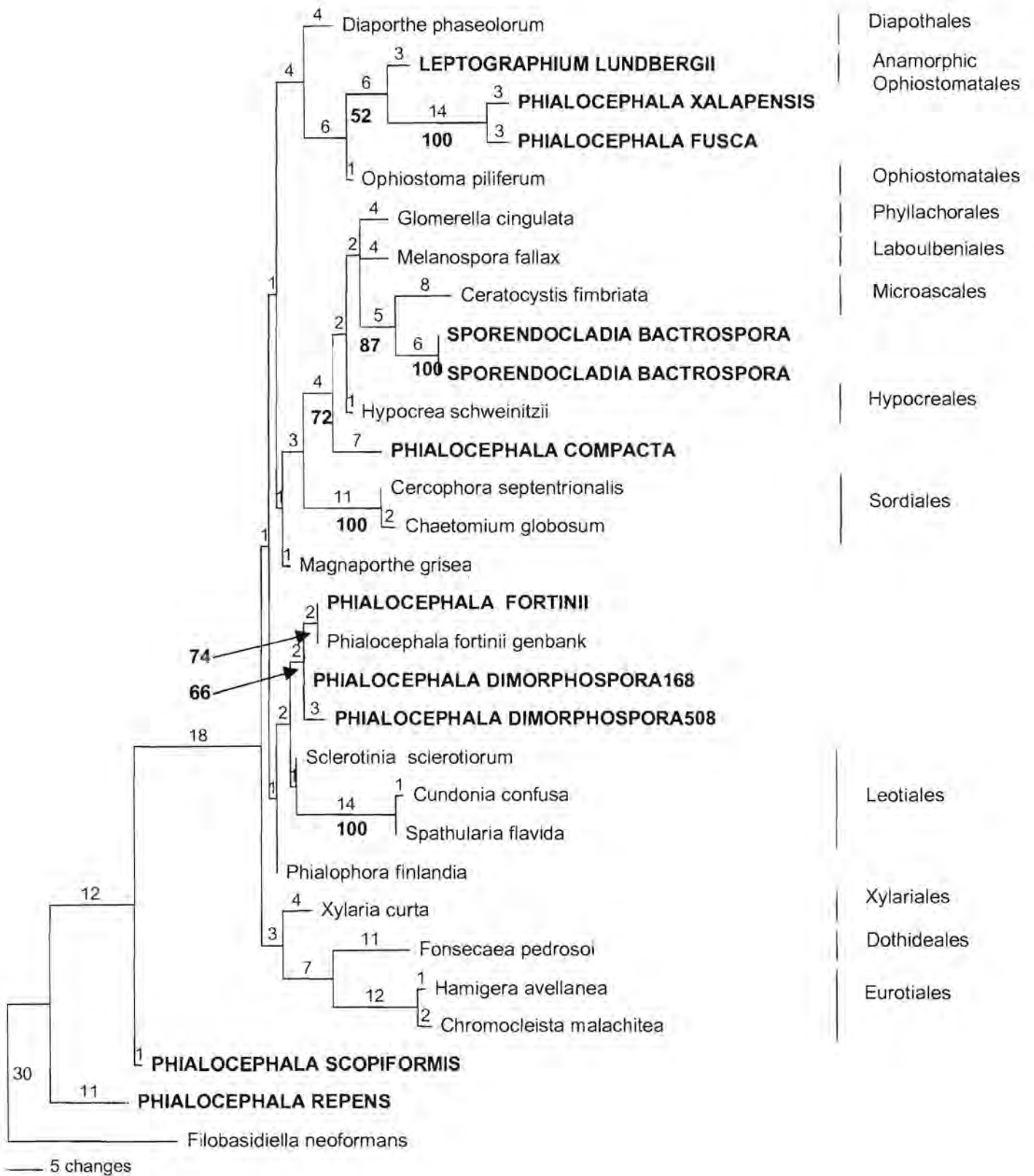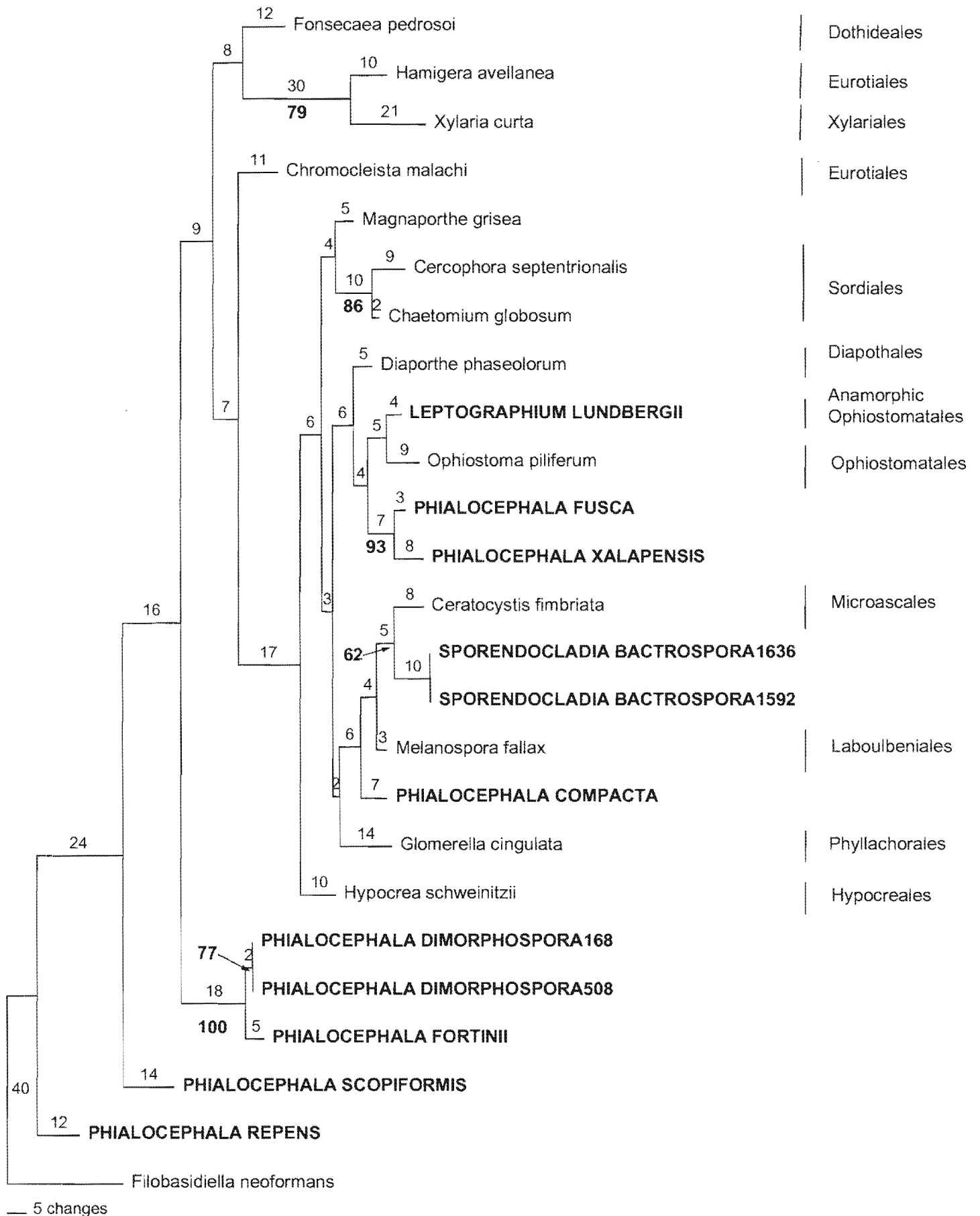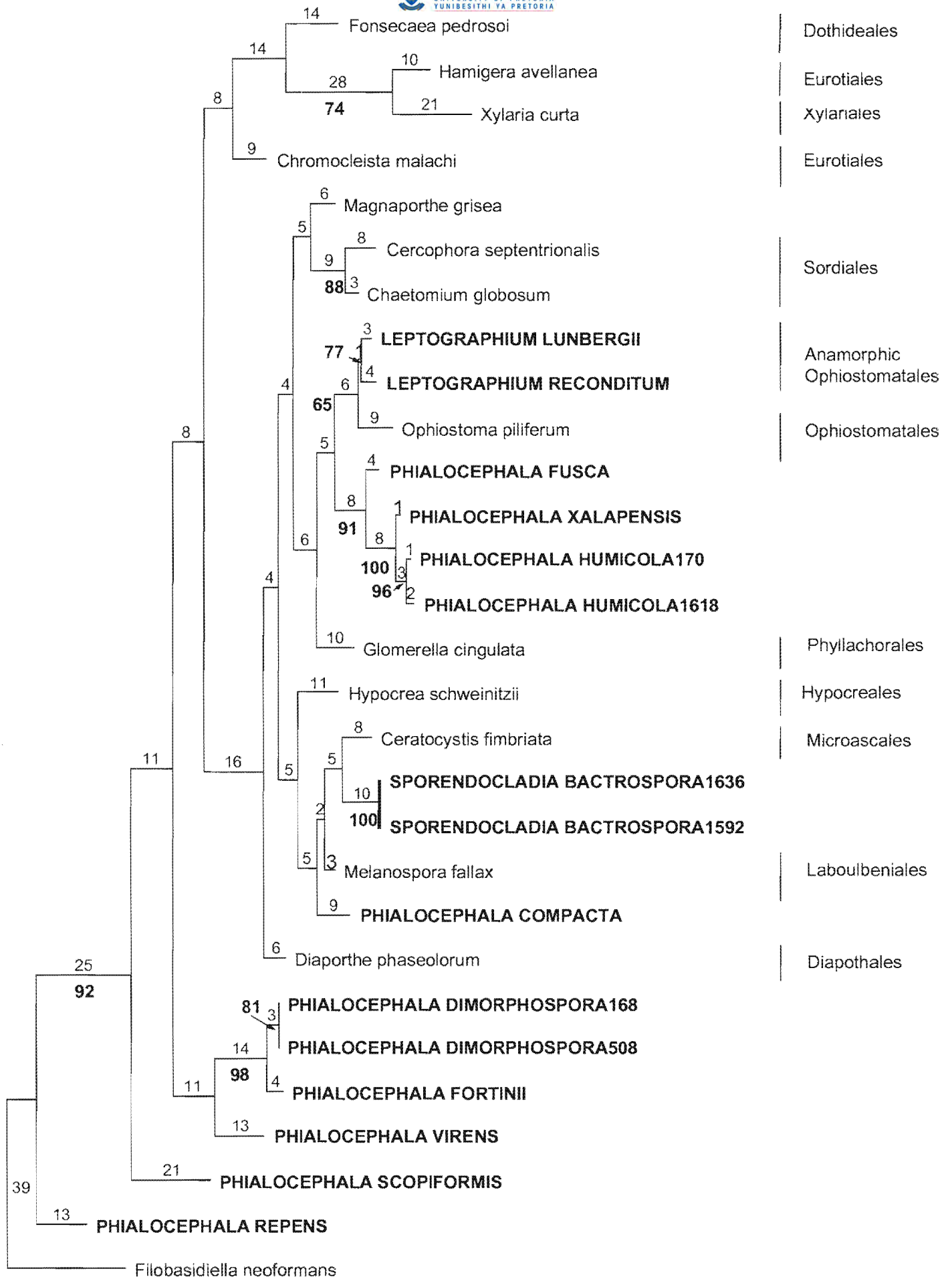
14 Fonsecaea pedrosoi — Dothideales

14

10 Hamigera avellanea — Eurotiales

28

74

21 Xylaria curta — Xylariales

8

9 Chromocleista malachi — Eurotiales

6 Magnaporthe grisea

5

8 Cercophora septentrionalis — Sordiales

9

88 3 Chaetomium globosum

3 **LEPTOGRAPHIUM LUNBERGII** — Anamorphic Ophiostomatales

77

4 **LEPTOGRAPHIUM RECONDITUM**

6

65

9 Ophiostoma piliferum — Ophiostomatales

4

5

4 **PHIALOCEPHALA FUSCA**

8

91

1 **PHIALOCEPHALA XALAPENSIS**

8

100

1 **PHIALOCEPHALA HUMICOLA170**

3

96 2 **PHIALOCEPHALA HUMICOLA1618**

6

10 Glomerella cingulata — Phyllachorales

4

11 Hypocrea schweinitzii — Hypocreales

8 Ceratocystis fimbriata — Microascales

5

5

10 **SPORENDOCLADIA BACTROSPORA1636**

2

100 **SPORENDOCLADIA BACTROSPORA1592**

5 3 Melanospora fallax — Laboulbeniales

9 **PHIALOCEPHALA COMPACTA**

6 Diaporthe phaseolorum — Diapothales

8

16

5

81 3 **PHIALOCEPHALA DIMORPHOSPORA168**

14 **PHIALOCEPHALA DIMORPHOSPORA508**

98

4 **PHIALOCEPHALA FORTINII**

11

13 **PHIALOCEPHALA VIRENS**

11

21 **PHIALOCEPHALA SCOPIFORMIS**

25

92

39

13 **PHIALOCEPHALA REPENS**

Filobasidiella neoformans

— 5 changes

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Summary

Phialocephala was established to accommodate phialidic fungi in the *Leptographium* complex. They are characterized by considerable variation in morphological characters. These morphological characteristics have been used as basis to compile dichomomous and synoptic keys to known species of *Phialocephala*. These keys are supported by comprehensive descriptions accompanied by both photographs and line drawings.

The second chapter deals with the generic placement of *Phialocephala humicola*. *Phialocephala humicola* display annellidic conidiogenesis and the subsequent elongation of the conidiogenese cells. Based on the sequence data obtained from the 28S subunit of the rRNA operon, this species represent a new genus. The new genus is associated with the Ophiostomatales and might be accommodated in *Leptographium*.

The third chapter of the thesis deals with polyphyletic nature pertaining to *Phialocephala*. This study was based on sequence data obtained from the 28S and 18S regions of the rRNA operon. The results indicate that *Phialocephala* represent at least three genera associated with the Leotiales, Ophiostomatales and Microascales, respectively.

This thesis represents a comprehensive study of the morphological and molecular characteristics of the fungi accommodated in *Phialocephala sensu lato*. This genus is clearly polyphyletic. Although not all described species were included in this study, it has shed more light on the diversity and taxonomic problems pertaining to this genus.

Opsomming

Phialocephala is totstand gebring om phialidiese fungi binne die *Leptographium* kompleks te akkomodeer. Hulle word gekenmerk deur geweldige variasie in morfologiese eienskappe. Hierdie eienskappe vorm die basis vir die sleutels wat vir die identifikasie van elke spesie ingesluit is. Die sleutels word ondersteun deur volledige beskrywings, foto's en lynsketse vir elke spesie.

Die tweede hoofstuk handel oor die posisie van *P. humicola* in die Ascomycotina. *Phialocephala humicola* word gekenmerk deur annelidiese konidiogenese en die verlenging van die konidiogenese selle gedurende die vorming van elke konidium. Basispaaropeenvolgingsdata verkry vanaf die 28S subeenheid van die ribosomale RNA operon, dui daarop dat hierdie spesie 'n nuwe genus verteenwoordig. Hierdie genus word geassosieer met die Ophiostomatales, maar kan moontlik ook in *Leptographium* val.

Die derde hoofstuk handel oor die verwantskappe tussen die verskillende *Phialocephala* spesies, gebaseer op molekulêre data. Hierdie data, vanaf die 18S en 28S subeenhede van die rRNA operon dui daarop dat *Phialocephala* ten minste drie genera voorstel. Hierdie drie genera word onderskeidelik met die Leotiales, Ophiostomatales en Microascales geassosieer.

In die tesis word 'n breedvoerige studie van beide die molekulêre en morfologiese eienskappe van *Phialocephala sensu lato* saamgevat. Dit is duidelik dat *Phialocephala* meer as een genus verteenwoordig. Ten spyte van die feit dat nie alle spesies in die tesis behandel is nie, word daar egter meer lig gewerp op die komplekse taksonomiese aard van die genus.