Ministry of Jihad-e-Agriculture
Agricultural Research & Education Organization

A Supplement to:

Rostaniha
(Botanical Journal of Iran)

A Publication of
Plant Pests & Diseases Research Institute

No. 3, 2004 ISSN: 1608-4306

A MONOGRAPH OF *VERTICILLIUM* SECTION *PROSTRATA*

BY:

RASOUL ZARE

AND

WALTER GAMS

Proprietor
Plant Pests & Diseases Research Institute

Executive director
Gh.A. Abdollahi (Associate Prof.) - Director, Plant Pests & Diseases Res. Inst.

Publication permit No.
124/12734

Address
Plant Pests & Diseases Research Institute
P.O. Box 1454, Tehran 19395, Iran
Tel.: + 98 21 2403012-16, 2403688
Fax: + 98 21 2403691
http://www.magiran.com/rostaniha
E-mail: rostaniha@magiran.com

Printed by
Press of the Deputy for Training and Academic Relations,
Agricultural Research & Education Organization,
Ministry of Jihad-e-Agriculture

A Supplement to:

Rostaniha

(Botanical Journal of Iran)

A Publication of
Plant Pests & Diseases Research Institute

No. 3, 2004 **ISSN: 1608-4306**

A MONOGRAPH OF *VERTICILLIUM* SECTION *PROSTRATA*

BY:

RASOUL ZARE

Department of Botany, Plant Pests & Diseases Research Institute
P.O. Box 1454, Tehran 19395, Iran
e-mail: simplicillium@yahoo.com

AND

WALTER GAMS

Centraalbureau voor Schimmelcultures
P.O. Box 85167, 3508 AD Utrecht, The Netherlands
e-mail: gams@cbs.knaw.nl

CONTENTS

PREFACE

In the genus *Verticillium* a heterogeneous assembly of morphologically little differentiated anamorph taxa has been compiled that belong to different families and orders of Ascomycota. We speak of verticillium-like, an expression in which 'verticillium' is rather a descriptive term than a formal genus.

While *Verticillium s. str.* comprises more or less plant-associated species related to *Glomerella* (? Phyllachorales), some diverse verticillium-like fungi belong to the Clavicipitaceae and other families in the Hypocreales. Although being aware of this situation, GAMS (1971 and subsequent papers) refrained from attributing them generic status, because of difficulties in morphological distinction. Thus the group of clavicipitaceous anamorphs retained its status as section *Prostrata* of *Verticillium* until 2001. With the advent of molecular techniques, fungal taxonomy is placed on a much sounder basis and the generic separation of unrelated elements can no longer be avoided.

Thanks to the assiduous work of RASOUL ZARE, a voluminous material of living strains could be reexamined both from the molecular and the morphological angles. Using only nuclear ribosomal DNA fragments, we were fortunate to recognize major lineages and make clear distinctions so that some additional genera and a larger number of species could be delineated than those recognized previously. The resulting breadth of the species concept is intermediate between the splitter approach in many papers by PETCH (mostly according to host organism) and the lumping adopted so far by GAMS.

The verticillium-like members of the Clavicipitaceae comprise many taxa that are important parasites of fungi (including slime moulds), arthropods, nematodes, and even rotifers. They play important roles in natural control of pathogenic and pest organisms and they can sometimes also be applied in biological control. The importance of the Clavicipitaceae is underlined in the book edited by WHITE *et al*. (2003), in which HODGE and GAMS & ZARE contributed review chapters on these anamorphs, where more ecological and applied information is included.

The previous publications by ZARE & GAMS were scattered in a series of articles in *Nova Hedwigia* and a brief survey in *Mycological Research*. It is appropriate to combine them here in one volume to make the whole text better accessible. We are greatly indebted to the copyright holders of these journals (E. Schweizerbart'sche Verlagsbuchhandlung and British Mycological Society) for granting permission to produce this modified reprint. We also greatly appreciate the contributions of our coauthors, G.-H. SUNG, J.W. SPATAFORA, K. HODGE and H.C. EVANS, which made the chapters 2 and 5 possible. The chapters in this book were originally published as:

Summary (p. 5): GAMS, W. & ZARE, R. 2002. New generic concepts in *Verticillium* sect. *Prostrata*. Mycol. Res. 106: 130–131.

Introduction (p. 8) and **Material & Methods** (p. 19): integrated parts of the 6 following papers.

Part 1 (p. 25): ZARE, R., GAMS, W. & CULHAM, A. 2000. A revision of *Verticillium* sect. *Prostrata*. I. Phylogenetic studies using ITS sequences. Nova Hedwigia 71: 465–480.

Part 2 (p. 37): SUNG, G.-H., SPATAFORA, J.W, ZARE, R., HODGE, K.T. & GAMS, W. 2001. A revision of *Verticillium* sect. *Prostrata*. II. Phylogenetic analyses of SSU and LSU nuclear rDNA sequences from anamorph and teleomorphs of the Clavicipitaceae. Nova Hedwigia 72: 311–328.

Part 3 (p. 51): GAMS, W. & ZARE, R. 2001: A revision of *Verticillium* sect. *Prostrata*. III. Generic classification. Nova Hedwigia 72: 329–337.

Part 4 (p. 58): ZARE, R. & GAMS, W. 2001. A revision of *Verticillium* sect. *Prostrata*. IV. The genera *Lecanicillium* and *Simplicillium* gen. nov. Nova Hedwigia 73: 1–50.

Part 5 (p. 110): ZARE, R., GAMS, W. & EVANS, H.C. 2001. A revision of *Verticillium* sect. *Prostrata*. V: The genus *Pochonia*, with notes on *Rotiferophthora*. Nova Hedwigia 73: 51–86.

Part 6 (p. 146): ZARE, R. & GAMS, W. 2001. A revision of *Verticillium* sect. *Prostrata*. VI. The genus *Haptocillium*. Nova Hedwigia 73: 271–292.

We are aware that not all taxa so far classified in sect. *Prostrata* have been adequately reclassified. The refined study of the groups revised here cannot be considered a complete version. Not all available strains have been thoroughly examined and the analysis of additional genetic markers is also expected to yield further refinements in the taxonomic structure. But we hope to have provided a sound basis for such future work.

WALTER GAMS
Utrecht, June 2004

SUMMARY

Section *Prostrata* of the genus *Verticillium* was introduced by GAMS (1971) to separate verticillium-like anamorphs of the Clavicipitaceae from other species of the genus that were known or supposed to have affinities with other families of the *Hypocreales*. Other sections of *Verticillium* were subsequently described by GAMS & VAN ZAAYEN (1982). MESSNER *et al*. (1996) showed that the most important plant pathogens of the genus, which still are most frequently investigated, have affinities with the *Phyllachorales*. Also the type species of the genus, *Verticillium luteo-album* (i.e. *V. tenerum*) has affinities with this order (ZARE *et al*. 2000 – part 1) and its connection with a '*Nectria inventa*' teleomorph supposed by PETHYBRIDGE (1919) is erroneous (ZARE *et al*. 2004). The inclusion of these plant pathogens together with the unrelated, often valuable biocontrol organisms of sect. *Prostrata* in one and the same genus *Verticillium* is undesirable for both scientific and practical reasons. Plant-associated *Verticillium* species recently been studied using RFLPs of the ITS region, β-tubulin and mitochondrial DNA (ZARE 2003) and ITS sequences (BARBARA & CLEWES 2003).

In morphological and molecular work, laid down in a PhD thesis (ZARE 2000), large numbers of isolates were analysed. The strains were first delimited according to RFLP patterns of amplicons of the ITS region, β-tubulin and mitochondrial DNA. The phylogenetic analysis of ITS sequences (ZARE *et al*. 2000 – part 1) then showed that species of section *Prostrata* should be divided into several genera. This conclusion was subsequently corroborated by the analysis of large and small subunits of the ribosomal DNA carried out for a wider array of clavicipitaceous fungi (SUNG *et al*. 2001 – part 2). GAMS & ZARE (2001 – part 3), therefore, distinguished several genera. *Lecanicillium* comprises the majority of the species of the former section *Prostrata*, mainly entomogenous and fungicolous taxa (revised by ZARE & GAMS 2001a – part 4). The same phylogenetic clade also includes the morphologically distinct genera *Beauveria*, *Microhilum* and *Paecilomyces tenuipes*, in relation to which *Lecanicillium* takes a paraphyletic position. A similar genus with generally simple (i.e. not verticillate), very slender phialides, *Simplicillium* (ZARE & GAMS 2001a – part 4), type species *S. lanosoniveum*, is a sister taxon and falls outside this clade. Both genera have teleomorphs

in *Torrubiella*, *Lecanicillium* also in *Cordyceps*. *Pochonia* (syn. *Diheterospora*) comprises parasites of nematode cysts and eggs (ZARE *et al*. 2001 – part 5). *Haptocillium* is introduced for verticillium-like taxa with apically adhesive conidia which facilitate parasitisim of free-living nematodes (ZARE & GAMS 2001b – part 6). In addition, *Rotiferophthora* is recognized as a distinct genus for parasites of bdelloid rotifers. Keys to the species of this genus and of *Harposporium* are provided by GAMS & ZARE (2003).

The most important of the 19 so far recognized species of *Lecanicillium* (ZARE & GAMS 2001a – part 4) are: *Lecanicillium lecanii*, which, however, is defined more narrowly and is restricted to parasites of tropical scale insects. Most other isolates previously identified as *Verticillium lecanii*, including the biocontrol strain Mycotal, are now classified in *L. muscarium*. Similar strains with considerably larger conidia are included in *L. longisporum*, which includes the biocontrol strain Vertalec. *Lecanicillium psalliotae* is also more narrowly circumscribed, comprising isolates with small numbers of sharply pointed falcate conidia produced on each phialide. It is distinguished from the similar and also common *L. dimorphum*, which in addition has numerous microconidia borne on aphanocladium-like denticles. Fast-growing isolates that were hitherto generally misidentified as *Aphanocladium album* also fall in *Lecanicillium* as *L. aphanocladii*, while *A. album sensu stricto* is unrelated with this whole group; it grows more slowly and is known only as a parasite of slime moulds.

The name *Pochonia* predates *Diheterospora*; this name change is deemed justifiable because of the changed generic concept (ZARE *et al*. 2001 – part 5). The most important of the six species of this genus are the well-known parasites of nematode cysts and eggs, *P. chlamydosporia* and *P. suchlasporia*, each with two varieties. A teleomorph of the former, *Cordyceps chlamydosporia*, was discovered on mollusc eggs in Ecuador (EVANS *in* ZARE *et al*. 2001 – part 5, page 117).

In *Haptocillium* seven species are now distinguished and delimited more narrowly than in former work by GAMS (1988) (ZARE & GAMS 2001b – part 6). *Haptocillium balanoides* in the strict sense seems to be rarer than *H. sphaerosporum*. The distinction of *Pochonia* and *Haptocillium*, necessary for phylogenetic reasons, also reflects the ecological difference between parasites of free-living nematodes (and rotifers) and nematode eggs and cysts.

It can be expected that refined studies of more numerous isolates from other parts of the world will reveal a number of additional species in all these genera. However, not all species of the former section *Prostrata* have yet found a satisfactory generic classification.

INTRODUCTION

Verticillium Nees is known to be a very heterogeneous genus. In the present classification, valuable biocontrol agents appear in one anamorph genus together with unrelated serious plant pathogens, such as *Verticillium albo-atrum* Reinke & Berthold and *V. dahliae* Kleb., although they belong to different orders of Ascomycetes. This situation is highly undesirable and, with the assistance of molecular methods, it is now possible to make the necessary distinctions in doubtful cases and to delimit a series of more natural genera.

Verticillium has been linked to several families of the Ascomycota including Clavicipitaceae, Hypocreaceae, and Nectriaceae in the Hypocreales, and taxa related to *Glomerella* in the Phyllachorales (GAMS & VAN ZAAYEN 1982, GAMS 1988, SAMUELS 1988, MESSNER *et al*. 1996, ZARE *et al*. 2000 – part 1). The genus is characterized by phialidic conidiophores produced directly from the mycelium as either erect or prostrate structures. The verticillate phialides are generally aculeate and are typically produced in whorls of three to five. Conidia are hyaline and range in morphology from cylindrical to ellipsoidal to falcate, according to species. In addition, some species produce varying forms of chlamydospores that are thought to serve as resting structures.

Verticillium sect. *Prostrata* W. Gams was introduced by GAMS (1971) for species producing prostrate conidiophores that are often poorly differentiated from the fine, white or yellowish mycelium. This section comprises some very frequent and ubiquitous saprotrophic and parasitic fungi, which can attack insects and other arthropods, nematodes and other invertebrates, and various fungi. The fungi so far included in *Verticillium* sect. *Prostrata*, anamorphs of Clavicipitaceae, are only distantly related to others accommodated in the same genus, which represent anamorphs of Phyllachorales and other families of the Hypocreales. For this reason the distinction of clavicipitaceous anamorphs from plant-pathogenic and plant-associated species of *Verticillium* has become an urgent problem.

This unrelatedness has been known for more than two decades, but the limited morphological differentiation of these anamorphs has so far not allowed a more accurate classification reflecting phylogenetic relationships. GAMS & VAN ZAAYEN (1982), when segregating further sections within *Verticillium*, were aware of this situation but felt unable to distinguish genera, because the morphological boundaries were not sufficiently clear. Even the prostrate conidiophores of *Verticillium* sect. *Prostrata*, which, in addition to the white fluffy colonies, were taken as major criteria characterizing this section, turned out to be unreliable, when species like *V. suchlasporium* W. Gams & Dackman were found to have mostly erect and well-differentiated conidiophores.

Verticillium sect. *Prostrata* includes the majority of entomogenous and nematophagous species and some fungicolous taxa within the genus. Hosts of the entomogenous (*s.l.*) species include species of Arachnida, Coleoptera, Homoptera, Diptera, Thysanoptera and Lepidoptera (GAMS 1971). Hosts of the fungicolous include a variety of Ascomycota and Basidiomycota, including fleshy basidiomata of Hymenomycetes and rusts of the Urediniomycetes (VAN ZAAYEN & GAMS 1982, LIM & WAN 1983, GAMS *et al.* 2004). Nematophagous species of *V.* sect. *Prostrata* have a fairly broad host range, but species specialize on either adults or cysts (BARRON 1977, GAMS 1988). Many species, however, are isolated from soil samples and plant litter and the exact nature of their nutritional mode and potential host affiliation is unknown. The host range of *V.* sect. *Prostrata* is therefore quite diverse and the patterns and processes by which this host range arose are largely unknown. Like other fungi of the Clavicipitaceae, species of *V.* sect. *Prostrata* are the subject of numerous biological control studies, including those of nematodes (MANKAU 1980, KERRY & CRUMP 1977), arthropods (SCHULER *et al.* 1991, VESTERGAARD *et al.* 1995), and fungal pathogens (SAKSIRIRAT & HOPPE 1991, VERHAAR *et al.* 1996). A more accurate understanding of their systematics and phylogenetic affinities is desirable for the design of biological control experiments and the application and introduction of such agents in nature.

Recognition of monophyletic lineages within *V.* sect. *Prostrata* is also complicated by the fact that several other anamorph genera, including *Tolypocladium*, *Engyodontium*, *Aphanocladium*, and *Acremonium*, are morphologically similar to *V.* sect. *Prostrata* and are hypothesized to be closely related, but the exact nature of their relationships remains disputed (reviewed in ZARE *et al*. 2000 – part 1, SUNG *et al.* 2001 – part 2).

The type species of *Verticillium* sect. *Prostrata* (GAMS 1971) is the anamorph of *Cordyceps militaris* (L. : Fr.) Link, with ellipsoid conidia partly adhering in chains, and partly in slimy heads; but the core of the section is formed by the complex species *Verticillium lecanii* (A. Zimmerm.) Viégas with cylindrical and *V. psalliotae* Treschow with falcate conidia. Several other species of *V.* sect. *Prostrata* have been linked to entomopathogenic (e.g., *Torrubiella confragosa* Mains) and fungicolous (e.g., *Cordyceps ophioglossoides* (Fr.) Link) teleomorphs of the Clavicipitaceae. The majority of species within the section, however, are only known from anamorphs and their relationship to teleomorphs of the Clavicipitaceae is speculative.

Most of the synonyms listed by GAMS (1971) for *V. lecanii* had originally been placed in *Cephalosporium*, a genus of doubtful application (GAMS 1971), which is usually regarded as a synonym of *Acremonium* Link.

The conidial heads of species of *Verticillium* sect. *Prostrata* can be rather dry and few-spored, and the conidia often characteristically move into a transverse position on the tip of the phialides (LA TOUCHE 1947). While most species of the section have conidia adhering in heads, some more species were included by GAMS (1971) that form dry conidial chains. These taxa have not yet been critically reexamined.

Other species with white, fluffy colonies forming consistently solitary phialides, such as *Simplicillium obclavatum* (syn. *Acremonium obclavatum* W. Gams) (in GAMS *et al*. 1984), are obviously closely related to this group but have been segregated into *Acremonium* sect. *Albolanosa* Morgan-Jones & W. Gams (MORGAN-JONES & GAMS 1982) because of the complete absence of verticillate branching. The remaining species originally placed in that section are now classified in *Neotyphodium* (GLENN *et al.* 1996), a genus introduced for mostly sparsely sporulating anamorphs of *Epichloë* in the Balansieae, to which the readily sporulating *A. obclavatum* is not closely related.

Some species that are often found as parasites of nematode eggs, produce an additional form of pluricellular resting spores termed dictyochlamydospores (e.g. Figs 5-3, 5-6). The ontogeny of these dictyochlamydospores was studied by CAMPBELL & GRIFFITHS (1975). Species with dictyochlamydospores were accommodated by BARRON & ONIONS (1966) in the genus *Diheterospora* Kamyschko. Such species are mainly parasites of nematode cysts (GAMS 1988). However, because the formation of dictyochlamydospores is erratic, in some species very common, in others rare or absent, GAMS (1971, 1988) did not consider this criterion suitable for a generic distinction. Furthermore, a wide variety of conidial shapes, ranging from subglobose to falcate, is seen in species that produce dictyochlamydospores as well as in species lacking them. A generic classification based on the fact of dimorphic sporulation also appeared to be an unreliable solution.

Dictyochlamydospores were also seen by GAMS (1988) in *Haptocillium balanoides* (syn. *Verticillium balanoides* (Drechsler) Dowsett *et al.*), but they could not be found again in older strains from culture collections. This species was characterized by apically truncate conidia with a thickened wall layer that facilitates external adhesion to eelworms, a situation comparable with the extreme modification seen in *Drechmeria coniospora* (Drechsler) W. Gams & H.B. Jansson (GAMS & JANSSON 1985, GAMS 2003), which is also recognized as belonging to the Clavicipitaceae (GERNANDT & STONE 1999); several debatable synonyms were listed for *V. balanoides* by Gams (1988) and some of them are now reinstated as species in part 6.

Highly differentiated, often flat, dictyochlamydospores are commonly produced by some parasites of bdelloid rotifers. BARRON (1991) introduced a separate genus, *Rotiferophthora*, for these species. Members of this genus, in addition, have a tendency to form intercalary phialides with a short lateral conidiiferous neck. This group and the species around *V. balanoides* comprise slow-growing fungi that are sometimes difficult to grow in culture. On morphological grounds, it is easy to envisage that *Rotiferophthora* is derived from nematophagous species with dictyochlamydospores. The *Rotiferophthora* species were keyed out by GAMS & ZARE (2003).

The genus *Aphanocladium* was introduced by GAMS (1971: 196) for *Acremonium album* Preuss, a characteristic mould commonly found on myxomycetes. The swollen, quickly collapsing conidiogenous cells, then called 'aphanophialides', were interpreted as phialides, although they only produce single conidia (GAMS 1973). Similar fungi with faster growing colonies, that were identified as the same species by GAMS (1971) but are more likely to represent *Aphanocladium aranearum* (Petch) W. Gams, are commonly found on agarics and other substrata. A molecular study by O'DONNELL (in GAMS *et al.* 1998) and our observations indicated that the fungi formerly included in *Aphanocladium* are heterogeneous, some of them related to *L. psalliotae*, the others belonging to a Hypocreales clade near *Verticimonosporium* Matsushima.

A further subdivision of *Verticillium* has been suggested by several authors who used molecular, biochemical and physiological evidence (JACKSON & HEALE 1985, JUN *et al.* 1991, BIDOCHKA *et al.* 1999, KOUVELIS *et al.* 1999, ZARE *et al.* 1999), without leading to a conclusive classification.

Molecular phylogenetics holds great promise in developing more robust phylogenetic hypotheses through the integration of anamorphic and teleomorphic fungi in systematic studies (TAYLOR 1993, 1995). Broad taxon sampling of representatives with diverse life histories will not only result in more accurate hypotheses of relationships (BLACKWELL & SPATAFORA 1994), but they will also result in a better understanding of the evolution of morphologies and ecologies, such as host affiliation and nutritional mode.

In a phylogenetic study of ITS rDNA, ZARE *et al*. (2000 – part 1) revealed at least three distinct groups of *V.* sect. *Prostrata* that were consistent with differences in conidium and phialide morphology, host affiliation, and, to a lesser extent, production of dictyochlamydospores. Their data also supported a phylogenetic affinity with the Clavicipitaceae, but few teleomorphs were included in their analyses. ZARE *et al.* (2000 – part 1) provided a thorough insight into the polyphyly of *V.* sect. *Prostrata* and laid the foundation for an eventual revision of its nomenclature. SUNG *et al.* (2001 – part 2) confirmed the trends recognized in the first study by analysing different genome sequences among a wider range of taxa of Clavicipitaceae. As a consequence, several genera have been distinguished in part 3.

The genus *Lecanicillium* W. Gams & Zare (GAMS & ZARE 2001 – part 3) has been introduced to accommodate entomogenous and fungicolous verticillium-like anamorphs of Clavicipitaceae previously classified in *Verticillium* section *Prostrata*. It comprises the major cluster distinguished within that section in molecular analyses by ZARE *et al.* (2000 – part 1) and SUNG *et al.* (2001 – part 2). In a selection of strains analysed by comparing sequences of the internal transcribed spacer region (ITS), the cluster received 99% Jackknife support as a monophylum (ZARE *et al.* 2000 – part 1), but when more taxa were compared it appeared paraphyletic as it also includes the genera *Beauveria* Vuill. and *Microhilum* H.-Y. Yip & A.C. Rath and several species of *Paecilomyces* Bain. (now *Isaria* Pers. : Fr., according to HODGE *et al.*, in press). These genera were so different morphologically that none of them appeared suitable to accommodate the verticillium-like anamorphs involved. GAMS & ZARE (2001 – part 3) therefore introduced the genus *Lecanicillium*, although it is paraphyletic in relation to the other genera of the cluster.

A morphologically similar group consists of four species with exclusively or predominantly solitary phialides. According to ITS sequences (ZARE *et al.* 2000 – part 1), these three species form a monophylum basal to the other taxa of *Lecanicillium* that could possibly be included in the genus; according to sequences of small and large subunits of ribosomal DNA (SSU and LSU) of a broader sample (SUNG *et al.* 2001 – part 2), this group falls outside the main cluster with 100% bootstrap support and is less related to *Lecanicillium* than the beauveria-like and paecilomyces-like taxa mentioned. Therefore we have decided to introduce a new genus, *Simplicillium*, for this group, despite its difficult morphological distinction (part 4).

The most representative members of *Lecanicillium* fall into two subgroups, species related to *Verticillium lecanii* (Zimm.) Viégas with ellipsoidal–cylindrical conidia, and those related to *V. psalliotae* Treschow with more or less fusiform–falcate conidia. This grouping is reflected in the phylogenetic cladograms (Figs 1-1, 4-1). The members of the aggregates around these two species were investigated in detail by examining large numbers of isolates obtained from diverse geographical areas and hosts. The recognized species are delineated based on an integration of morphological observations with molecular groupings. Most species of *Lecanicillium* are clearly distinct, both morphologically and at the molecular level.

GODDARD (1913), in the oldest description of *Pochonia chlamydosporia*, the type species of this genus, used the generic name *Verticillium*. He was obviously aware of the fact that the phialidic anamorph gives more information about natural affinities of such fungi than the chlamydosporic synanamorph. BARRON & ONIONS (1966) used the name *Diheterospora* Kamyschko (KAMYSCHKO 1962) for this group, disregarding the fact that the genus was not validly published in 1962. No generic type species was indicated in the original work rendering the genus invalid (Art. 37 of the International Code of Botanical Nomenclature), only BARRON & ONIONS (l.c.) designated a type, thus validating the genus. GAMS (1971) therefore concluded that *Diheterospora* dated from 1966 and is predated by *Pochonia*. GAMS (1971, 1988) did not regard the distinction of this genus from *Verticillium* sect. *Prostrata* as workable, because it was based only on the presence or absence of dictyochlamydospores. The recent molecular studies clearly show the justification of a separate genus. The circumscription of this genus deviates considerably from the previous concept of *Diheterospora* and, therefore, we advocate the change to the simpler and nomenclaturally correct name, *Pochonia*, rather than proposing conservation of the name *Diheterospora* (GAMS & ZARE 2001 – part 3). Other generic names used for representatives of this group are inappropriate: *Stemphyliopsis* A.L. Smith was based on an albino mutant of *Stemphylium*. *Dictyoarthrinopsis* Bat. & Cif. was probably based on a fungus with little-differentiated chlamydospore masses, unrelated to the dictyochlamydospores of *Pochonia*.

In the new genus *Haptocillium* we accommodate nematophagous species so far classified in the genera *Acrostalagmus* Corda and *Verticillium* Nees (formerly also in *Cephalosporium* Corda and *Spicaria* Harting). DRECHSLER (1941) described three of these species, *Acrostalagmus bactrosporus*, *A. obovatus*, and *Cephalosporium balanoides* on the nematodes *Plectus parvus* and *Acrobeloides buetschlii*; a fourth species that he described from nematodes, *Spicaria coccospora*, is of uncertain identity (see below under *H. sinense*). Five years later, DRECHSLER (1946) added one more species, *Acrostalagmus zeosporus*. DRECHSLER generally did not grow his fungi in pure culture and no type material is preserved from his studies (A.Y. ROSSMAN, pers. comm.), but his meticulous drawings serve as iconotypes. BARRON (1989) added a further species, *Verticillium coronatum* G.L. Barron, that probably belongs to this group. But this species escaped our attention and no material could be examined. This species would be unique in the genus in having mostly five apical adhesive buds on the ovoid conidia, which measure 3.5–5.0 × 2.2–2.5 µm.

SUBRAMANIAN (1977) schematically combined *Acrostalagmus bactrosporus* and *A. obovatus* into *Verticillium* and *Cephalosporium balanoides* into *Acremonium*. DOWSETT *et al*. (1982) redescribed *Cephalosporium balanoides* Drechsler carefully and transferred it to *Verticillium*. GAMS (1988) subsequently revised the group of nematophagous *Verticillium* species from a morphological point of view. He compiled much ecological information on these fungi that is not recapitulated here. Gams recognized the close affinity of the species with adhesive conidia and, adopting a rather broad species concept, subsumed the four species described by Drechsler under one name, *Verticillium balanoides*. GLOCKLING & DICK (1997) contested GAMS's wide species concept, but did not provide any concrete arguments to disprove it. They studied some fresh isolates of similar fungi obtained from nematodes and produced a key, in which they did not distinguish between species having adhesive conidia and species lacking them. A few isolates studied by these authors were available to us, but unfortunately no representative isolate was available of the new species *Verticillium seriatum* Glockling & Dick, which seems to be very close to *V. coccosporum* (Drechsler) W. Gams. GLOCKLING & DICK (l.c.) identified an isolate (preserved as CBS 101433) as *V. bactrosporum* (Drechsler) Subram., which was reclassified by ZARE *et al*. (2001 – part 5) as *Pochonia microbactrospora* because it lacked adhesive conidia. The original *Acrostalagmus bactrosporus* Drechsler (DRECHSLER 1941) obviously is distinct from that species because DRECHSLER clearly illustrated conidia attached to the nematode with the adhesive end. Another of GLOCKLING & DICK's isolates, described here as *Haptocillium rhabdosporum*, differs from DRECHSLER's diagnosis of *A. bactrosporus* by having larger conidia. We do not have material that exactly matches his description of *A. bactrosporus*, although *H. campanulatum* seems to be closest to it.

KEY TO THE GENERA TREATED AND SOME RELATIVES (modified from GAMS & ZARE 2003). Genera treated here are printed in bold.

0. Conidiophores erect and well-differentiated (stipe usually thick-walled); dictyochlamydospores absent ....former *Verticillium* section *Albo-erecta* and other groups (not treated here)
See also *Pochonia suchlasporia* and *Verticillium pseudohemipterigenum* (part 5)
0´. Conidiophores usually prostrate, sometimes also erect but not thick-walled, often hardly differentiated from vegetative hyphae; (dictyo-)chlamydospores present or not **1**

1. Conidiogenous cells with swollen, often almost globose venter and sharply delimited slender neck... **2**
1´. Conidiogenous cells aculeate or with moderately inflated venter or of reduced shape **4**

2. Phialides bearing curved phialoconidia on one or several necks; generally parasitizing nematodes or bdelloid rotifers.. ....*Harposporium* (key in GAMS & ZARE 2003)
2´. Phialides bearing globose to cylindrical conidia, usually from single necks **3**

3. Conidiophores with rather dense clusters of phialides; soilborne or entomogenous species, rarely associated with nematodes.. *Tolypocladium*
3´. Conidiophores with scattered terminal and lateral phialides; on aquatic flies and rotifers ....*Culicinomyces*

4. Intercalary conidiogenous cells (mostly phialides) with short conidiiferous necks commonly produced **5**
4´. Intercalary conidiogenous cells absent (or rarely formed in *Haptospora*) **8**

5. Intercalary phialides mostly produced singly, frequently in verticillate end-branches of the conidiophore; a conspicuous oil globule present in each conidium; parasites of rotifers; dictyochlamydospores present, often flattened ....*Rotiferophthora* (key in GAMS & ZARE 2003)
5´. Several intercalary conidiogenous cells produced below a terminal one; dictyochlamydospores absent... **6**

6. Conidiogenesis phialidic with single openings **7**

6´. Conidiogenesis polyblastic; conidia globose, with a conspicuous basal slime pad; parasitizing rotifers .... *Pseudomeria mucosa*

7. Conidiophores erect; obclavate conidia in heads, with adhesive tip; parasitizing nematodes or ciliated protozoa .... *Drechmeria*
7´. Conidiophores more or less prostrate; obovoid conidia in chains .... *Pleurodesmospora*

8. Conidiogenous cells flask-shaped; discrete dictyochlamydospores absent **9**
8´. Conidiogenous cells hardly swollen, aculeate, often in whorls; dictyochlamydospores often present **14**

9. Conidiogenous cells phialidic, with a flaring collarette; conidia with basal appendage .... *Haptospora*
9´. Conidiogenous cells phialidic or with solitary conidia, apparently blastic, lacking a discernable collarette; conidia lacking a basal appendage **10**

10. Conidia adhering in regular dry chains .... *Isaria* (*Paecilomyces*)
10´. Conidia adhering in heads or formed singly **11**

11. Conidiophores synnematous or mononematous; conidia with a distinct, chromophilic slime layer or covered by a finely warted epispore, often somewhat fusiform.. *Hirsutella*
11´. Conidiophores mononematous; conidia thin- and smooth-walled **12**

12. Conidiogenous cells single, with only the tips protruding from the nematode; in vitro single swollen phialides supported by slender stalks .... *Plesiospora*
12´. Complex conidiophores appearing outside the host animal, more or less verticillate ... **13**

13. Conidia with a distal adhesive surface, appearing as a wall thickening; parasites of nematodes .... ***Haptocillium***
13´. Conidia lacking an adhesive structure; some species parasitizing rotifers .... *Tolypocladium*

14. Conidiophores erect and with differentiated stipe (usually somewhat thick-walled) .... see ***Pochonia suchlasporia***
14´. Conidiophores usually prostrate, sometimes also erect, but hardly differentiated from vegetative hyphae **15**

15. Conidiogenesis polyblastic, with conidia either on sympodially produced denticles of terminal conidiogenous cells or on densely crowded, rapidly collapsing denticles laterally along intercalary cells of prostrate fertile hyphae; dictyochlamydospores absent **16**

15´. Conidiogenesis phialidic; phialides aculeate, more or less persistent, each producing several conidia; dictyochlamydospores present or absent **17**

16. Conidiiferous denticles persistent, mostly in terminal position, sometimes inserted next to discrete conidiogenous cells .... see *Beauveria* and *Microhilum* (entomogenous taxa)

16´. Conidiiferous denticles scattered along cells of fertile hyphae, soon collapsing ('aphanophialides'); colonies deeply woolly .... aphanocladium-like species of ***Lecanicillium***

17. Colonies slow-growing, reaching 5-15 mm diam. in 10 d; parasites of free-living nematodes or rotifers **18**

17´. Colonies growing faster, reaching 15-40 mm diam. in 10 d; growing on insects or fungi; if attacking nematodes, then parasitizing cysts or eggs **19**

18. Parasites of bdelloid rotifers; intercalary phialides with a lateral neck normally present below terminal, flask-shaped or elongate phialides; conidia adhering in heads; a conspicuous oil globule present in each conidium; dictyochlamydospores commonly present, often flattened .... see *Rotiferophthora*

18´. Parasites of free-living nematodes; conidia balanoid, campanulate to cylindrical, subglobose to irregularly angular, mostly terminally adhesive (visible as a wall thickening at the upper, more or less truncated end), produced in heads or short chains or both; sesquiphialides absent; dictyochlamydospore-like structures sometimes present .... ***Haptocillium***

19. Phialides exclusively solitary (if verticillate, conidia narrowly acerose); dictyochlamydospores absent .... ***Simplicillium***

19´. Phialides at least partly in whorls **20**

20. Conidia subglobose to short-ellipsoidal, sometimes short-falcate, often cyanophilic; dictyochlamydospores spherical or irregularly shaped, often present in the aerial mycelium or in the agar; mostly parasites of nematode cysts or saprotrophic, soilborne; crystals absent in the medium .... ***Pochonia***

20´. Conidia short- or long-ellipsoid to cylindrical or falcate, not conspicuously cyanophilic; chlamydospores or dictyochlamydospores absent; crystals abundantly produced in the medium; sporulation with aculeate phialides predominant; denticles with blastoconidia, if present, densely scattered along the cells of fertile hyphae; on various substrata (mostly entomogenous, fungicolous, or soilborne) .... ***Lecanicillium***

MATERIAL AND METHODS

Fungal strains (tabulated and listed in the subsequent parts) were obtained from diverse geographical areas and from different hosts/substrata. Strains were maintained on potato-carrot agar (PCA) slants at 4°C. The majority of the isolates preserved at CBS and IMI culture collections and some fresh isolates obtained from H.C. EVANS were studied, both morphologically and with molecular methods. In this way, additional teleomorph connections were ascertained. The large number of isolates available was first screened with RFLP methods (see ZARE *et al*. 2000 – part 1).

Morphological studies

Colony growth rate and morphology (shape, colour, height) were recorded on streak-inoculated cultures grown on potato-dextrose agar (PDA, Oxoid). Conidiophore structure and branching, conidia, phialides and resting structures were recorded on PCA. Undisturbed cultures were examined in open Petri dishes under a compound microscope to observe the overall structure and branching pattern. The temperature optima for ex-type or representative isolates were assessed in incubators with 3°C intervals on malt extract agar for streak-inoculated cultures growing on 2% malt extract agar (MEA, made of malt extract from a brewery, adjusted to 2% sugar concentration and pH 7) in 90 mm diam. plastic Petri dishes incubated at 21, 24, 27, 30, 36, and 40°C for 10 days in the dark. If values reached at different temperatures did not differ by more than 2–3 mm, the temperature optimum is noted as a range. Growth rates on MEA were often less than those on PDA; therefore, the growth rates used in colony descriptions can deviate somewhat from those given for temperature relationships. The formation of crystals could be clearly seen on potato-carrot agar (PCA) after 1–2 weeks. Other features were observed and recorded on PDA plates inoculated with agar blocks (2 × 2 × 1 mm) taken from 10–20-day-old PCA plates. To record changes with age, PDA plates were checked 3 times at 10-day intervals. Colony diameter and height, pigmentation and pigment diffusion into the agar, were recorded each time. Unless otherwise indicated, all colony descriptions in parts 3–6 are from colonies grown at 24 ± 1°C. For microscopic examination, cultures on PCA were incubated at 24 ± 1°C in the dark. Observations were made from the fifth day onwards. Conidiophore branching and conidial arrangement were generally observed in open Petri dishes with a 10× objective of the compound microscope. For this purpose colonies grown on PCA or soil extract agar (SEA) are most suitable. Slide preparations were mounted in lactic acid-cotton blue. Structures were measured from freshly prepared slides under a 100 × lens of a BH2 Olympus microscope using a camera lucida. Twenty or more conidia were measured for each strain after 10 days. Phialide length and width, hyphal width, presence and location of resting structures, and production of crystals were also recorded. Photographs were taken on a Leitz microscope equipped with Nomarski differential interference contrast (DIC).

Molecular studies

Glucose-Yeast Medium (GYM, MUGNAI *et al.* 1989) was used to produce mycelium for DNA extraction (for details see ZARE *et al.* 1999). Harvested cultures were stored at –20°C and then lyophilized. Biomass was prepared for extraction by grinding up the freeze-dried material in an alcohol-sterilized mortar and pestle.

DNA extraction: DNA was extracted from both dried herbarium specimens and live cultures. Crude DNA extracts were prepared using the method of RAEDER & BRODA (1985) or modified CTAB method (GARDES & BRUNS 1993, SPATAFORA *et al.* 1998). To assess the quality of the extracted DNA, the DNA was diluted 1:9 in loading buffer (PATERSON & BRIDGE 1994) and loaded on 1% (w/v) SeaKem LE agarose (FMC BioProducts, Rockland, USA) gels in TBE buffer (0.089 M Tris-Base, pH 8.3, 0.089 M boric acid, 0.002 M EDTA in a minigel tank (Hybaid Ltd).

Polymerase chain reaction and DNA sequencing: ITS regions, including parts of the small and large nuclear rDNA genes and the central 5.8S rDNA gene, were amplified and cleaned using Quiagen PCR cleanup columns (Quiagen Ltd, Crawley, UK) according to the manufacturer's instructions. Primers ITS1F (GARDES & BRUNS 1993) and ITS4 (WHITE *et al.* 1990) were used for PCR amplification and sequencing, as previously described (ZARE *et al.* 1999). A PE

Biosystems ABI Prism 377 Automated DNA Sequencer was used, with dRhodamine dye terminators incorporated by Amplitaq DNA polymerase FS from PE Biosystems. The dideoxy-nucleotide chain termination method of sequencing (SANGER *et al.* 1977) was applied. The PCR products were sequenced in both directions. Primers ITS3 and NL4a (G. HAGEDORN, unpubl.) were applied to sequence ITS-2 in *H. rhabdosporum*.

To integrate *V.* sect. *Prostrata* into a database of clavicipitalean fungi (part 2), 1150 bp of the small subunit (SSU) and 950 bp of the large subunit (SSU) nuclear ribosomal DNA (nrDNA) were amplified in the conventional polymerase chain reactions (PCR) (MULLIS & FALOONA 1987). The SSU rDNA was amplified with primers NS1 and NS4 (WHITE *et al*. 1990). The LSU rDNA was amplified with primers LR0R and LR5 (VILGALYS & SUN 1994). PCRs were performed in 50-µl reactions as follows: [94ºC (1 min), 50–52ºC (30 sec), 72–73ºC (1 min)] × 35–40 cycles. Success of PCRs was confirmed by agarose gel electrophoresis of 5 µl of the reaction mix. PCR products of SSU and LSU rDNA were purified using QIAquick PCR purification kits (Qiagen Inc., Valencia, CA). Purified PCR products were visualized on a 1% agarose gel stained with ethidium bromide and quantified using Gibco-BRL low DNA Mass Ladder. The purified product was sequenced using ABI Prism BigDye Terminator Cycle Sequencing chemistry with AmpliTaq DNA polymerase, FS on an ABI Prism Model 377 (version 2.1.1) automated DNA sequencer (Perkin-Elmer) at the Central Services Laboratory of the Center for Gene Research and Biotechnology at Oregon State University. The template strands of purified PCR-products were directly sequenced utilizing the primers NS1, SR7, NS3, NS4 for SSU rDNA and LR0R, LR5 for LSU rDNA (WHITE *et al*. 1990, VILGALYS & SUN 1994).

Phylogenetic analyses: For ITS region the programs EditSeq and MegAlign, parts of the DNASTAR Lasergene 1994 software package for Macintosh, were used for editing and aligning the sequence files. The alignments were initially constructed using the CLUSTAL option in MegAlign and adjusted manually. Gaps or indels (insertions/deletions) were coded as extra character states using MacClade

(MADDISON & MADDISON, 1992). The indels were treated as single events and coded as binary characters in subsequent analyses. Parts of the sequences in which homology could not be ascertained were excluded from further analysis. The beginnings and ends of the 18S, 28S and 5.8S rRNA genes and spacers were determined according to the sequences reported by MUGNIER (1994).

For the small and large subunits of ribosomal DNA, DNA sequences were edited in SeqEd Ver. 1.0.3, manually aligned using a color font, and appended to a preexisting data set of the Clavicipitaceae and other perithecial fungi (SPATAFORA *et al*. 1998). The phylogenetic analyses were performed with PAUP* 4.0 (SWOFFORD 1998). Parsimony analyses were conducted on the combined data set of SSU and LSU nrDNA using the following heuristic search options: 100 replicates of random sequence addition, TBR (Tree bisection-reconnection) branch swapping, and MULTRE in effect. Insertions and deletions (indels) were minimized in alignments and gaps were treated as missing data in the analyses. Ambiguously aligned sequence regions were excluded from the data matrix before analysis. Weighted parsimony analyses were performed using a step-matrix to weight nucleotide transformations based on the reciprocal of the observed transition : transversion (TN:TV) ratio. TN:TV ratios were estimated a-priori from an average of pairwise comparisons calculated in PAUP using the 'PAIRWISE Base Differences' command. Relative support for the resulting trees was determined by 2500 bootstrap replications on informative characters only with the previously mentioned search options except that only one tree was retained during each replication (MONCALVO *et al*. 2000). The phylogenetic trees generated from the combined data set with SSU and LSU nrDNA data sets were rooted with *Xylaria curta* and *X. hypoxylon*.

To test alternative phylogenetic hypotheses for *V.* sect. *Prostrata*, i.e., monophyly of *V.* sect. *Prostrata*, constraint topologies were constructed in MacClade 3.0 (MADDISON & MADDISON 1992). Constraint topologies forced the monophyly of *V.* sect. *Prostrata*, but left all other nodes of the tree as unresolved. These topologies were used as starting trees in maximum parsimony analyses; search options were as described above except that maxtrees was set to 1000. The most parsimonious trees recovered from the constraint analyses were statistically compared to the trees recovered from the maximum and weighted parsimony analyses using the Templeton WSR test implemented in PAUP* 4.0b3 (SWOFFORD 1998). Host affiliation was mapped in part 2 onto one of the most parsimonious trees using MacClade 3.0 (MADDISON & MADDISON 1992) with equal weights for all character state transformations.

SPECIAL SECTION

PART ONE:
PHYLOGENETIC STUDIES USING ITS SEQUENCES

The strains examined are summarized in Table 1-I, with additional sequences downloaded from GenBank in Table 1-II.

Results of ITS analyses

Variation of amplicon length: The total length of ITS-1–5.8S–ITS-2 across *Verticillium s. l.* varied from 476 bp in IMI 130213 (*V. nubilum*, clade A) to 558 bp in CBS 248.83 (*V. suchlasporium* var. *catenatum*, clade D). The 5.8S gene is very conserved both in sequence and in length, ranging from 158 bp in *Cephalosporium curtipes* var. *uredinicola* CBS 154.61 to 205 bp in *V. lamellicola* CBS 116.25. *Rotiferophthora* species, which are close to clade D, had a comparatively longer 5.8S gene (200–207 bp). Size variation was much greater in the ITS region: for ITS-1 from 126 bp (in *V. tricorpus*) to 189 bp (in *V. antillanum*) and ITS-2 from 145 bp (in *V. zeosporum*) to 188 bp (in *V. suchlasporium* var. *catenatum*). *Rotiferophthora* species had relatively long ITS-1 (204–215 bp) and ITS-2 sequences (156–186 bp).

Sequence divergence: Sequence variations were calculated using PAUP version 4.0b4a (SWOFFORD, 1998). Up to 60% sequence variation was observed in the ITS region across *Verticillium s. l.*, rendering alignment problematic, particularly of the species included in Clade A. Only the 5.8S gene was highly conserved (Table 1-III), serving as a basis for the alignment. Average pairwise sequence divergence of the ITS region within *Verticillium s. l.* was 10–36% in ITS-1 and 5–43% in ITS-2, while these values were much smaller for the 5.8S gene, ranging from 2 to 10% (Table 1-III). The highest differences were found between *Verticillium* clade A and the remaining clades, indicating their distant relationship. *Rotiferophthora* and clade D are closest to each other, with only 8–16 and 8–21% sequence difference in their ITS-1 and ITS-2, respectively.

Table 1-I. Strains examined. Because of unavailable binomials in more appropriate genera, several isolates are cited under obsolete genera like *Acrostalagmus* or *Cephalosporium*. *A.* = *Acremonium*, *Acrost.* = *Acrostalagmus*, *Aph.* = *Aphanocladium*, *Ceph.* = '*Cephalosporium*', *Eng.* = *Engyodontium*, *Plect.* = *Plectosphaerella*, *R.* = *Rotiferophthora*, *V.* = *Verticillium*.

Taxon	Accession code	Host/Substratum	Origin, date, collector	Other information	Genbank accession
Clade A					
V. luteo-album (Link) Subram.	IMI 017438	*Polygonum sieboldii*	UK, 1947, S.J. Hughes		AJ292421
V. luteo-album	IMI 182719	dead butterfly	UK, IMI 1974, H.J. O'Neil		AJ292420
V. nigrescens Pethybr.	IMI 044575	*Solanum tuberosum*	UK, 1944, R.V. Harris		AJ292440
V. nubilum Pethybr.	IMI 150213	mushroom compost	Scotland, 1967		AJ292463
V. theobromae (Turconi) E. Mason & S. Hughes	IMI 172699	*Musa* sp.	Caribbean, IMI 1973, J. Burden		AJ292422
Clade B					
A. obclavatum W. Gams	CBS 311.74	air above sugarcane field	India, CBS 1974, Karnal	ex-type, IMI 185383	AJ292394
Aph. dimorphum J.D. Chen	CBS 365.86	*Agaricus bisporus*	China, J.D. Chen	ex-type, NRRL 26542	AJ292429
Aph. araneorum (Petch) W. Gams	IMI 090008	*Agaricus bisporus*	UK, 1962, E.W. Mason	CBS 121.286	AJ292430
Aph. aranearum	CBS 370.77	*Agaricus bisporus*	?, CBS 1977, Fontaine		AJ292431
Ceph. aphidicola Petch	IMI 129174	*Brachycaudus helichrysi* (Aphididae)	UK, IMI 1973, R.A. Hall		AJ292387
Ceph. aphidicola	IMI 282532	*Cossus cossus* (Lepidoptera)	Italy, IMI 1984, A. Kovacs		AJ292435
Ceph. aphidicola	Z039	*Zygaenidia pullula*	Italy, 1982, P. Pirtri		AJ292433
Ceph. aphidicola	IMI 068689	*Trialeurodes vaporariorum* (Homoptera)	UK, IMI 1957, N.W. Hussey	ex neotype, CBS 143.53, ATCC 28300, MUCL 9713	AJ292388
Ceph. curtipes var. *uredinicola* Sukap. & Thirum.	CBS 154.61	uredinium of *Olivea* [illegible] (Uredinales)	India, 1961, M.J. Thirumalachar	ex-type, ATCC 11495, IMI 090216, HACC 105	AJ292404
Ceph. curtipes var. *uredinicola*	CBS 894.85		India, CBS 1985	ATCC 16541, HACC 188	AJ292405
Ceph. lanosoniveum van Beyma (*Torrubiella* sp.)	CBS 704.86	*Hemileia vastatrix*	Venezuela, CBS 1956, R. Fokes	ex-type of *Ceph. pimentinum* [illegible]	AJ292396
Ceph. lanosoniveum (*Torrubiella* sp.)	IMI 317442	*Hemileia vastatrix*	Jamaica, IMI 1987, C. Prior	CBS 101267	AJ292395
Ceph. longisporum Petch	IMI 179172	*Macrosiphoniella sanborni* (Aphididae)	UK, IMI 1973, R.A. Hall		AJ292384
Ceph. longisporum	IMI 021167	*Icerya purchasi* (Coccidae)	Sri Lanka, 1921, T. Petch	ex-type, NCTC 1862, MUCL 9702, CBS 126.27	AJ292385
Eng. aranearum (Cavara) W. Gams	CBS 309.85	spider	Spain, CBS 1985, W. Gams		AJ292391
V. antillanum Castañeda & G. Arnold	CBS 350.85	agaric	Cuba, CBS 1985, R.F. Castañeda	ex-type	AJ292392
V. cinnamomeum (Petch) W. Gams	CBS 725.73a	Aleurodes	Ghana, CBS 1973, H.C. Evans		AJ292464
V. fusisporum W. Gams	CBS 164.70	*Coltricia perennis*	Netherlands, 1958, W. Gams	ex-type	AJ292428
V. lamellicola F.E.V. Smith	CBS 116.25	*Agaricus bisporus*	UK, 1925, F.E.V. Smith	ex-type	AJ292393
V. lecanii (A. Zimmerm.) Viégas (*T. confragosa* Mains)	IMI 304813	scale insect (Coccidae)	W. Indies, IMI 1986, C. Prior		AJ292383
V. lecanii (*T. confragosa*)	IMI 304807	*Coccus viridis*	W. Indies, IMI 1985, C. Prior	CBS 101247	AJ292382
V. psalliotae Treschow	IMI 165660	soil	UK, IMI 1972, J.B. Appleton	CBS 101270	AJ292389
V. psalliotae	CBS 100173	nymph of *Ixodes* tick	USA, CBS 1997, G.J. Samuels		AJ292390

V. saksenae Kushwaha	IMI 179841	forest soil	India, IMI 1981, S.C. Agrawal	ex-type, CBS 532.81, ITCC 1890	AJ292432
Verticillium sp.	CBS 402.78	leaf litter of *Acer saccharum*	USA, CBS 1978, G.A. Kuter		AJ292434
Verticillium sp. 1	CBS 639.85	rhizosphere of *Pseudotsuga menziesii*	Germany, CBS 1985, H. Schonhar		AJ292386
Verticillium sp. 1	CBS 100890	contaminant in mushroom spawn	USA, CBS 1988, J. Kremser		AJ292427
Clade C					
V. balanoides (Drechsler) Dowsett, Reid & Hopkins	CBS 522.80	*Ditylenchus triformis*	Germany, CBS 1980, U. Dürschner		AJ292413
V. balanoides	CBS 250.82	nematode in soil	Winnipeg, CBS 1982, J. Reid	ex-neotype	AJ292414
V. campanulatum Glockling	IMI 356015	dung of nematode	England, 1992, S. L. Glockling	ex-type, CBS 101240	AJ292416
V. sinense K.G. Zhang, L. Cao & Z.Q. Liang	CBS 567.95	nematode near root of *Actinidia deliciosa*	China, 1991, K.Q. Zhang	ex-type	AJ292417
V. sinense	CBS 282.90	bacteriovorous nematode from soil	Netherlands, CBS 1990, P.H. van den Boogert		AJ292415
	Verticillium sp.	CBS 101431	nematode, rabbit dung	Japan, 1996, S. Glockling	AJ292418
V. zeosporum	CBS 335.80	nematode in soil	Iraq, CBS 1980, I.S. Dirrinlagh	IMI 239515	AJ292419
Clade D					
Aph. album (Preuss) W. Gams	CBS 401.70	myxomycete	Netherlands, 1968, W. Gams	ex-epitype, NRRL 26536	AJ292401
R. minutispora S.L. Glockling	CBS 101436	rotifer, soil	Japan, 1996, S. Glockling		AJ292411
R. angustispora (Barron) Barron	CBS 101437	rotifer, decaying straw	England, 1997, S. Glockling		AJ292412
V. bulbillosum W. Gams & Malla	CBS 145.70	root of *Picea abies*	Denmark, 1970, D.S. Malla	ex-type	AJ292410
V. chlamydosporium Goddard var. *chlamydosporium*	CBS 103.65	soil under *Brassica napus* (rape seed)	Germany, 1963, W. Gams	ex-neotype, ATCC 10289	AJ292397
V. chlamydosporium var. *catenulatum* (Barron) W. Gams	CBS 504.66	soil, Canada	G.L. Barron, 1963	ex-type, IMI 113161, ATCC 16683, OAC 10250	AJ292398
V. chlamydosporium var. [illegible] (*Cordyceps* sp.)	IMI 331575	beetle larva (Coleoptera)	Ecuador, 1974, H.C. Evans		AJ292399
V. suchlasporium W. Gams & Dackm. var. *suchlasporium*	CBS 251.83	eggs of *Heterodera avenae*	Sweden, 1983, C. Dackman	ex-type	AJ292402
V. gonioides Drechsler	CBS 891.72	*Pulcherricium caeruleum*	Germany, 1972, J.A. Stalpers	ex-neotype	AJ292409
V. pseudohemipterigenum H.C. Evans & Y. Jun	I 96-1013	*Coccus viridis*	Trinidad, 1985, C. Prior	ex-type, IMI 331562	AJ292403
Verticillium sp. cf. *Acrost. bactrosporus* Drechsler	CBS 101433	rotifer, pine needles	Japan, 1996, S. Glockling		AJ292408
V. suchlasporium var. *catenatum* W. Gams & Dackman	CBS 789.85	eggs of *Lymantria dispar* (gipsy moth)	USA, G.C. Carroll		AJ292407
V. suchlasporium var. *catenatum*	CBS 248.83	eggs of *Heterodera avenae*	Sweden, 1983, C. Dackman	ex-type	AJ292406
V. cf. *suchlasporium*	CBS 464.88	eggs of *Heterodera avenae*	Scotland, CBS 1988, L. Lopez Llorca	red pigment on PDA	AJ292400

Table 1-II. Additional sequences obtained from GenBank.

Species	GenBank accession	Reference and author
Claviceps sorghicola	AJ011591	Pazoutova et al. (unpublished)
Cordyceps militaris (L. : Fr.) Link	AF153264.1	Park et al. (unpublished)
Plectosphaerella cucumerina (Lindfors) W. Gams	AF132805	Harrington et al. (2000)
P. cucumerina	L36640	O'Donnell & Gray (1995)
Verticillium dahliae Klebahn	Z29511	Morton et al. (unpublished)
V. tricorpus Isaac	L28679	Moukhamedov et al. (unpublished)
Epichloë glyceriae Schardl & Leuchtm.	L78302.1	Schardl et al. (1997)
E. typhina (Pers.) Tul. & C. Tul.	L78298.1	Schardl et al. (1997)

Table 1-III. Inter- and intra-cluster mean average distance matrix in the 5.8S gene, and the ITS-1 and ITS-2 regions. Values in bold face indicate intra-cluster distances. Values in parentheses indicate minimum and maximum recorded. Distances are in percentage difference.

5.8S	1	2	3	4	5
1. *Verticillium* cluster A	**(0–) 8 (–16)**				
2. Cluster B	(8–) 17 (–17)	**(0–) 3 (–7)**			
3. Cluster C	(2–) 6 (–9)	(2–) 6 (–7)	**(0–) 2 (–4)**		
4. Cluster D (excl. *Rotif.*)	(8–) 10 (–17)	(3–) 6 (–12)	(3–) 5 (–11)	**(0–) 2 (–8)**	
5. *Rotiferophthora*	(5–) 8 (–10)	(5–) 8 (–10)	(3–) 4 (–6)	(2–) 3 (–9)	**3**

ITS1	1	2	3	4	5
1. *Verticillium* cluster A	**(0–) 15 (–23)**				
2. Cluster B	(29–) 34 (–44)	**(0–) 10 (–29)**			
3. Cluster C	(28–) 34 (–40)	(23–) 29 (–36)	**(5–) 12 (–19)**		
4. Cluster D (excl. *Rotif.*)	(28–) 35 (–38)	(23–) 30 (–41)	(21–) 27 (–33)	**(0–) 12 (–33)**	
5. *Rotiferophthora*	(32–) 35 (–38)	(30–) 35 (–40)	(33–) 35 (–36)	(8–) 15 (–28)	**14**

ITS2	1	2	3	4	5
1. *Verticillium* cluster A	**(0–) 35 (–51)**				
2. Cluster B	(24–) 43 (–60)	**(0–) 7 (–24)**			
3. Cluster C	(27–) 42 (–57)	(15–) 21 (–31)	**(2–) 8 (–14)**		
4. Cluster D (excl. *Rotif.*)	(29–) 41 (–60)	(14–) 19 (–26)	(8–) 14 (–27)	**(0–) 14 (–21)**	
5. *Rotiferophthora*	(30–) 43 (–58)	(17–) 20 (–23)	(12–) 16 (–21)	(7–) 13 (–19)	**5**

Phylogeny of the ITS region: A Blast search starting with the sequence for *V. chlamydosporium* var. *chlamydosporium*, CBS 103.65, led to a number of sequences in GenBank. Three of these, belonging to the clavicipitaceous species *Claviceps purpurea* (AJ011591), *Epichloë typhina* (L78298.1), and *Epichloë glyceriae* (L78302.1), were used as outgroups (Fig. 1-1). In another analysis some species of Cluster A were used as outgroups (Fig. 1-2).

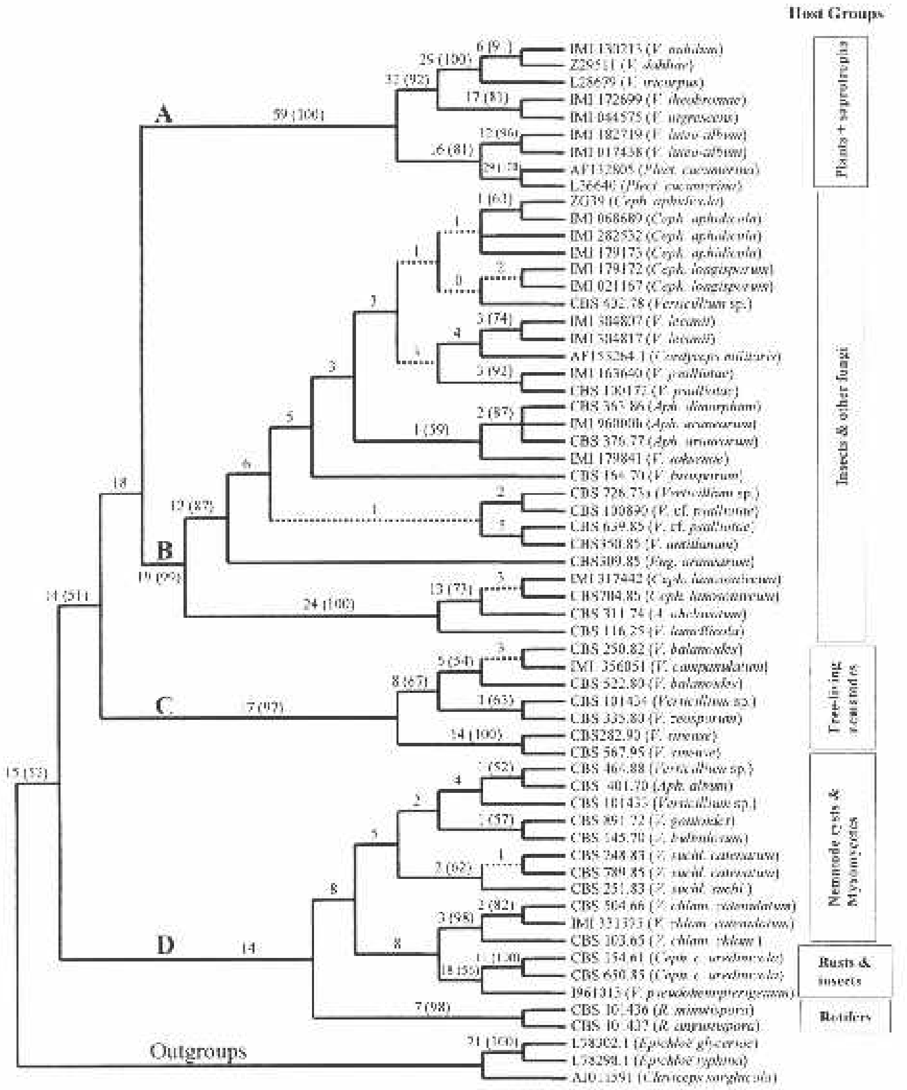


Fig. 1-1. One of 240 equally parsimonious trees recovered using sequences of ITS region and 5.8S gene, based on *Epichloë* and *Claviceps* as outgroup. Dashed lines indicate branches that collapsed in the strict consensus tree. Plain figures indicate branch lengths and figures in brackets show the Jackknife support values. CI = 0.52, HI = 0.47, RI = 0.82, RC = 0.42, tree length = 909.

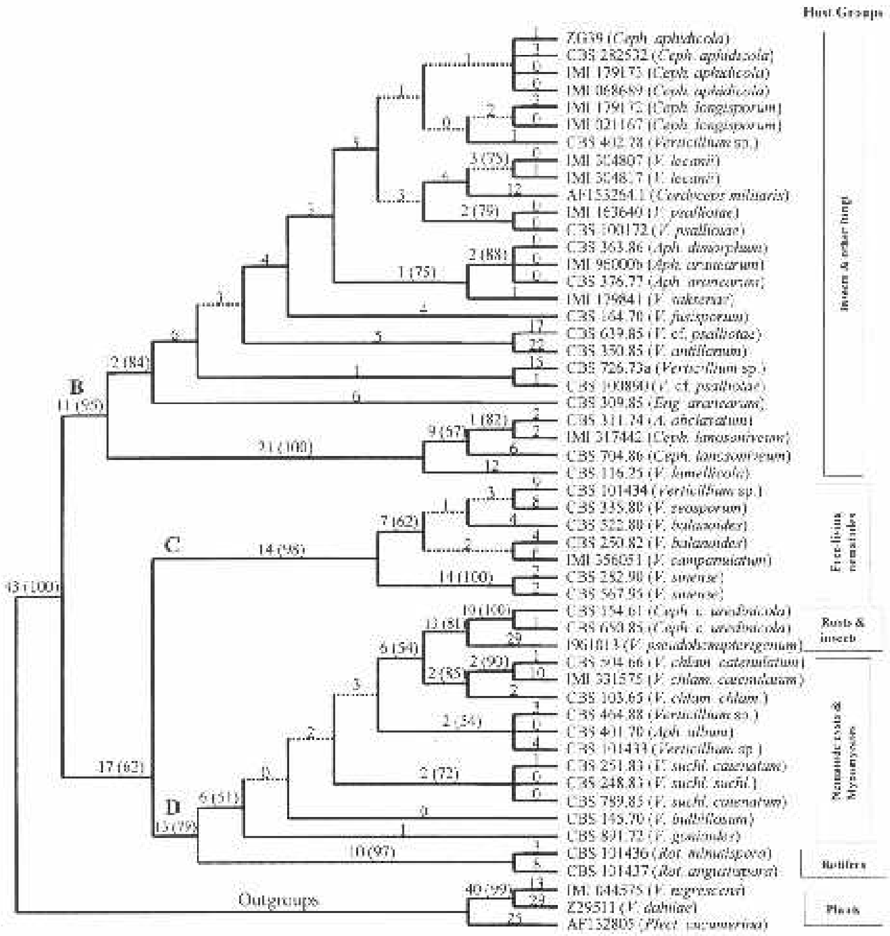


Figure 1-2. One of 720 equally parsimonious trees recovered using sequences of ITS region and 5.8S gene, based on species of Clade A as outgroup. Dashed lines indicate branches that collapsed in the strict consensus tree. Plain figures indicate branch lengths and figures in brackets show the Jackknife support values. CI = 0.60, HI = 0.39, RI = 0.83, RC = 0.50, tree length = 584.

A heuristic search with all strains of Table 1-I yielded 240 equally parsimonious trees when rooted on *Epichloë* and *Claviceps*. A strict consensus tree of these was calculated (Fig. 1-1). Rooting the tree on species of Cluster A led to 720 equally parsimonious trees with a very similar topology (Fig. 1-2). Four major clades (A–D) were distinguished in all these trees (Fig. 1-1).

Clade A comprises species of *Verticillium* in the most current sense, i.e., the type species and the mainly plant-associated species of section *Nigrescentia*. It also has 100% Jackknife support. Long branches (59 steps) suggest some distance in the relationship of this clade with the remaining clusters. *Plectosphaerella cucumerina* is placed adjacent to *V. luteo-album*. It seems that all these fungi are correctly classified in the Phyllachorales (PALM *et al.* 1995, MESSNER *et al.* 1996).

Clade B, comprising *V. lecanii* and *V. psalliotae*, has 99% Jackknife support. Isolates previously identified as *V. lecanii* could be subdivided into several taxa that are referred to here with older names available in *Cephalosporium*. *Aphanocladium aranearum* and *A. dimorphum* are very closely related to *V. psalliotae*. *Engyodontium aranearum* (CBS 309.85), which was included in the analysis because of some similarity with *Aphanocladium dimorphum*, was also found to be nested within this clade.

Clade C comprises the nematophagous species with adhesive conidia. It has 97% Jackknife support. From the tree topology it is clear that these species are no closer to the species that often attack cysts and eggs of plant-pathogenic nematodes (Clade D) than to the plant and insect pathogens of Clades A and B.

Clade D comprises mainly species that occur frequently as parasites of nematode cysts. The clade as a whole has less than 50% Jackknife support. The two species *V. chlamydosporium* and *V. suchlasporium* are obviously distinct though closely related. Species of *Rotiferophthora*, which are morphologically similar to this group, form a distinct subclade. In clade D, two species, *Cephalosporium curtipes* var. *uredinicola* and *V. pseudohemipterigenum*, appear topologically close to the nematode parasites, but their rather great branch length suggests that they are distantly related. These two species are fungicolous and entomogenous, respectively, and because of their falcate conidia they were initially expected to be closer to *V. psalliotae*. The presence of scanty chlamydospores in isolates labelled *C. curtipes* var. *uredinicola* distinguishes them from *V. psalliotae*. The correct classification of this species and *V. pseudohemipterigenum* cannot yet be resolved.

Discussion

Most phylogenetic studies of fungi have used sequences from the ribosomal RNA genes and spacers. The ITS regions are frequently used to analyse relationships at the species level (LIECKFELDT & SEIFERT 2000). The present study was intended to elucidate the relationships among taxa traditionally accommodated in *Verticillium*, with particular reference to its section *Prostrata*. In addition, a number of strains of plant-associated taxa (*Verticillium* sect. *Nigrescentia*) and of a few other groups (e.g. section *Verticillium*) and some species of uncertain affinities, were included. A sound taxonomy should be based on both morphological and molecular sequence data (possibly of several genome fractions). In most cases, the available molecular and morphological data supported each other. But in some cases (e.g. *V. pseudohemipterigenum*, *Cephalosporium curtipes* var. *uredinicola*), because of insufficient numbers of strains and inadequate information, no clear-cut conclusions could be drawn.

The species presently placed in *Verticillium* form at least four distinct clades, A–D (Fig. 1-1), which are also separable on morphological grounds (Table 1-IV). It is obvious that not all verticillium-like anamorphs of Clavicipitaceae can be accommodated in these clusters and the similar hypocrealian anamorphs have not yet been tackled. The ITS sequences show a great extent of variation (up to 60%). Jackknife support corroborates the distinction of at least three of the four clades. A possibility of

Table 1-IV. Summary of morphological features distinguishing molecular clades.

Clades/ Features	Conidio-phores	Conidia	Resting Structures	Associated with
Clade A	erect	(sub)cylindrical, non-adhesive	dark resting mycelium, chlamydospores, micro sclerotia	plants, soil
Clade B	prostrate	(sub)cylindrical, obclavate, subglobose, oval, falcate, fusiform, non-adhesive	none	insects, fungi
Clade C	prostrate	balanoid, rarely elongate adhesive	none*	nematodes
Clade D**	prostrate or erect	subglobose, oval, falcate, subcylindrical, non-adhesive	dictyochlamydospores	nematode cysts and eggs

* Dictyochlamydospores were reported for some members by Gams (1988) but they were not seen again.

**Excluding *Aph. album*, *Rotiferophthora* spp., *Ceph. curtipes* var. *uredinicola*, and *V. pseudohemipterigenum*.

retaining these four groups in *Verticillium* and recognizing them as sections could be envisaged, but it appears by far preferable to us to subdivide the genus into a number of more naturally circumscribed genera. This is desirable because of the classification of associated teleomorphs in different orders of ascomycetes and great biological differences.

The teleomorphs of clade A are probably members of the order Phyllachorales (PALM *et al.* 1995, MESSNER *et al.* 1996). The remaining taxa discussed here belong to the Clavicipitaceae. *Torrubiella confragosa* Mains is known to be the teleomorph of *V. lecanii* (MAINS 1949, EVANS & SAMSON 1982), the most representative member of clade B. But *Cordyceps militaris* also belongs to this cluster, showing that the distinction between these two teleomorph genera is by no means sharp while, on the other hand, *Cordyceps* shows considerable heterogeneity. A species of *Cordyceps* (H.C. EVANS, pers. comm.) has been proven to be the teleomorph of *V. chlamydosporium*, representative of clade D.

No teleomorph is yet known for clade C, although its affinity with the Clavicipitaceae is probable. Many more verticillium-like anamorphs are known that are not included in the present study; many of them have affinities with genera of the Hypocreales, an order from which the Clavicipitaceae emerge in a not completely resolved manner (GLENN *et al.* 1996, GAMS *et al.* 1998).

The host–pathogen relationships clearly reflect the clusters distinguished: Clade B comprises entomogenous and fungicolous species, Clade C contains endoparasites of nematodes that attach themselves to their host by means of adhesive conidia, and species of Clade D mainly parasitize nematode cysts and eggs. All these groups differ strongly from the plant–associated and soil–borne species of Clade A.

Morphological criteria are not always sufficient to make clear-cut distinctions between the clusters distinguished. The criteria introduced by GAMS & VAN ZAAYEN (1982) for the distinction of sections seem to hold for a very crude subdivision, but in order to differentiate clusters within the former section *Prostrata*, additional criteria must be sought. The conidia provided with an adhesive upper end turn out to be a significant criterion distinguishing Clade C. Species of Clade D had already been segregated by BARRON & ONIONS (1966) as a separate genus, *Diheterospora*, using the presence of dictyochlamydospores as the major criterion. Because of the unreliability of this feature, GAMS (1971, 1988) did not recognize this generic distinction, but now the distinction appears to be phylogenetically significant and points to the importance of examining fresh and optimally preserved cultures in morphological studies. The cluster now includes species that never form any dictyochlamydospores, and therefore secondary criteria must be found to properly identify its species. The range of conidial shapes, short-oval to bluntly short-falcate or polyhedric differs from the cylindrical to fusiform or falcate conidia of Clade B. Moreover, young conidia of Clade D are often distinctly cyanophilic and crystals are usually absent.

Interestingly, Clade B includes species with verticillate conidiophores in addition to some with strictly solitary phialides, so far classified as *Acremonium obclavatum* W. Gams and *Cephalosporium lanosoniveum* van Beyma. The hyper-parasites of rust fungi, identified either as *Cephalosporium curtipes* var. *uredinicola* Sukap. & Thirum. or *Verticillium epiphytum* Hansf. are very similar to *V. psalliotae* in Clade B. They produce scanty chlamydospores in culture and do probably not belong to Clade B, but are closer to Clade D. Their classification will require further studies using additional representative isolates and more kinds of sequences.

The presence of strongly reduced, short and swollen, evanescent conidiogenous cells that produce only single, obovate conidia was proposed by GAMS (1971) as the criterion for distinguishing the genus *Aphanocladium*. The type species, *A. album* (Preuss) W. Gams, was originally described from myxomycetes, while other isolates identified as that species were obtained from various, mostly fungal, substrata. These isolates are here identified as *A. aranearum* (Petch) W. Gams. Its strains grow faster and produce a deep, fluffy aerial mycelium, while the mycelium of *A. album* shows some tendency of undulate growth. Observations by K. O'DONNELL (unpubl.) and the present findings point to a sharp bipartition among these two groups of *Aphanocladium*. *Aphanocladium album*, CBS 401.70, from Myxomycetes appears in our study close to Clade D, while the second group around *A. aranearum* is a neighbour of *V. psalliotae*. It is not surprising that *Aphanocladium dimorphum* J.D. Chen with its two types of conidia, falcate and obovoid, and differentiated and evanescent conidiogenous cells links the two taxa.

Engyodontium aranearum was included in the present study because of its similarity to *Aphanocladium dimorphum*. Sequence comparisons suggest that this species is closely related to clade B as well.

The species of Clade D, consisting of parasites of nematode cysts, deserve generic distinction. The combination of dictyochlamydospores (or at least irregularly swollen hyphae or solitary chlamydospores) and rather rapid growth are sufficient evidence for this cluster. But in the absence of any resting structure, the subglobose to short–ellipsoid or bluntly falcate, often cyanophilic conidia are indicative of this affinity.

BARRON (1991) and GLOCKLING (1998) presented arguments that *Rotiferophthora* is a distinct genus. Members of this genus are strictly parasitic on rotifers. Two species, *R. angustispora* and *R. minutispora*, were included in the present study. Comparison of sequences derived from the ITS region and the 5.8S rRNA gene suggests a close relationship between *Rotiferophthora* and nematophagous *Verticillium* species and places the genus on a subclade within clade D.

Verticillium pseudohemipterigenum with its compact whorls of phialides does not seem to be closely related to any of the other groups distinguished here. Sequence comparison of the ITS region including the central 5.8S was insufficient to resolve its position.

Species of the sections *Verticillium* and *Nigrescentia* are apparently closely related to each other, both using morphological and molecular criteria. *Verticillium luteo-album* has so far only been reported as a saprotroph (HUGHES 1951), sometimes in association with other fungi (PETHYBRIDGE 1919, BOOTH 1959, TSUNEDA *et al*. 1976). This species can be distinguished easily from the species of sect. *Nigrescentia* by its brick-red colony colour. ITS sequences also clearly distinguish this species from other taxa of sect. *Nigrescentia*. This species is closer to *Plectosphaerella cucumerina*. PETHYBRIDGE (1919) found it in association with perithecia of *Nectria inventa* Pethybr., a connection that appears doubtful in view of the present molecular findings and those by MESSNER *et al*. (1996), which suggest that species of *Verticillium* sect. *Nigrescentia* and *V. luteo-album* (K. O'DONNELL, pers. comm.) are related to the phyllachoralean genus *Glomerella*. In summary, four distinct clusters recognizable by molecular and morphological features are identified. These groups correlate with host range.

PART TWO:
PHYLOGENETIC ANALYSES OF SSU AND LSU NUCLEAR rDNA SEQUENCES FROM ANAMORPHS AND TELEOMORPHS OF THE CLAVICIPITACEAE

The strains analysed are listed in Table 2-I.

Results

The combined SSU and LSU nrDNA dataset included 2040 aligned nucleotide positions, with SSU rDNA comprising 1100 and the LSU rDNA comprising 940 positions. One-hundred and ten SSU rDNA positions and 128 LSU rDNA positions were excluded due to either ambiguously aligned regions or an excess of missing data near the 5′ and 3′ ends. The final data set included 1802 nucleotide positions of which 347 position -146 from the SSU rDNA and 201 from the LSU rDNA were identified as parsimony-informative. The alignment is available from Treebase as study accession number S573, matrix accession number M866. Maximum parsimony analysis of the 93 taxa dataset yielded 329 equally most parsimonious trees of 1710 steps. For each of these trees, the consistency index (CI) was 0.322 and the retention index (RI) was 0.690. Although a large number of trees were recovered in these analyses, many of the nodes amongst the major genera and groups of the Clavicipitaceae were resolved in the strict consensus tree (Fig. 2-1). One of the most parsimonious trees from the maximum parsimony analyses was chosen at random and is shown in Fig. 2-2 for the purpose of displaying branch length and mapping host affiliations.

Maximum parsimony analyses of the combined SSU and LSU rDNA data support the inclusion of all isolates sampled from *V.* sect. *Prostrata* in the Clavicipitaceae except for *V. incurvum*. However, the monophyly of the Clavicipitaceae is weakly supported by bootstrap values and is characterized by a relatively short branch. The clavicipitalean isolates of *V.* sect. *Prostrata* did not form a monophyletic group and were placed in at least three separate parts of the Clavicipitaceae clade.

Table. 2-I. List of cultures and specimens used in this study.

Taxon	Specimen voucher	Host/subtratum	GenBank accession number[a]	
			SSU	LSU
ANAMORPHS				
Aphanocladium album (Preuss) W. Gams	CBS 401.70	Myxomycete	AF339568	AF339518
Beauveria bassiana (Bals.) Vuill.	IFO 4848	Insect	AB027336	AB027382
Beauveria bassiana	NRRL 28020	*	AF049144	AF049164
Beauveria brongniartii (Sacc.) Petch	IFO 5299	(Type of *Isaria fogane*)	AB027335	AB027381
Beauveria caledonica Bissett & Widden	ARSEF 2567	Saprobe	AF339570	AF339520
Engyodontium aranearum (Cavara) W. Gams	CBS 309.85	Spider (Arachnida)	AF339576	AF339526
Engyodontium aranearum	ARSEF 2929	Spider (Arachnida)	AF339575	AF339525
Harposporium helicoides Drechsler	ARSEF 5354	Nematode (Nematoda)	AF339577	AF339527
Hirsutella thompsonii Fisher	ATCC 24874	Citrus rust mite (Arachnida: Acari)	U32406	AF339528
Metarhizium anisopliae (Metch.) Sorok.	IFO 5940	*Tomaspis saccharina* (Homoptera)	AB027337	AB027383
Metarhizium anisopliae var. *majus*	ARSEF 3145	*Oryctes rhinoceros* (Coleoptera: Scarabaeidae)	AF339579	AF339530
Metarhizium anisopliae var. *frigidum*	ARSEF 4606	*	AF339578	AF339529
Metarhizium flavoviride W. Gams & Rozsypal var. *minus*	ARSEF 2037	*Nilaparvata lugens* (Homoptera: Delphacidae)	AF339580	AF339531
Microhilum oncoperae H.Y. Yip & Rath	ARSEF 4358	*Oncopera intricata* (Lepidoptera: Hepialidae)	AF339581	AF339532
Neotyphodium coenophialum (Morgan-Jones & W. Gams) Glenn et al.	ATCC 62374	*Festuca arundinacea* (Poaceae)	U45942	U57651
Paecilomyces javanicus (Friederichs & Bally) A. Brown & G. Smith	ARSEF 322	*Litodactylus leucogaster* (Coleoptera: Curculionidae)	AF339582	AF339533
Paecilomyces lilacinus (Thom) Samson	ARSEF 2181	Tylenchida, egg mass of *Meloidogyne* sp. (Nematoda)	AF339583	AF339534
Paecilomyces tenuipes (Peck) Samson	OSC 76403	Lepidoptera	U45880	U47838
Paecilomyces tenuipes		Lepidoptera	AB027334	AB027380
Rotiferophthora angustispora (Barron) Barron	CBS 101437	Rotifer (Rotifera), decaying straw	AF339584	AF339535
Verticillium sect. *Prostrata*				
'*Acremonium*' *obclavatum* W. Gams	CBS 311.74	Air above sugarcane field	AF339567	AF339517
'*Cephalosporium*' *lanosoniveum* (van Beyma) W. Gams	CBS 704.86	*Hemileia vastatrix* (Uredinales)	AF339602	AF339553
'*Cephalosporium*' *lanosoniveum*	IMI 317442	*Hemileia vastatrix* (Uredinales)	AF339603	AF339554
Verticillium antillanum Castañeda & G. Arnold	CBS 350.85	Agaric (Agaricales)	AF339585	AF339536
Verticillium aranearum (Petch) W. Gams	CBS 726.73a	Spider (Arachnida)	AF339586	AF339537
Verticillium balanoides (Drechsler) Dowsett, Reid & Hopkins	CBS 335.80	Nematode in soil (Nematoda)	AF339589	AF339540
Verticillium balanoides	CBS 522.80	*Ditylenchus triformis* (Nematoda)	AF339590	AF339541
Verticillium balanoides	CBS 250.82	Nematode in soil (Nematoda)	AF339588	AF339539
Verticillium bulbillosum W. Gams & Malla	CBS 145.70	Root of *Picea abies*	AF339591	AF339542
Verticillium campanulatum Glockling	IMI 356051	Dung of nematode (Nematoda)	AF339592	AF339543
Verticillium cf. *bactrosporum* (Drechsler) Subram.	CBS 101433	Rotifers (Rotifera) in litter	AF339587	AF339538

Taxon	Specimen voucher	Host/substratum	GenBank accession number	
			SSU	LSU
Verticillium chlamydosporium var. *catenulatum* (Barron) W. Gams	CBS 504.66	Soil	AF339593	AF339544
Verticillium epiphytum Hansf.	CBS 154.61	Uredinium of rust (Uredinales)	AF339597	AF339548
Verticillium epiphytum	CBS 384.81	*Hemileia vastatrix* (Uredinales)	AF339596	AF339547
Verticillium fusisporum W. Gams	CBS 164.70	*Coltricia perennis* (Hymenomycetes)	AF339598	AF339549
Verticillium gonioides (Drechsler) W. Gams & Stalpers	CBS 891.72	*Pulcherricium caeruleum*	AF339599	AF339550
Verticillium incurvum W. Helfer	CBS 460.88	*Ganoderma lipsiense* (Hymenomycetes)	AF339600	AF339551
Verticillium lamellicola F.E.V. Smith	CBS 116.25	*Agaricus bisporus* (Agaricales)	AF339601	AF339552
Verticillium lecanii (*Torrubiella confragosa*)	IMI 304807	*Coccus viridis* (Homoptera)	AF339604	AF339555
Verticillium lecanii (Zimm.) Viégas	NRRL 28023	?	AF049156	AF049176
Verticillium lecanii	CBS 126.27	*Icerya purchasi* (Homoptera: Coccidae)	AF339605	AF339556
Verticillium psalliotae Treschow	CBS 363.86	*Agaricus bisporus* (Agaricales)	AF339608	AF339559
Verticillium psalliotae	CBS 332.81	Forest soil	AF339609	AF339560
Verticillium psalliotae	CBS 639.85	Rhizosphere of *Pseudotsuga menziesii*	AF339610	AF339561
Verticillium psalliotae	IMI 163640	Soil	AF339607	AF339558
Verticillium psalliotae	CBS 100172	Nymph of *Ixodes* (Arachnida: Acari)	AF339606	AF339557
Verticillium pseudohemipterigenum H.C. Evans & Y. Jun	ARSEF 5687	Homoptera	AF339611	AF339562
Verticillium pseudohemipterigenum	I 95-1015	*Coccus viridis* (Homoptera)	AF339612	AF339563
Verticillium sinense K.Q. Zhang, L. Cao & Z.Q. Liang	CBS 567.95	Nematode (Nematoda) near root of *Actinidia deliciosa*	AF339594	AF339545
Verticillium sinense	CBS 121.95	Bark near root of *Actinidia deliciosa*	AF339595	AF339546
Verticillium sp. **(later identified as *Lecanicillium attenuatum*)**	CBS 402.78	Leaf litter of *Acer saccharum*	AF339614	AF339565
Verticillium sp. **(later identified as *Pleurodesmospora coccorum*)**	CBS 101284	Spider (Arachnida)	AF339613	AF339564
Verticillium suchlasporium W. Gams & Dackm. var. *catenatum*	CBS 464.88	Eggs of *Heterodera avenae* (Nematoda)	AF339615	AF339566
Verticillium sect. *Nigrescentia*				
Verticillium dahliae Kleb.			AF104926	AF104926
TELEOMORPHS				
Perithecial Ascomycota				
Aphysiostroma stercorarium Barrasa, Martínez & Moreno			U32398	U47820
Cercophora septentrionalis N. Lundq.			U32400	U47823
Chaetomium globosum Kunze : Fr.			U20379	U47825
Colletotrichum gloeosporioides (Penz.) Penz. & Sacc.			M55640	Z18999
Daldinia concentrica (Bull. : Fr.) Ces. & De Not.			U32402	U47828
Diaporthe phaseolorum (Cooke & Ellis) Sacc.			L36985	U47830
Diatrype disciformis (Hoffm. : Fr.) Fr.			U32403	U47829
Glomerella cingulata (Stoneman) Spauld. & H. Schrenk			L48427	U48428
Haematonectria haematococca (Berk. & Br.) Samuels & Nirenb.			U32413	AF049134
Hypocrea schweinitzii (Fr.) Sacc.			L36985	U47833

Taxon	Specimen voucher	Host/subtratum	GenBank accession number	
			SSU	LSU
Hypomyces polyporinus Peck			U32410	AF049133
Microascus trigonosporus C. W. Emmons & B. O. Dodge			L36987	U47835
Nectria cinnabarina (Tode : Fr.) Fr.			U32412	U00748
Neocosmospora vasinfecta E. F. Smith			U32414	U47836
Neurospora crassa Shear & B. O. Dodge			X04971	M38154
Ophiostoma piliferum Syd. & P. Syd.			U20377	U47837
Petriella setifera (J. C. Schmidt) Curzi			U32421	U48421
Sphaerostilbella aureonitens (Tul.) Seifert et al.			U32415	U00755
Xylaria curta Fr.			U32417	U47840
Xylaria hypoxylon (L.: Fr.) Grev.			U20378	U47841
Clavicipitaceae				
Atkinsonella hypoxylon (Peck) Diehl		Poaceae	U44034	U57087
Atricordyceps harposporifera Samuels	ARSEF 5472	Millipede? (Diplopoda)	AF339569	AF339519
Balansia aristidae Atkinson		Poaceae	U44035	U57677
Balansia henningsiana (Moell.) Diehl		Poaceae	U44036	U57678
Claviceps paspali F. Stevens & J. G. Hall		Poaceae	U32401	U47826
Claviceps purpurea (Fr. : Fr.) Tul.		Poaceae	U44040	U57085
Cordycepioideus bisporus Stifler		termite (Isoptera)	AF009651	AF009654
Cordyceps bifusispora O. E. Eriksson	ARSEF 5690	Lepidoptera	AF339571	AF339521
Cordyceps capitata (Fr.) Link		*Elaphomyces* (Euascomycetes)	AB027318	AB027364
Cordyceps coccidiicola Y. Kobayasi & D. Shimizu		scale insect (Homoptera)	AB031195	AB031196
Cordyceps cochlidiicola Y. Kobayasi & D. Shimizu		moth (Lepidoptera)	AB027331	AB027377
Cordyceps gunnii (Berk.) Sacc.	OSC 75404	Lepidoptera	AF339572	AF339522
Cordyceps militaris (L. : Fr.) Link		moth (Lepidoptera)	AB027333	AB027379
Cordyceps ophioglossoides (Ehrenb. : Fr.) Link		*Elaphomyces*	AB027321	AB027367
Cordyceps roseostromata Y. Kobayasi & D. Shimizu	ARSEF 4871	Lepidoptera	AF339573	AF339523
Cordyceps scarabaeicola Y. Kobayasi & D. Shimizu	ARSEF 5689	Coleoptera: Scarabaeidae	AF339574	AF339524
Epichloe amarillans J. F. White		Poaceae	U35034	U57680
Epichloe typhina (Pers.) Tul.		Poaceae	U32405	U17396
Myriogenospora atramentosa (Berk. & M.A. Curtis) Diehl		Poaceae	U44155	U57084

To test the monophyly of *V.* sect. *Prostrata*, two topological constraint analyses were performed. One topological constraint that forced the monophyly of *V.* sect. *Prostrata* including *V. incurvum* (constraint 1), while the second constraint forced the monophyly of *V.* sect. *Prostrata* without *V. incurvum* (constraint 2). Maximum parsimony analyses using constraint 1 and constraint 2 topologies as starting trees resulted in trees that were 75 and 55 steps longer, respectively. The most parsimonious trees from both constraint 1 and constraint 2 were both rejected as being significantly worse explanations of the data than the trees from both the maximum and weighted parsimony analyses ($P = < 0.0001$). This finding is consistent with the observation that the ITS-5.8S sequences of *V. incurvum* could not be aligned with the remaining taxa of *Verticillium*.

Discussion

Familial relationships of *Verticillium* sect. *Prostrata*: *Verticillium* is known to be phylogenetically related to many families of perithecial ascomycetes (GAMS 1971, GAMS & VAN ZAAYEN 1982, SAMUELS 1988, ZARE *et al*. 2000 – part 1). In these analyses, the specimens sampled from *V.* sect. *Prostrata* grouped within the Clavicipitaceae with the exception of *V. incurvum*, which grouped more closely with members of the Hypocreaceae. The clavicipitaceous affinity of *V.* sect. *Prostrata* is consistent with hypotheses from morphological studies (GAMS 1971, GAMS & VAN ZAAYEN 1982) and phylogenetic analyses using the ITS rDNA (ZARE *et al*. 2000 – part 1). Although the Clavicipitaceae were inferred to be monophyletic, their monophyly was not strongly supported by the data. The clavicipitaceous region of the rDNA tree is characterized by relatively short basal branches that received low bootstrap support (Fig. 2-2). The monophyly of the family is, however, consistent with morphology. The Clavicipitaceae are united by the synapomorphies of long cylindrical asci with a conspicuously thickened apex and filiform ascospores that typically disarticulate into part-spores (DIEHL 1950, MAINS 1958, ROGERSON 1970, KOBAYASI 1982, SPATAFORA & BLACKWELL 1993).

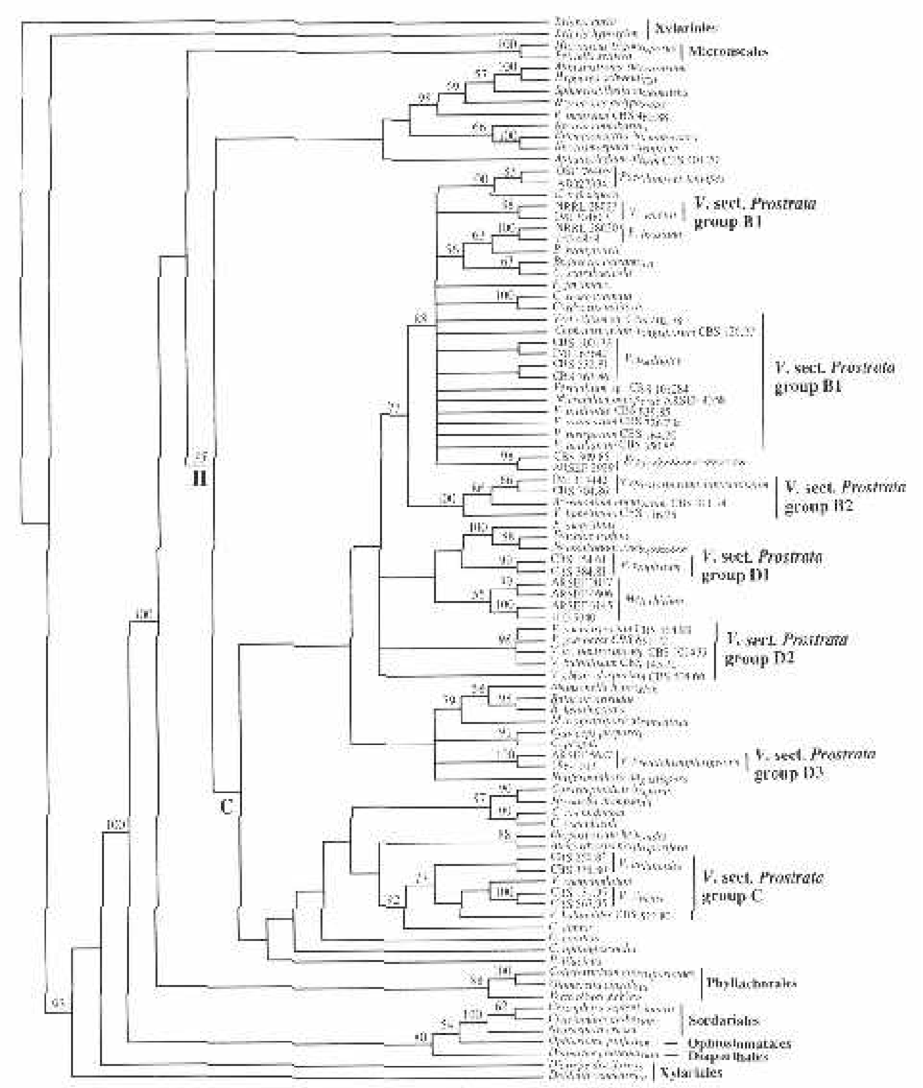


Fig. 2-1. Strict consensus tree of 329 most parsimonious trees from the maximum parsimony analyses of the combined SSU and LSU rDNA dataset. Species represented by multiple isolates are differentiated by accession number; accession numbers for species represented by a single isolate are listed in Table 1. Bootstrap values of >50% are noted above their respective nodes. The three major groups of *Verticillium* sect. *Prostrata* are designated to the right of the species names following the convention of ZARE *et al.* (2000 – part 1); subgroups are designated numerically, i.e., B1, D1–D3. The nodes representing the Hypocreales and Clavicipitaceae are noted by H and C, respectively.

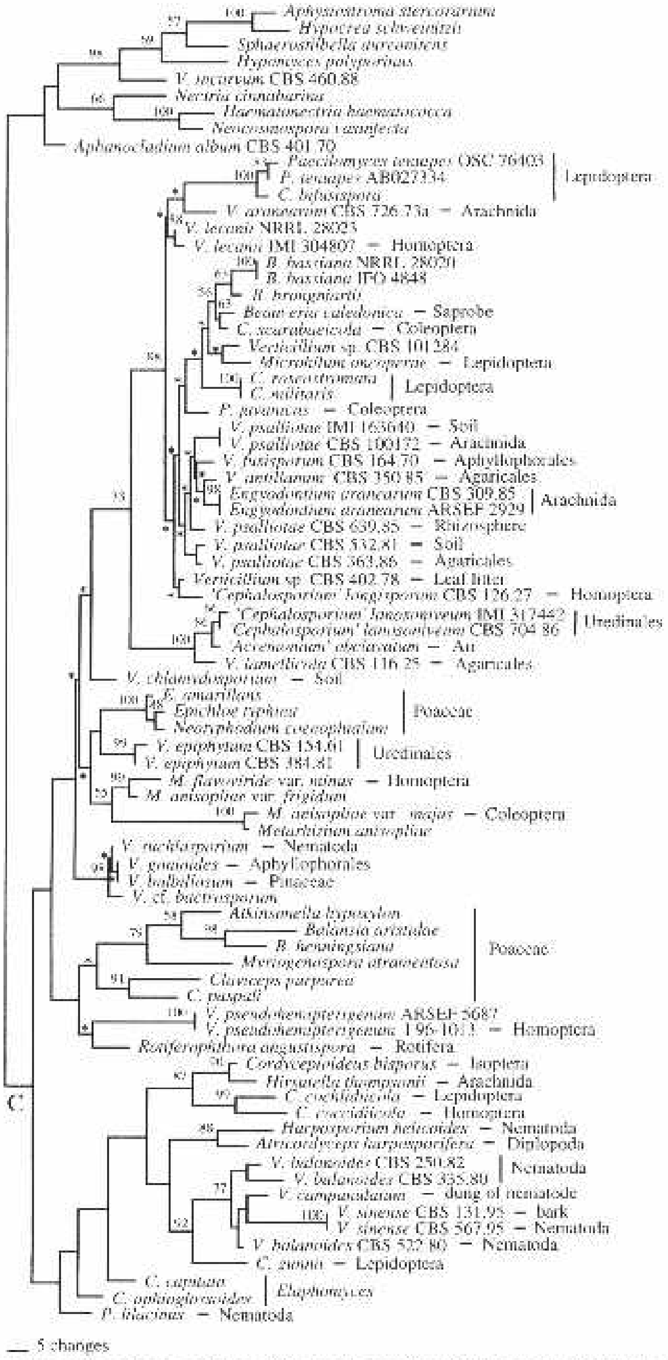


Fig. 2-2. A phylogram of one of the 329 most parsimonious trees. Only the Hypocreales section of the tree is shown to emphasize the Clavicipitaceae and *Verticillium* sect. *Prostrata*. Tree descriptors are as in Figure 2-1 with the exception that nodes which collapse in the strict consensus are designated by asterisks. Host or substratum is provided to the right of species names.

These data are also consistent with a distant relationship between fungi of *V.* sect. *Prostrata* and those of *V.* sect. *Nigrescentia* (ZARE *et al*. 2000 – part 1). In a previous study of the SSU rDNA, the plant pathogen *V. dahliae* Kleb. was shown to be closely related to the Phyllachoraceae (MESSNER *et al*. 1996). This relationship was not strongly supported by the data and the node in question was characterized by long branch lengths. Here we included sequence data from both the SSU and LSU rDNA and a larger sampling of perithecial ascomycetes, which may serve to disrupt long branch attraction (FELSENSTEIN 1985, GRAYBEAL 1998). These analyses increased the support for a close relationship between *V. dahliae* and *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and its teleomorph *Glomerella cingulata* (Stonem.) Spauld. & Schrenk and the inclusion of *V. dahliae* in the Phyllachoraceae (Fig. 2-1). These data also strengthened the hypothesis of the disparate relationship between *V.* sect. *Prostrata* and *V.* sect. *Nigrescentia* (ZARE *et al*. 2000 – part 1).

Polyphyly of *Verticillium* sect. *Prostrata*: Although *V.* sect. *Prostrata* was confirmed as being included in the Clavicipitaceae, the monophyly of *V.* sect. *Prostrata* was rejected. The most parsimonious trees from the analyses of constraint topologies, which forced the monophyly of *V.* sect. *Prostrata* with and without *V. incurvum*, were rejected as significantly worse explanations of the data (Templeton WSR test $P = < 0.0001$). These results suggest that *V.* sect. *Prostrata* is polyphyletic and does not represent a natural group of fungi within the Clavicipitaceae. At least three, and possibly six, separate groups of *V.* sect. *Prostrata* were resolved within the Clavicipitaceae. We emphasize groups, rather than clades, out of convenience as several of the groups were not supported as monophyletic in the maximum parsimony analyses (Figs 2-1, 2-2), but represent closely related sets of species. Also, certain morphological and ecological traits are discussed in the context of these groups. We follow the convention of ZARE *et al.* (2000 – part 1) and designate the groups of *V.* sect. *Prostrata* as B–D with group A representing *V. dahliae* of *V.* sect. *Nigrescentia*. Groups B and D are not supported as monophyletic. Group B comprises two closely related groups that are designated B1 and B2. Group D includes three separate lineages of *V.* sect. *Prostrata* (D1–D3), a finding consistent with ZARE *et al*. (2000 – part 1), that were part of a poorly resolved region of the tree (Figs 2-1, 2-2).

Group B1 includes the type of *V.* sect. *Prostrata*, the anamorph of *C. militaris*, and morphologically similar species of *V. lecanii*, *V. psalliotae*, *V. fusisporum*, *V. aranearum*, and *V. antillanum*; group B2 includes *V. lamellicola*, '*Cephalosporium*' *lanosoniveum*, and '*Acremonium*' *obclavatum*. Species in group B are characterized by phialides produced on prostrate conidiophores, nonadhesive conidia that vary in morphology from oval to cylindrical to falcate, and the lack of dictyochlamydospore production. Group C includes the nematophagous species *V. balanoides*, *V. campanulatum*, and *V. sinense*. It is distinguished from the other groups of *V.* sect. *Prostrata* by the basal inflation of phialides, which are similar to *Tolypocladium*, and adhesive conidia that are balanoid and rarely elongated.

Group D is grossly paraphyletic in these analyses and includes three lineages that are part of an unresolved region of the tree (Fig. 2-1). Group D1 consists of the rust parasite *V. epiphytum*, which produces falcate conidia that are morphologically similar to *V. psalliotae*, but differs from the latter species in its sparse production of thick-walled, cyanophilic chlamydospores. Group D2 comprises the species *V. chlamydosporium*, *V. suchlasporium*, *V.* cf. *bactrosporum*, and *V. gonioides*. It is not resolved as monophyletic, largely due to the numerous placements of *V. chlamydosporium* among the most parsimonious trees (Figs 2-1, 2-2). These species occur primarily on nematode cysts and eggs and are characterized by the often prominent production of dictyochlamydospores and nonadhesive conidia that are oval to subglobose to truncate. *Verticillium suchlasporium* is unusual among *V.* sect. *Prostrata* in that it forms erect conidiophores. Group D3 consists of the homopteran pathogen *V. pseudohemipterigenum*, which produces a compact whorl of phialides on erect conidiophores that distinguishes it from other species of the group (HYWEL-JONES *et al*. 1997). The considerable morphological differences that exist among the fungi of group D may serve to divide them into more natural groups in future analyses (ZARE *et al.* 2000 – part 1), however, the data presented here do not robustly address this issue.

Discrepancies exist whether some species of *V.* sect. *Prostrata* (e.g., *V. balanoides*) are capable of dictyochlamydospore production, and considerable debate exists over the taxonomic utility of dictyochlamydospore production among species of *Verticillium*. Because of its inconsistent formation in culture, GAMS (1971, 1988) regarded the dictyochlamydospore as a poor taxonomic character, unsuitable for a subdivision of *V.* sect. *Prostrata*. In contrast, KAMYSCHKO (1962) erected *Diheterospora* and BATISTA & FONSECA (1965) *Pochonia*, largely on the basis of dictyochlamydospores with type species that turned out to be identical with *V. chlamydosporium*. This taxonomy was followed by BARRON & ONIONS (1966) and BARRON (1985) who considered the dictyochlamydospore as a valuable character in the taxonomy of nematophagous fungi and parasites of rotifers (e.g. *Rotiferophthora*). These analyses suggest that while production of dictyochlamydospores may distinguish some taxa, i.e., group D2, their production may be polymorphic for some verticilliate fungi of the Clavicipitaceae and the ability to produce such structures may have been lost and gained several times. A more accurate character state reconstruction of this trait is not possible until its distribution is confirmed for more anamorphs of the Clavicipitaceae.

Relationships of *V.* sect. *Prostrata* with anamorphs and teleomorphs of the Clavicipitaceae: Isolates of *V.* sect. *Prostrata* integrate with anamorphs and teleomorphs of the Clavicipitaceae at several points in the rDNA phylogeny. Importantly, not only was *Verticillium* revealed to be polyphyletic, but *Cordyceps* was also inferred not to be monophyletic. It consisted of two separate clades; one that included *C. militaris* and relatives and will be referred to as *Cordyceps sensu stricto*, and one that included the parasites of *Elaphomyces* (e.g., *C. ophioglossoides*) and pathogens of a diversity of arthropods and will be referred to as the *C. ophioglossoides* clade. Furthermore, the grass endophytes of the Clavicipitaceae did not form a monophyletic group, although their monophyly could not be rejected by parsimony analyses of constraint topologies that forced their monophyly (Templeton WSR, P >>0.05). *Verticillium* sect. *Prostrata* group B is included with teleomorphs of *Cordyceps s. s.* and the closely related anamorphic species of *Beauveria*, *Engyodontium*, *Microhilum*, and *Paecilomyces* (*Isaria*). The teleomorphs of the clade include *C. militaris*, the type species of *Cordyceps*, and morphologically similar and closely related *Cordyceps* species that are pathogens of Lepidoptera (e.g., *C. bifusispora* O.E. Erikss.) and Coleoptera (e.g. *C. scarabaeicola* Y. Kobay.). Of particular interest are the isolates of *Verticillium* that are known to be linked with teleomorphs of *Torrubiella*, a genus hypothesized as closely related to *Cordyceps* because of its ecology as an entomopathogen and its ascus and ascospore morphology (MAINS 1949, KOBAYASI 1982). *Verticillium lecanii* (isolate IMI 304807) was established from an isolate of *Torrubiella confragosa* pathogenic on scale insects (JUN *et al*. 1991). *Verticillium aranearum* has also been linked to *Torrubiella* (*T. alba* Petch), although the culture sampled here was not isolated from a teleomorphic specimen. Another link to *Torrubiella* may exist in *V.* sect. *Prostrata* group B2, the most closely related clade to *Cordyceps s.s.* '*Cephalosporium*' *lanosoniveum* CBS 740.86 was isolated from a teleomorph tentatively identified as *Torrubiella* sp. (H.C. EVANS, pers. comm.). These results suggest that *Torrubiella* may display its closest phylogenetic affinity among the brightly pigmented, fleshy stromatic species of *Cordyceps s. s.*

In the strict consensus tree (Fig. 2-1), *V.* sect. *Prostrata* group C is resolved as being related to teleomorphs of the *C. ophioglossoides* clade. (e.g., *C. capitata*, *C. gunnii*) and the closely related anamorphs of *Hirsutella*, *Harposporium*, and *Paecilomyces* (Figs 2-1, 2-2). Teleomorphs of this clade are represented by darkly pigmented, stromatic species that show considerable diversity in morphology and host affiliation to the point that two isolates, *Cordycepioideus bisporus* and *Atricordyceps harposporioides*, are not currently classified in *Cordyceps*. The *C. ophioglossoides* clade is not strongly supported by the data (Fig. 2-2) and the current sampling certainly underrepresents the phylogenetic diversity of teleomorphs of the Clavicipitaceae. However, *C. gunnii*, an Australian parasite of lepidopteran larvae (KOBAYASI 1941, 1982), is well supported as a teleomorph closely related to *V.* sect. *Prostrata* group C (Fig. 2-2). An anamorph has not been established by culture methodology for *C. gunnii* and these data do not establish that *C. gunnii* is the teleomorph of any member of this group of *V.* sect. *Prostrata*. Rather, these results provide predictive value for future sampling of teleomorphs that may further illuminate teleomorph–anamorph relationships and connections within the Clavicipitaceae.

Production of *Verticillium* anamorphs is known for *C. ophioglossoides*, a parasite of *Elaphomyces*, but the phialides lack the basal inflation as in *V.* sect. *Prostrata* group C and these data do not confidently establish a close relationship between *C. ophioglossoides* and *V.* sect. *Prostrata* group C.

Although *V.* sect. *Prostrata* group D is confirmed as a member of the Clavicipitaceae, its relationships to teleomorphs and other anamorphs of the family are poorly resolved (Figs 2-1, 2-2). Group D1 (*V. epiphytum*) is included in the clade containing the teleomorphs and anamorphs of *Epichloë* and group D3 (*V. pseudohemipterigenum*), along with the nematophagous species and *Rotiferophthora angustispora*, grouped most closely with the teleomorphs of *Atkinsonella*, *Balansia* and *Claviceps*. None of these relationships were strongly supported by the data (Fig. 2-2). All of these teleomorphs are symbionts of the Poaceae (ROGERSON 1970, CLAY 1988) and some are linked to anamorphs that are morphologically similar to *V.* sect. *Prostrata* (e.g., *Acremonium*, *Neotyphodium*) (DIEHL 1950, MORGAN-JONES & GAMS 1982, GLENN *et al*. 1996, SCHARDL *et al*. 1991, 1997). The similarity in anamorphs, however, exists for many teleomorphs of the Clavicipitaceae and is by itself not proof of a close relationship. Group D2 of *V.* sect. *Prostrata* and *Metarhizium*, an entomopathogenic anamorph, are also placed in this poorly resolved region of the tree, but again their relationship is not strongly supported by the data (Fig. 2-2) and remains speculative.

Evolution of host association: In the classification of *V.* sect. *Prostrata* and Clavicipitaceae, host affiliation has been regarded as an important taxonomic character (DIEL 1950, GAMS & VAN ZAAYEN 1982, ZARE *et al*. 2000 – part 1). In an attempt to better understand the evolution of host-jumping, host association was mapped onto one of the most parsimonious trees (Fig. 2-2). Because of the lack of support for much of the basal nodes of the Clavicipitaceae, and the presence of multiple most parsimonious trees, we present these results as working hypotheses and emphasize only the more strongly supported resolutions (Fig. 2-2).

Verticillium sect. *Prostrata* group B1 includes pathogens of insects, mites, and fungi. Its placement within *Cordyceps s.s.* expands the host range of this clade beyond that of Lepidoptera and Coleoptera and bolsters arguments that the inclusion of anamorphic taxa in phylogenetic analyses improves our understanding of the evolution of host affiliation and life histories. Pathogens and parasites of fungi are located in four regions of the rDNA tree of the Clavicipitaceae (Fig. 2-2), a pattern consistent with multiple origins of fungal pathogens on distantly related groups of fungi, i.e., homobasidiomycetes, rusts, and ascomycetous truffles. However, these data also support independent origins of fungal pathogens on the same host (*Hemileia vastatrix*), as two clades (B2 and D1) of rust pathogens are resolved in these analyses (Fig. 2-2). NIKOH & FUKATSU (2000) proposed that the parasites of *Elaphomyces* originated from a host-jump from arthropods onto truffles. While these data are consistent with their finding, they do not provide unequivocal support for the polarity of a single arthropod to *Elaphomyces* host-jump (Fig. 2-2).

At least two separate groups of nematophagous fungi exist among the species of *Verticillium*, suggesting that two independent origins of parasitism of nematodes may have occurred within the Clavicipitaceae (Fig. 2-2). However, the poorly resolved basal nodes of the Clavicipitaceae and the paraphyly of *V.* sect. *Prostrata* group D3 do not allow us to distinguish between the hypotheses of two independent origins of parasitism of nematodes and a single origin followed by multiple losses or host-jumps. Furthermore, many isolates of nematophagous *Verticillium* species are isolated from plant or fungal material and the extent to which they may exist saprotrophically in nature is unknown.

Conclusion: The data presented here illustrate the need for the inclusion of anamorphs and teleomorphs in common phylogenetic analyses. Gene phylogenies provide testable phylogenetic hypotheses of anamorph–teleomorph relationships and their evolutionary history, despite disparate morphologies and ecologies. These data supported the inclusion of most isolates of *V.* sect. *Prostrata* in the Clavicipitaceae, but rejected the monophyly of these fungi within the family. The *Verticillium* morphology is distributed throughout much of the Clavicipitaceae, but differences in phialide and conidium morphology, and to a lesser extent dictyochlamydospore production are consistent with certain groups of *V.* sect. *Prostrata*. Finally, many of the basal nodes in the rDNA phylogeny of the Clavicipitaceae were characterized by short branch lengths and poor support. Molecular analyses that include increased sampling of additional loci and taxa of the Clavicipitaceae and their anamorphic fungi are needed to more confidently resolve these nodes and better define monophyletic, infrafamilial clades.

PART THREE:
GENERIC CLASSIFICATION

Results

In both phylogenetic analyses presented in parts 1 & 2, a major clade becomes evident that comprises most of the entomogenous species. According to SUNG *et al*. (2001 – part 2), this cluster not only includes the majority of species previously classified in *Verticillium* sect. *Prostrata* but also the genus *Beauveria* with polyblastic conidiogenesis, the entomogenous species generally classified in *Paecilomyces* (now *Isaria*, HODGE *et al*. 2004) characterized by swollen phialides and catenate conidia, and the unispecific genus *Microhilum* with polyblastic conidiogenesis. The species of *Verticillium* sect. *Prostrata* with catenate conidia (GAMS 1971) have not yet been characterized with molecular methods and cannot be considered here. Within group B1 of SUNG *et al*. (2001 – part 2) the morphologically most similar species, *V. lecanii* and '*Cephalosporium*' *longisporum* CBS 126.27 appear not to be very closely phylogenetically related, but they roughly span the array of species that we would include in one genus (with the exclusion of *Beauveria* and *Microhilum*). Our main concern is to delineate workable and reasonably natural genera that can also be recognized on morphological grounds. A genus is needed to accommodate the mostly entomogenous species with slender phialides. To facilitate practical usage, this genus will have to exclude the three groups mentioned, and possibly others, even though they are probably derived from it. We therefore take the risk of introducing a new, obviously paraphyletic genus, *Lecanicillium*. Based on the ITS-5.8S sequences, the species comprised in *Lecanicillium* form a monophyletic cluster with 99% Jackknife support, as evidenced in the cladogram of Fig. 3-1 (ZARE *et al*. 2000 – part 1).

It is somewhat surprising that no other available generic name was found among the numerous synonyms of the included taxa, most of which were originally described in *Cephalosporium* Corda, a generic name of doubtful application that is now abandoned.

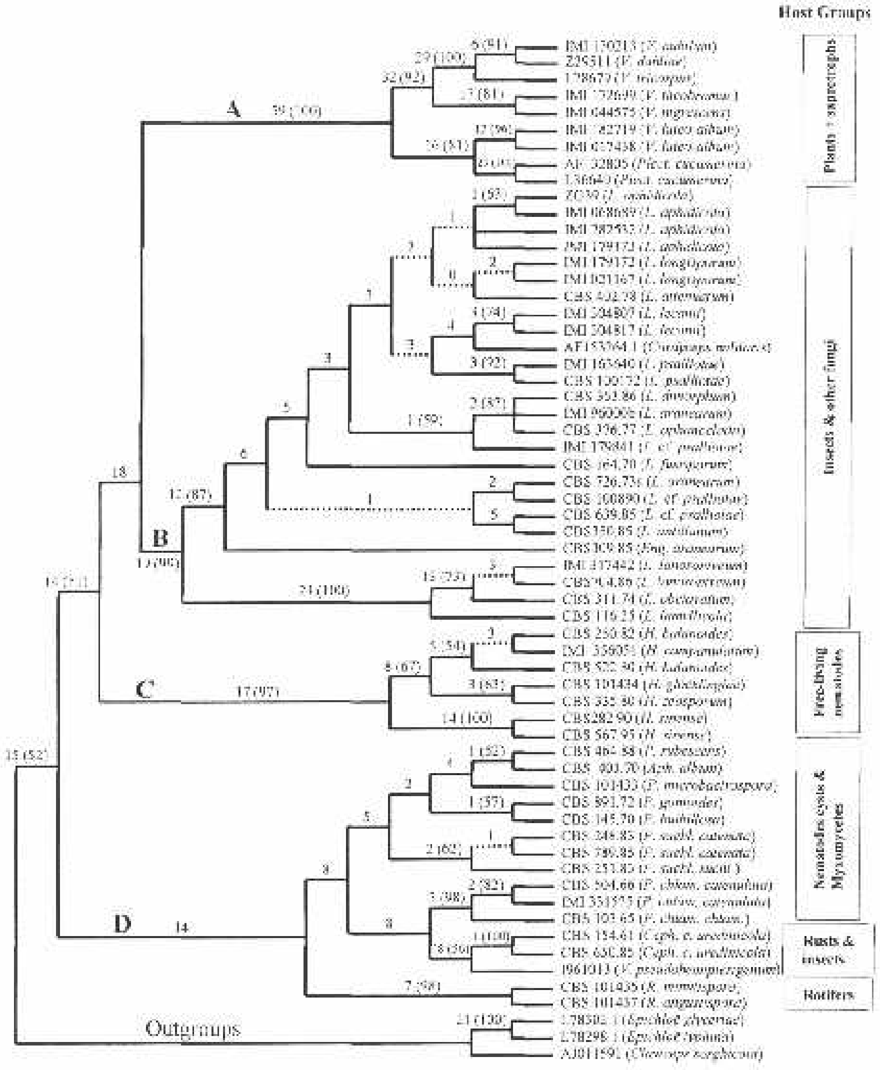


Fig. 3-1. One of 240 equally parsimonious trees recovered using sequences of ITS region and 5.8S gene (adapted from ZARE *et al*. 2000 – part 1). Dashed lines indicate branches that collapsed in the strict consensus tree. Plain figures indicate branch lengths and figures in brackets show the Jackknife support values. CI = .52, HI = 0.47, RI = 0.82, RC = 0.42, tree length = 909. (*V.* = *Verticillium*, *Plect.* = *Plectosphaerella*, *L.* = *Lecanicillium*, *Eng.* = *Engyodontium*, *H.* = *Haptocillium*, *P.* = *Pochonia*, *Aph.* = *Aphanocladium*, *Ceph. c.* = *Cephalosporium curtipes* var., *R.* = *Rotiferophthora*). The relevant new combinations are given in parts 4-6.

A surprising finding from molecular work was the heterogeneity of fungi classified in *Aphanocladium* W. Gams (GAMS *et al*. 1998, ZARE *et al.* 2000 – part 1). Many strains hitherto misidentified as *A. album* (Preuss) W. Gams were reclassified as *A. aranearum* (Petch) W. Gams (ZARE *et al*. 2000 – part 1). These strains form exclusively short, early collapsing conidiogenous cells, often with a swollen base, or just minute conidiiferous denticles, scattered laterally along the cells of aerial hyphae, termed 'aphanophialides' by GAMS (1971). Such strains are linked to *V. psalliotae* Treschow by an intermediate taxon, identified thus far as *Aphanocladium dimorphum* J.D. Chen. Therefore this group of species, with the exclusion of *A. album s. stricto*, is included in *Lecanicillium*.

Microhilum oncoperae H.-Y. Yip & A.C. Rath (YIP & RATH 1989) is a member of the same clade. It seems morphologically sufficiently distinct from the species here included in *Lecanicillium*, because of its polyblastic conidiogenesis on mostly discrete, terminal or lateral conidiogenous cells with persistent, gradually narrowing denticles. These denticles are reminiscent of those seen in *Beauveria*, though they are less sharply separated from the supporting venter and not diverted in a zig-zag manner. *Microhilum* is also similar to the unrelated *Spicellum roseum* Nicot & Roquebert (SEIFERT *et al*. 1997), which has less conspicuous denticles.

Engyodontium aranearum (Cavara) W. Gams *et al.*, which is characterized by partially polyblastic conidiogenesis, obviously forms part of the *Lecanicillium* clade. The type species of this genus, *E. parvisporum* (Petch) de Hoog, of which no living culture is available, has a different kind of dense polyblastic conidiogenesis and is probably not congeneric. However, before more species of *Engyodontium* are examined, no nomenclatural conclusions can be drawn on species of this genus.

An unsupported branch within (according to ITS) or next to *Lecanicillium* (according to other ribosomal sequences) comprises two species with exclusively solitary phialides (*Acremonium obclavatum* and '*Cephalosporium*' *lanosoniveum*) and *V. lamellicola* with sometimes verticillate phialides. Their separation from the remaining species at the generic level is hardly defensible on morphological grounds and no decision about their status is taken at the moment.

Verticillium epiphytum Hansf., despite its similarity with *V. psalliotae*, appears on another subclade near species of *Pochonia*. This species deviates from *Lecanicillium* by its thick-walled cyanophilic chlamydospores, a feature not seen in any *Lecanicillium* species. Therefore *V. epiphytum* is not included either in *Lecanicillium* or in *Pochonia*.

More verticillium-like anamorphs, formerly placed in sect. *Prostrata*, remain that do not properly fit into these genera and still await a reliable reclassification. For the time being, they are still referred to as species of *Verticillium.*

LECANICILLIUM W. Gams & Zare, Nova Hedwigia 72: 332, 2001.

Conidiophores commonly arising from aerial hyphae, usually prostrate and little differentiated from the subtending hyphae. Conidiogenous cells discrete aculeate phialides and/or aphanophialides, verticillate or solitary. Conidia adhering in slimy heads or fascicles, sometimes inserted transversely on the tip of the phialide, sometimes forming straight or imbricate chains, short- to long-ellipsoidal to falcate with pointed ends, not conspicuously cyanophilic. Chlamydospores, dictyochlamydospores or swollen hyphal portions absent. Octahedral or prismatic crystals commonly present in the agar medium. Colonies usually fast-growing, reaching 15-30 mm diam. in 10 d on PDA or MEA. Species mostly entomogenous or fungicolous.

Type species: *Lecanicillium lecanii* (Zimmerm.) Zare & W. Gams.

Lecanicillium lecanii (Zimmerm.) Zare & W. Gams, Nova Hedwigia 72: 333, 2001.

≡ *Cephalosporium lecanii* Zimmerm., Over een schimmelepidemie der groene luizen, korte berichten uit 's Lands Plantentuin. Teysmania 9: 243, 1899 (basionym).
≡ *Verticillium lecanii* (Zimmerm.) Viégas, Rev. Inst. Café São Paulo 14: 754, 1939.

Neotype: IMI 331550, designated by GAMS & ZARE (2001), derived from a single ascospore of *Torrubiella confragosa* Mains isolated from *Coccus viridis* in Indonesia.

Teleomorph: *Torrubiella confragosa* Mains, Mycologia 41: 303–310, 1949.

A detailed account of the species and the necessary new combinations is given in part 4.

POCHONIA Batista & O.M. Fonseca, Publ. Inst. Micol. Recife 462: 5, 1965.

= *Diheterospora* Kamyschko ex G.L. Barron & Onions, Canad. J. Bot. 44: 866, 1966 [*Diheterospora* Kamyschko, Botan. Mater. (Not. Syst. Sect. Crypt. Inst. Bot. Acad. Sci. USSR) 15: 138, 1962, nom. inval. Art. 37.1].

Colonies rather fast-growing, reaching 15–40 mm diam. in 10 days. Conidiophores usually prostrate and little differentiated from vegetative hyphae, sometimes erect. Conidiogenous cells phialides, verticillate or solitary. Conidia subglobose, ellipsoidal to rod-shaped, isodiametric-polyhedral, or falcate with blunt ends, adhering in globose heads or chains. Dictyochlamydospores often produced on the surface of the colony or submerged in the agar. Crystals absent.

Type species: *Pochonia humicola* Batista & O.M. Fonseca = *P. chlamydosporia* (Goddard) Zare & W. Gams.

Species accommodated under *Pochonia* mainly parasitize nematode cysts and mostly produce dictyochlamydospores or at least some irregularly swollen hyphae. The species can be more or less easily distinguished from each other on the basis of conidial shape. A detailed account of the species is given in part 5.

Pochonia chlamydosporia (Goddard) Zare & W. Gams, var. ***chlamydosporia***, Nova Hedwigia 72: 334, 2001.

≡ *Verticillium chlamydosporium* Goddard, Bot. Gazette 56: 275, 1913 (basionym).

≡ *Diheterospora chlamydosporia* (Goddard) G.L. Barron & Onions, Canad. J. Bot. 44: 866, 1966.

Neotype: CBS 103.65 = ATCC 16289, isolated from soil under *Brassica napus* in Germany by W. Gams, designated by GAMS (1988).

Pochonia chlamydosporia var. ***catenulata*** (Kamyschko ex G.L. Barron & Onions) Zare & W. Gams, Nova Hedwigia 72: 334, 2001.

≡ *Diheterospora catenulata* Kamyschko [Botan. Mater. (Not. Syst. Sect. Crypt. Inst. Bot. Acad. Sci. USSR) 15: 138, 1962] ex G.L. Barron & Onions, Canad. J. Bot. 44: 868, 1966 (basionym).

≡ *Verticillium catenulatum* (Kamyschko ex G.L. Barron & Onions) W. Gams, *Cephalosporium*-artige Schimmelpilze, p. 190, 1971.

≡ *Verticillium chlamydosporium* var. *catenulatum* (Kamyschko ex G.L. Barron & Onions) W. Gams, Netherlands J. Pl. Pathol. 94: 134, 1988.

Contrary to the statement in BARRON & ONIONS (1966), KAMYSCHKO (1962) did not designate a type species for the genus; therefore her description of the genus was not recognized as valid by GAMS (1971). The genus was validated only by BARRON & ONIONS (1966) and is therefore predated by *Pochonia*. The most important species, *P. chlamydosporia*, is in recent literature generally referred to as a species of *Verticillium* and the name *Diheterospora* is not very widely used. Therefore we do not propose it for conservation. Changing the name is also defensible to emphasize the changed genus concept, in which the dictyochlamydospores are subordinate in importance.

Aphanocladium album Preuss *s. str.*, a species that is morphologically similar to *Aphanocladium aranearum*, appears closely related to *P.* cf. *suchlasporia* CBS 464.88 according to the ITS sequences. Other ribosomal sequences do not support its inclusion in *Pochonia* (GAMS *et al*. 1998, SUNG *et al.* 2001 – part 2). Therefore the genus *Aphanocladium* is retained for this parasite of slime moulds for the time being.

HAPTOCILLIUM W. Gams & Zare, Nova Hedwigia 72: 334, 2001.

Colonies slow-growing, reaching 5–15 mm diam. on PDA after 10 days. Conidiophores erect or prostrate, bearing verticillate or solitary phialides which are more or less swollen near the base. Conidia balanoid, campanulate to cylindrical, subglobose to irregularly angular, mostly terminally adhesive (adhesive element visible as a wall thickening at the upper, more-or-less truncate end), produced in heads or short chains or both. Dictyochlamydospores sometimes present. Mostly parasites of free-living nematodes, to which the conidia adhere. A detailed account of several more species to be distinguished is given in part 6.

Type species: *Haptocillium balanoides* (Drechsler) Zare & W. Gams.

Haptocillium balanoides (Drechsler) Zare & W. Gams, Nova Hedwigia 72: 335, 2001.

≡ *Cephalosporium balanoides* Drechsler, Phytopathology 31: 786, 1941 (basionym).
≡ *Acremonium balanoides* (Drechsler) Subramanian, Kavaka 5: 98, 1978 ('1977').
≡ *Verticillium balanoides* (Drechsler) Dowsett, Reid & Hopkin, Mycologia 74: 690, 1982.
≡ *Tolypocladium balanoides* (Drechsler) J. Bissett, Canad. J. Bot. 61: 1313, 1983.

Neotype and ex-neotype culture: CBS 250.82, isolated from nematodes in soil by J. Reid in Winnipeg, Canada. Designated by GAMS (1988).

ROTIFEROPHTHORA G.L. Barron, Canad. J. Bot. 69: 495, 1991.

The genus is characterized by its specialized parasitic lifestyle on bdelloid rotifers, frequent production of intercalary phialides, mostly applanate dictyochlamydospores, and the presence of one or more oil droplets in the conidia (BARRON 1985, 1991, GLOCKLING 1998). The colonies of the included species grow very slowly.

Unclassified species

Among the species of sect. *Prostrata* characterized by DNA sequences, *Verticillium epiphytum* Hansf. (HANSFORD 1943) is not included either in *Pochonia* or in *Lecanicillium*, see above. *Verticillium pseudohemipterigenum* H.C. Evans & Y. Jun (in HYWEL-JONES *et al*. 1997) is of uncertain affinity and therefore is not placed under any of the above genera. At the end of the chapter you may enter: For an expanded key to the genera see page 16.

PART FOUR:
THE GENERA *LECANICILLIUM* AND *SIMPLICILLIUM*

Species of *Lecanicillium* form slender aculeate phialides, mostly from procumbent or prostrate aerial hyphae, singly or in terminal and intercalary whorls. Erect conidiophores with one or several whorls of phialides may also occur, but the stipes are not differentiated from the subtending hyphae in wall thickness. Contrasting with *Acremonium* species, the phialide base is usually narrower than the subtending hypha. Occasionally an aculeate cell (originally differentiated as a phialide) becomes septate and produces secondary phialides. Conidia are generally elongate, adhering in heads or fascicles at the tips of the phialides, often at right angles to the phialide at maturity (LA TOUCHE 1947). Conidia adhering in fascicles at right angles to the phialide tip are seen only in *Lecanicillium* and *Simplicillium*; when present, they distinguish these genera from other verticillium-like anamorphs. In a few species or isolates, solitary conidia are formed on each phialide. Primary elongate conidia are occasionally septate, but this feature seems to have little taxonomic significance. Conidia are sometimes dimorphic. This can have two reasons: either the primary conidia are longer than those formed subsequently on the same phialide tip, or the smaller conidia are formed singly on a different kind of conidiogenous cells. These conidiogenous cells are either scattered laterally along the cells of fertile hyphae, they are short with a swollen base and narrow tip and soon collapse into inconspicuous denticles (aphanophialides in the sense of GAMS 1971), or short conidiiferous denticles appear on nodes of conidiogenous cells as they occur in *Lecanicillium tenuipes* (syn. *Engyodontium aranearum* (Cavara) W. Gams *et al.*) (GAMS *et al*. 1984). Dimorphic modes of sporulation are thus displayed by this species and by *L. dimorphum*, which both have in addition falcate phialoconidia borne on verticillate aculeate phialides. *Lecanicillium dimorphum* thus forms a link between *L. psalliotae* and *L. aphanocladii* (the former *Aphanocladium aranearum*), which only forms microconidia. Dictyochlamydospores characteristic of *Pochonia* and related genera and simple chlamydospores are absent in *Lecanicillium*.

The delimitation of *Lecanicillium* against mononematous taxa of *Hirsutella* (MINTER *et al*. 1983) is not sharp and species of *Hirsutella* have not yet been included in the phylogenetic studies. But species of this genus usually show a more sigmoid tapering of the phialides and the conidia are mostly enclosed in conspicuous slime masses. Many more isolates of the genera studied should be examined morphologically and with sequences of additional DNA elements. But we believe that this study can serve as a basis for further refinement of the taxonomy.

A cladogram based on sequences of ITS-1–5.8S–ITS-2 of the ribosomal DNA, obtained using PAUP version 4.0b4a (SWOFFORD 1998) is shown in Fig. 4-1. The RFLP patterns of the isolates examined are summarized in Table 4-I.

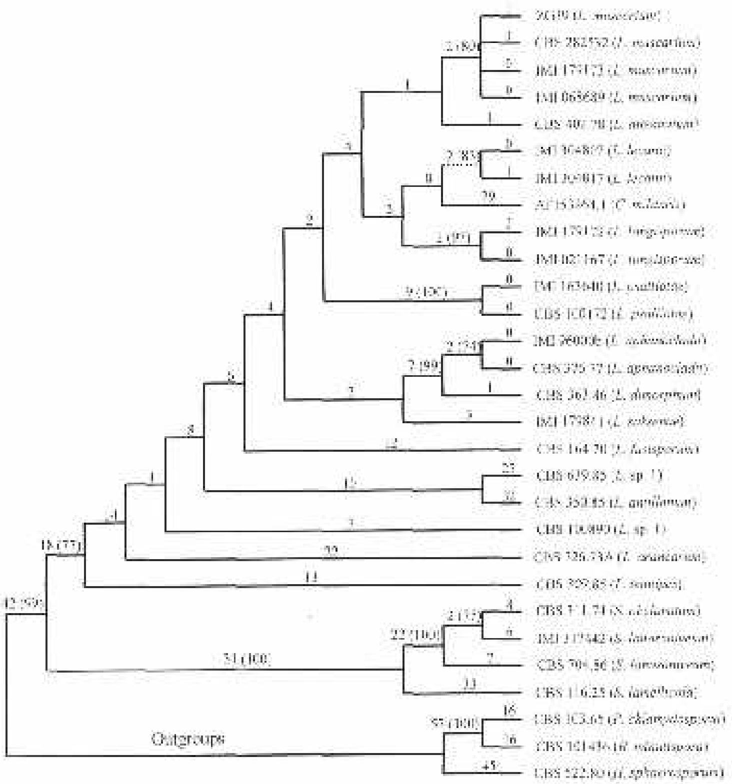
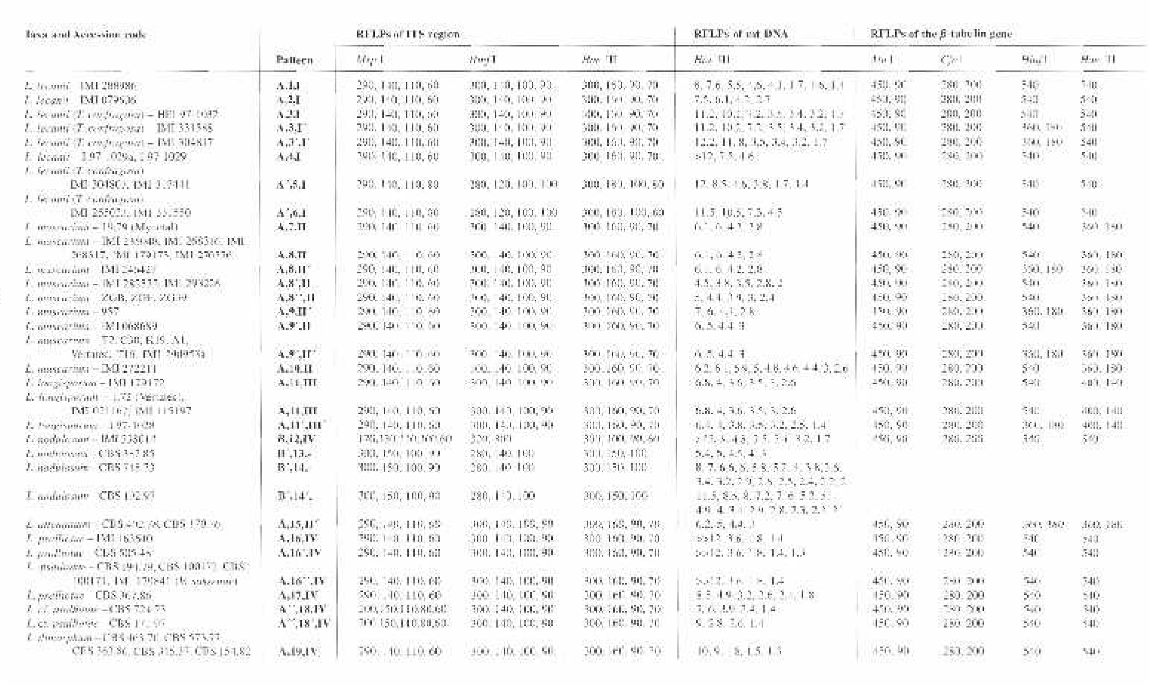


Fig. 4-1. One of the two equally parsimonious trees recovered using sequences of ITS region and 5.8S gene. Dashed lines indicate branches that collapsed in the strict consensus tree. Plain numbers above lines indicate branch lengths, and numbers in brackets show the Jackknife support values (CI = 0.67, HI = 0.32, RI = 0.69, RC = 0.46), tree length = 545. *L.* = *Lecanicillium*, *S.* = *Simplicillium*, *C.* = *Cordyceps*, *V.* = *Verticillium*, *R.* = *Rotiferophthora*, *H.* = *Haptocillium*.

Table 4-I. RFLP patterns of the taxa of *Lecanicillium* (*L.*) and *Simplicillium* (*S.*) investigated. Symbols for different patterns in the second column: ITS patterns = Capital letters, mtDNA = Arabic figures, β-tubulin gene = Roman figures.



Taxa and Accession code	Pattern	RFLPs of ITS region *Msp* I	*Hinf* I	*Hae* III	RFLPs of mt DNA *Hae* III	RFLPs of the β-tubulin gene *Alu* I	*Cfo* I	*Hinf* I	*Hae* III
L. lecanii – IMI 288986	A,1,I	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	8, 7.6, 5.5, 4.6, 4.1, 1.7, 1.6, 1.1	450, 90	280, 200	540	540
L. lecanii – IMI 079536	A,2,I	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	7.5, 6.1, 4.2, 2.7	450, 90	280, 200	540	540
L. lecanii (*T. confragosa*) – HFI 97.1037	A,2,I	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	11.2, 10.2, 7.2, 3.5, 3.4, 3.2, 1.7	450, 90	280, 200	540	540
L. lecanii (*T. confragosa*) – IMI 331556	A,3,I'	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	11.2, 10.2, 7.2, 3.5, 3.4, 3.2, 1.7	450, 90	280, 200	360, 180	540
L. lecanii (*T. confragosa*) – IMI 304817	A,3',I'	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	12.2, 11, 8, 3.5, 3.4, 3.2, 1.7	450, 90	280, 200	360, 180	540
L. lecanii – 1.97.1029a, 1.97.1029	A,4,I	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	≥12, 7.5, 1.6	450, 90	280, 200	540	540
L. lecanii (*T. confragosa*) IMI 304807, IMI 333411	A',5,I	290, 140, 110, 80	280, 120, 100, 100	300, 180, 100, 60	12, 8.5, 4.6, 3.8, 1.7, 1.4	450, 90	280, 200	540	540
L. lecanii (*T. confragosa*) IMI 255022, IMI 333550	A',6,I	290, 140, 110, 80	280, 120, 100, 100	300, 160, 100, 60	11.5, 10.5, 7.5, 4.5	450, 90	280, 200	540	540
L. muscarium – 19.79 (Mycotal)	A,7,II	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	6.2, 6, 4.3, 2.8	450, 90	280, 200	540	360, 180
L. muscarium – IMI 235648, IMI 268316, IMI 268317, IMI 179173, IMI 270336	A,8,II	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	6.1, 6, 4.3, 2.8	450, 90	280, 200	540	360, 180
L. muscarium – IMI 246427	A,8,II'	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	6.1, 6, 4.2, 2.8	450, 90	280, 200	360, 180	360, 180
L. muscarium – IMI 285537, IMI 293226	A,8',II	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	4.5, 4.8, 3.5, 2.8, 2	450, 90	280, 200	540	360, 180
L. muscarium – ZGB, ZGE, ZG19	A,8'',II	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	5, 4.4, 3.9, 3, 2.4	450, 90	280, 200	540	360, 180
L. muscarium – 957	A,9,II'	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	7, 6, 4.1, 2.8	450, 90	280, 200	360, 180	360, 180
L. muscarium – IMI 068689	A,9',II	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	6, 5, 4.4, 3	450, 90	280, 200	540	360, 180
L. muscarium – T2, C30, KJS, A1, Vertalec, 716, IMI 290953	A,9',II'	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	6, 5, 4.4, 3	450, 90	280, 200	360, 180	360, 180
L. muscarium – IMI 272211	A,10,II	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	6.2, 6.1, 5.8, 5, 4.8, 4.6, 4.4, 3, 2.6	450, 90	280, 200	540	360, 180
L. longisporum – IMI 179172	A,11,III	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	6.8, 4, 3.6, 3.5, 3, 2.6	450, 90	280, 200	540	400, 140
L. longisporum – 1.72 (Vertalec), IMI 021167, IMI 115197	A,11,III	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	6.8, 4, 3.6, 3.5, 3, 2.6	450, 90	280, 200	540	400, 140
L. longisporum – 1.97.1028	A,11',III'	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	6.5, 4, 3.8, 3.6, 3.2, 2.5, 1.4	450, 90	290, 200	360, 180	400, 140
L. nodulosum – IMI 338014	B,12,IV	170,150,110,100,60	320, 300	300, 160, 90, 60	≥15, 5, 4.5, 3.5, 3.4, 3.2, 1.7	450, 90	280, 200	540	540
L. nodulosum – CBS 357.85	B',13,-	300, 150, 100, 90	280, 140, 100	300, 150, 100	5.4, 5, 4.5, 4.3				
L. nodulosum – CBS 715.73	B',14,-	300, 150, 100, 90	280, 140, 100	300, 150, 100	8, 7, 6.6, 6, 5.8, 5.7, 4, 3.8, 3.6, 3.4, 3.2, 2.9, 2.8, 2.5, 2.4, 2.2, 2				
L. nodulosum – CBS 102.97	B'',14',-	300, 150, 100, 90	280, 140, 100	300, 150, 100	11.5, 8.6, 8, 7.2, 7, 6, 5.2, 5, 4.9, 4.3, 3, 2.9, 2.8, 2.3, 2.1, 2				
L. attenuatum – CBS 402.78, CBS 170.76	A,15,II'	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	6.2, 5, 4.4, 3	450, 90	280, 200	360, 180	360, 180
L. psalliotae – IMI 163540	A,16,IV	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	≥≥12, 3.6, 1.8, 1.4	450, 90	280, 200	540	540
L. psalliotae – CBS 505.48	A,16',IV	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	≥≥12, 3.6, 1.8, 1.4, 1.3	450, 90	280, 200	540	540
L. psalliotae – CBS 591.79, CBS 100172, CBS 100171, IMI 179841 (*V. subsessile*)	A,16'',IV	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	≥≥12, 3.6, 1.8, 1.4	450, 90	280, 200	540	540
L. psalliotae – CBS 367.86	A,17,IV	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	8.5, 4.9, 3.2, 2.6, 2.1, 1.8	450, 90	280, 200	540	540
L. cf. *psalliotae* – CBS 724.73	A'',18,IV	290,150,110,80,60	300, 140, 100, 90	300, 160, 90, 70	7, 6, 4.9, 2.4, 1.4	450, 90	280, 200	540	540
L. cf. *psalliotae* – CBS 1[illegible].97	A'',18',IV	290,150,110,80,60	300, 140, 100, 90	300, 160, 90, 70	9, 5.8, 3.6, 1.4	450, 90	280, 200	540	540
L. dimorphum – CBS 363.86, CBS 373.73, CBS 363.86, CBS 215.77, CBS 154.82	A,19,IV	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	10, 9, 1.8, 1.5, 1.3	450, 90	280, 200	540	540

Taxa and Accession code	RFLPs of ITS region				RFLPs of mt DNA	RFLPs of the *β*-tubulin gene			
	Pattern	*Msp* I	*Hinf* I	*Hae* III	*Hae* III	*Alu* I	*Cfo* I	*Hinf* I	*Hae* III
L. dimorphum – CBS 363.86	A,19',IV	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	10, 6, 5.2, 2.6, 1.2	450, 90	280, 200	540	540
Lecanicillium sp. 1 type 1 – CBS 639.85, CBS 716.??	C,20,V	200,140,110,80,60	280, 240, 90	290, 190, 160	>12, 2.5, 1.2	450, 90	280, 200	320, 220	260, 240
Lecanicillium sp. 1 type 2 – CBS 101890	C',21,VI	270, 140, 110, 60	290, 240, 90	300, 160, 90, 70	11, 10, 2.5	450, 90	290, 200	330, 210	540
L. aphanocladii – IMI 956006, CBS 451.93, CBS 581.66, CBS 274.76, CBS 336.77, CBS 365.76, CBS 458.82, CBS 797.84, CBS 771.83, CBS 798.83	A,22,IV	290, 140, 110, 60	300, 140, 100, 90	300, 160, 90, 70	12.5, 1.9	450, 90	280, 200	540	540
L. tenuipes – CBS 309.85	D,23,IV	260, 140, 120, 70	290, 140, 90, 60	310, 150, 90, 70	11, 9, 3.3, 2.8, 2.6, 1.6	450, 90	280, 200	540	540
L. attenuatum – CBS 726.73A	E,24,IV	270, 180, 140	290, 240, 90	290, 160, 90, 70	11, 5.2, 2, 2.5, 1.5	450, 90	280, 200	540	540
L. evansii – CBS 726.73B	F,25,VII	400, 190	300, 290, 90	400, 90, 80	9, 4.8, 4.3, 2.5, 1.6, 1.2	240, 120, 90	280, 120, 80	400, 140	210, 160, 130
L. evansii – CBS 264.39, CBS 711.84, CBS 631.82, CBS 100570	F,25',VII	400, 190	300, 290, 90	400, 90, 80	7.1, 5, 4.8, 2.5, 1.2	240, 120, 90	280, 120, 60	400, 140	210, 160, 130
Lecanicillium sp. 2 – CBS 352.77	G,26,VIII	270, 200, 150	200, 140, 120, 90	540, 90	7.1, 6, 4.2, 2.5	450, 90	280, 200	320, 220	290, 170, 140
L. antillanum – CBS 350.85	H,27,IX	190, 50,110,40,20	280, 150, 130, 90	350, 150, 80, 50	10, 8.5, 4, 2.8, 2, 1.8	250, 140, 80	180, 150, 90	540	300, 160, 80
L. fusisporum – CBS 164.70	I,28,X	290, 140, 120, 60	300, 220, 90	290, 160, 50, 50	9.5, 6, 5.2, 1.9, 1.4	280, 120, 90	280, 280	540	540
L. fusisporum – CBS 16?.70, CBS 166.70	I',28',X'	290, 140, 120, 60	300, 220, 90	290, 180, 50, 70	9.5, 5, 2.5, 2.6, 1.4	280, 120, 90	280, 120, 80	540	390, 180
L. acerosum – CBS 418.81	J,29,-	190, 140, 110	240, 230, 100	450, 150	6.5, 5, 4.5, 4, 3.5, 3.2, 2.8, 2.5, 2.3, 1.8, 1.4				
S. lanosoniveum – CBS 123.42, 1?7-100?, CBS 305.89	K,30,XI	290, 140, 110, 60	250, 140, 120, 90	260, 220, 90, 60	9, 5.5, 3.6, 2, 1.8	540	280, 200	540	360, 140
S. lanosoniveum (*Torrubiella* sp.) CBS 704.86, IMI 331??	K,30,XI'	290, 140, 110, 60	250, 140, 120, 90	260, 220, 90, 60	9, 5.5, 3.6, 2, 1.8	540	280, 200	540	400, 140
S. lanosoniveum – CBS 311.92	K,30',XI'	290, 140, 110, 60	250, 140, 120, 90	260, 220, 90, 60	12, 9, 6.5, 5.5, 3.6, 2, 1.8	540	280, 200	540	400, 140
S. lanosoniveum (*Torrubiella* sp.) – IMI 332451	K',30',XI''	290, 170, 140	250, 140, 120, 90	260, 220, 90, 60	5.5, 3.6, 2.9, 2.1, 2, 1.4	510	200, 150, 120	510	210, 150, 150
S. lanosoniveum – IMI 317421	K,31,XI'	290, 140, 110, 60	250, 140, 120, 90	260, 220, 90, 60	9, 5.5, 3.6, 2, 1.8	540	280, 200	540	400, 140
S. lanosoniveum – IMI 255094	K',31',XII	290, 170, 140	250, 140, 120, 90	260, 220, 90, 60	8, 5.2, 3.6, 2, 1.8	540	280, 200	360, 180	400, 140
S. lanosoniveum (*Torrubiella* sp.) – IMI 327112	K',31'',XII	290, 170, 140	250, 140, 120, 90	260, 220, 90, 60	8, 5.2, 4.5, 4, 2, 1.8	540	280, 200	360, 180	400, 140
S. lanosoniveum (*Torrubiella* sp.) – IMI 331542	K'',31''',XII	290, 170, 140	250, 250, 100	260, 220, 100, 50	9.5, 5.5, 3.6, 2, 1.8	540	280, 200	360, 180	400, 140
S. obclavatum – CBS 250.76, CBS 311.74	L,32,XII	290, 180, 150	250, 250, 90, 50	280, 220, 90, 70	10, 5.4, 3.6, 2.1, 1.9	540	280, 200	540	340, 200
S. lamellicola – IMI 294792, IMI 33????, IMI 23?1?0, CBS 595.?1, CBS 3?? 70A, CBS 302.70B, CBS 138.37	M,33,II	280, 190, 180	250, 250, 90, 50	280, 220, 80, 60	6, 5, 3.9, 3.8, 2.8, 2.6	450, 90	280, 200	540	300, 180
S. lamellicola – CBS 412.?0A, CBS 150.82, CBS 116.25, IMI 09????	M,33',II	280, 190, 180	250, 250, 90, 50	280, 220, 80, 60	6, 5, 3.9, 3.8, 2.8, 2.8	450, 90	280, 200	540	300, 180
S. lamellicola – CBS 302.70C	M,33'',II	280, 150, 180	250, 250, 90, 70	280, 220, 80, 60	8, 6, 3.9, 3.8, 2.8	450, 90	280, 200	540	300, 180
S. wallacei (*Torrubiella wallacei*) IMI 33? 349	M',34,II	260, 190, 100, 80	330, 250, 90, 70	260, 170, 90, 80	9, 7.4, 5.2, 4.8, 3.6, 3.4, 2.8, 2.4, 2.3, 1.9, 1.7	450, 90	280, 200	540	300, 180
V. pseudosimplicium – IMI 32?363, I 96-01?	N,35,XIII	620	200, 200, 100, 60	300, 150, 100, 70	8, 7, 2, 1, 2.2, 1.5	450, 90	200, 200	540	360, 180
Aphanocladium album – CBS 401.78	O,36,XIV	220, 140, 110, 70	190, 180, 120, 90	290, 150, 100, 90	7.5, 6.2, 4.1, 2.4	320, 220	280, 200	290, 190	400, 140
Aphanocladium album – CBS 852.72	O,36',XIV	220, 140, 110, 70	190, 180, 120, 90	290, 150, 100, 90	>12, 12, 9, 8, 7.1, 6.4, 4.3, 2.8	320, 220	280, 200	330, 190	400, 140

KEY TO THE SPECIES OF *LECANICILLIUM* AND *SIMPLICILLIUM*

1. Conidiogenous cells at least partly aphanophialides **2**
1´. Conidiogenous cells only phialides **3**

2. Conidiogenous cells only aphanophialides; conidia oval to ellipsoidal .... 10. *L. aphanocladii*
2´. Both phialides and aphanophialides present; conidia formed on aphanophialides ellipsoidal, those on phialides in heads, more or less falcate .... 9. *L. dimorphum*
If microconidia borne laterally along geniculate conidiogenous cells, . see 11. *L. tenuipes*

3. Conidia subglobose, ellipsoidal to cylindrical or obclavate (never fusiform, falcate or curved); usually in globose heads or fascicles (often at right angle to the phialide tip), sometimes in dry imbricate chains **4**
3´. Conidia at least partly falcate or fusiform, straight or curved (sometimes also partly subglobose to subcylindrical); solitary or in heads **10**

4. Phialides always solitary **5**
4´. Phialides at least partly verticillate **6**

5. Conidia obclavate, held in imbricate chains, 2.5–3.5 × 1.0–1.2 µm .... 19. *S. obclavatum*
5´. Conidia short-ellipsoidal, held in small globose heads, 1.5–3 × 0.7–1.3 µm .... 18. *S. lanosoniveum*

6. Conidia subglobose to short-ellipsoidal, large numbers adhering in heads or irregular chains; hyphal portions sometimes with conspicuously thickened walls .... 6. *Cordyceps militaris*
[other verticillium-like species with catenate conidia not treated]
6´. Thick-walled hyphal portions and chain formation absent **7**

7. Conidia cylindrical with tapering base, usually solitary, sometimes up to 2–4 per phialide .... 5. *L. attenuatum*
7´. Conidia ellipsoidal to cylindrical with symmetrically rounded ends, usually formed in large numbers (> 5) **8**

8. Conidiophores bearing up to six phialides in whorls from nodose swellings; hyphae stout (up to 4 µm wide), diminishing to 2 µm and becoming inflated at intervals into globose swellings; conidia ellipsoidal, 2.5–4.5 × 1.2–1.5 µm.........4. *L. nodulosum*
[If synnemata form on PDA, see *Hirsutella liberiana* Mains 1949]
8´. Hyphae not stout (< 2 µm wide) or nodose **9**

9. Phialides short, 11–20 × 1.3–1.8 µm, strongly tapering, secondarily produced phialides relatively common; conidia short-ellipsoidal to subcylindrical, 2.5–3.5 × 1–1.5 µm, usually very homogeneous in shape .... 1. *L. lecanii*

9´. Phialides longer, 20–35 × 1.1–1.7 µm, moderately tapering, secondarily produced phialides uncommon; conidia ranging from short ellipsoidal to narrowly cylindrical, of varying sizes, 2.5–5.5(–6) × 1–1.5(–1.8) µm .... 2. *L. muscarium*

9´´. Phialides 20–40 × 1.2–2.7 µm, moderately tapering, secondarily produced phialides uncommon; conidia cylindrical, 5.0–10.5 × 1.5–2.5 µm................3. *L. longisporum*

10(3). Phialides usually solitary, rather long (25–60 µm); conidia usually only of one type, slightly falcate (of varying sizes) with rather blunt ends, forming globose heads; terminal and thick-walled chlamydospores usually present (sometimes scanty); colony reverse usually yellow on PDA; mainly tropical and fungicolous on leaf-inhabiting fungi .... *Verticillium epiphytum* (see part 5)

10´. Phialides at least partly verticillate, generally shorter, in some species slightly flask-shaped; chlamydospores absent; colony reverse not yellow......................... **11**

11. Dense single (or double) whorls of somewhat flask-shaped phialides borne on short, erect conidiophores, bearing unilaterally curved solitary conidia in the shape of an orange segment, (3.5–)4.5–6.5 × 1.5–2 µm.. .... *V. pseudohemipterigenum* (see part 5)
[If conidia almost clavate with a sharply pointed end, produced singly or in pairs .... *Torrubiella hemipterigena*]
(see HYWEL-JONES *et al*. 1997)

11´. Phialides scattered over more or less prostrate conidiophores; besides macroconidia also microconidia present... **12**

12. Macroconidia longer than 10 µm **13**

12´. Macroconidia shorter than 10 µm.. **14**

13. Macroconidia sharply pointed, straight, rarely slightly curved, very long, 15.5–20 × 1.5–2.2 µm; microconidia cylindrical with rather pointed ends, 4.5–7.5 × 1.0–1.5 µm17. *L. acerosum*

13´. Macroconidia curved or flexuous, gradually tapering at both ends, 11–16 × 0.8–1.5 µm; microconidia ellipsoidal, 2.3–3.5 × 0.8–1.2 µm................ 15. *L. antillanum*

14. Conidia straight to slightly curved.. **15**

14´. Conidia curved **17**

15. Colonies with pink to vinaceous-red reverse; conidia solitary or few on each phialide, fusiform, straight, 3–5 × 1.3–2.0 µm.................................. 16. *L. fusisporum*

15´. Colonies with yellowish cream to brown reverse; conidia numerous on each phialide, usually straight to slightly curved **16**

16. Colonies usually with brown reverse, rather slow-growing, reaching 10–15(–30) mm diam. in 10 d; phialides narrow and delicate, 15–50 × 0.7–1 µm; macroconidia narrowly acerose, 4.5–9.0 × 0.8–1.2 µm, usually straight, microconidia 2–3 × 0.7–1.0 µm, with rather pointed ends .... 20. *S. lamellicola*

16´. Colonies with creamish brown reverse, growing faster, reaching 35–38 mm diam. in 10 days; phialides (14–)17–25(–29)× 0.7–1.2 µm; macroconidia fusiform, (7.0–)8.5–10.5(–12.5) × 1.0–1.5 µm, microconidia (3.0–)4.0–5.5(–6.5) × 0.7–1.2 µm.. 21. *S. wallacei*

16´´. Colonies with cream to yellowish cream reverse, rather fast-growing, reaching 24–28 mm diam. in 10 d; phialides wider, 18-30 × 1-1.5 µm; macroconidia straight to slightly curved, some with blunt, others with pointed ends, usually 1-celled, occasionally 1-septate, 6–9 × 1.5–2.2 µm .... 8. *Lecanicillium* sp. 1

17. Colonies high (4–8 mm); conidia fasciculate at right angles to the phialide tip, often apparently solitary, curved, with pointed ends, 5–10 × 1.2–1.7 µm............. **18**

17´. Colonies thin (2–4 mm); conidia in globose to subglobose heads, curved and of various sizes **20**

18. Ellipsoidal microconidia not prominently present and not produced on different conidiogenous cells; mostly red to purple pigment diffusing into the agar; mostly occurring in soil or associated with *Agaricus* species .... 7. *L. psalliotae*

18´. Microconidia prominently present, formed on different conidiogenous cells **19**

19. Microconidia produced on aphanophialides scattered among fertile aerial hyphae; reverse brownish cream (rarely pinkish); on various substrata.................................... .... 9. *L. dimorphum*

19´. Microconidia produced on denticles along nodes of somewhat geniculate conidiogenous cells; reverse uncoloured; on spiders... 11. *L. tenuipes*

20. Conidia curved with blunt ends and very short, 2.3–3.5 × 0.7–1.2 µm .... 13. *Lecanicillium* sp. 2

20´. Conidia curved with rather pointed ends and longer than 4 µm **21**

21. Macroconidia slightly curved, varying in size, 5–8 × 0.7–1.5 µm; ellipsoidal or subglobose conidia absent .... 11. *L. aranearum*

21´. Conidia distinctly curved, varying in size from falcate macroconidia, 4.5–7.5 × 0.8–1.2 µm, to ellipsoidal microconidia, 2.0–3.0 × 0.8–1.2 µm............ 12. *L. evansii*

LECANICILLIUM W. Gams & Zare, Nova Hedwigia 72: 332, 2001.

= *Verticillium* sect. *Prostrata* W. Gams, *Cephalosporium*-artige Schimmelpilze, p. 173, 1971 (*pro parte*).

Teleomorphs: *Torrubiella, Cordyceps*.

Etymology: *lecani-*, referring to the major component, *Verticillium lecanii*, *-cillium*, suffix taken from *Verticillium*.

Conidiophores commonly arising from aerial hyphae, usually prostrate and little differentiated from the subtending hyphae. Erect short conidiophores bearing one or two whorls of phialides, in prostrate conidiophores numbers of phialide whorls or single phialides practically unlimited. Phialides discrete, aculeate, i.e. tapering to a narrow tip, in which collarette and periclinal wall thickening are hardly visible, verticillate or solitary; some taxa also forming short, basally swollen, rapidly collapsing aphanophialides which bear single conidia. Conidia adhering in slimy heads or fascicles (often positioned at a right angle with the phialide tips), sometimes forming chains, short- to long-ellipsoidal to falcate with pointed ends, not conspicuously cyanophilic. Chlamydospores, dictyochlamydo-spores or swollen hyphal portions absent. Octahedral (sometimes also prismatic) crystals commonly present in the agar medium. Colonies usually fast-growing, reaching 15-30 mm diam. in 10 d at 20°C on PDA or MEA. Species mostly entomogenous or fungicolous.

Type species: *Lecanicillium lecanii* (Zimmerm.) Zare & W. Gams.

GAMS (1971) listed many names as synonyms under *V. lecanii*. PETCH (1925, 1931, 1932, 1939) was particularly productive in distinguishing taxa of this complex on minor details of host or morphology, as reviewed by BAŁAZY (1973). Cultural work on numerous isolates made it particularly difficult to distinguish species within a broad array of variable isolates. Nevertheless, among the strains previously subsumed under *V. lecanii* four consistent clusters could be discerned with molecular methods, which can also be recognized morphologically with some degree of confidence. They are distinguished here as the species, *L. lecanii*, *L. muscarium*, *L. longisporum*, and *L. nodulosum*. The former *Cephalosporium lanosoniveum* was found distinct from *L. lecanii* even at genus level. Strains formerly classified as *V. psalliotae* form at least three clusters, *L. psalliotae*, *L. dimorphum* and *Verticillium epiphytum* Hansf. (or *Cephalosporium curtipes* var. *uredinicola* Sukap. & Thirum.). Because of the scanty presence of thick-walled chlamydospores, and a high degree of sequence divergence compared with the remaining *Lecanicillium* species, *V. epiphytum* and similar isolates obtained from rust fungi do not seem to belong to this genus (see also parts 1, 2 and 5).

1. ***Lecanicillium lecanii*** (Zimmerm.) Zare & W. Gams, Nova Hedwigia 72: 333, 2001 (part 3, p. 54). Figs 4-2, 4-3

≡ *Cephalosporium lecanii* Zimmerm., Over een schimmelepidemie der groene luizen, korte berichten uit 's Lands Plantentuin, Teysmania 9: 243, 1899 (basionym).
≡ *Verticillium lecanii* (Zimmerm.) Viégas, Rev. Inst. Café São Paulo 14: 754, 1939.

Teleomorph: ***Torrubiella confragosa*** Mains, Mycologia 41: 305, 1949.

Among the synonyms cited by GAMS (1971), the following selection can be associated with the present species with some confidence. Only *Hirsutella confragosa* is so far convincingly connected with this species.

?= *Acrostalagmus coccidicola* Guéguen, Champignons paras. de l'homme: 252. Paris, 1904.
≡ *Cephalosporium coccidicolum* (Guéguen) Petch, Trans. Br. Mycol. Soc. 10: 162, 1925.
?= *Hyalopus yvonis* Dop, Bull. Scient. Fr. Belg. 39: 135, 1905; Rev. Mycol. (Toulouse) 28: 18, 1906.
?= *Cephalosporium coccorum* Petch, Trans. Br. Mycol. Soc. 10: 175, 1925.
= *Hirsutella confragosa* Mains, Mycologia 41: 303, 1949 (described for the anamorph of *Torrubiella confragosa*).

Colonies reaching 15–25 mm diam. in 10 days, rather compact, yellowish white, with deep yellow reverse. Phialides relatively short, 11–20(–30) × 1.3–1.8 µm, aculeate and strongly tapering, produced singly or in whorls of up to 6 directly on prostrate hyphae, or on short, more or less erect conidiophores, sometimes also produced secondarily on previous phialides. Conidia formed in heads at the apex of the phialides, typically short-ellipsoidal, 2.5–3.5(–4.2) × 1–1.5 µm, homogeneous in size and shape. Octahedral crystals present. Temperature optimum: 21–24°C (13–15 mm diam.). No growth at 33°C.

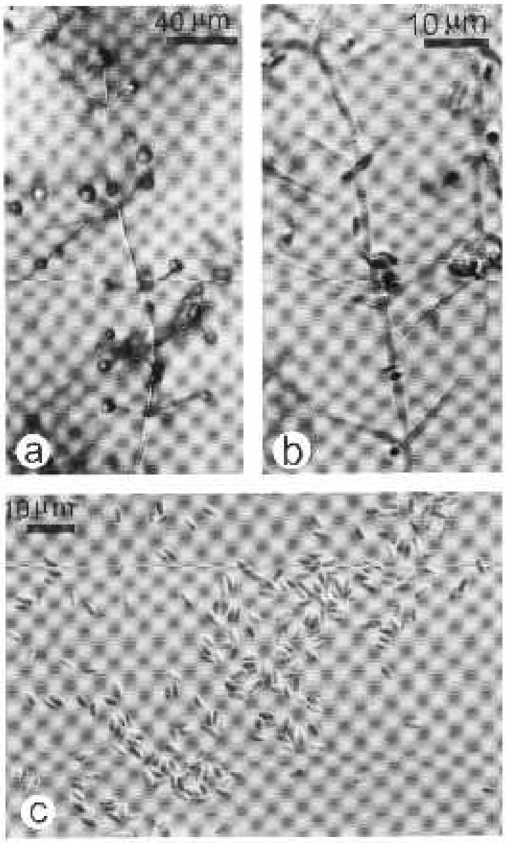


Fig. 4-2. *Lecanicillium lecanii*. a, b. Conidiophores and conidial heads, c. conidia. a–c. IMI 304817, DIC.

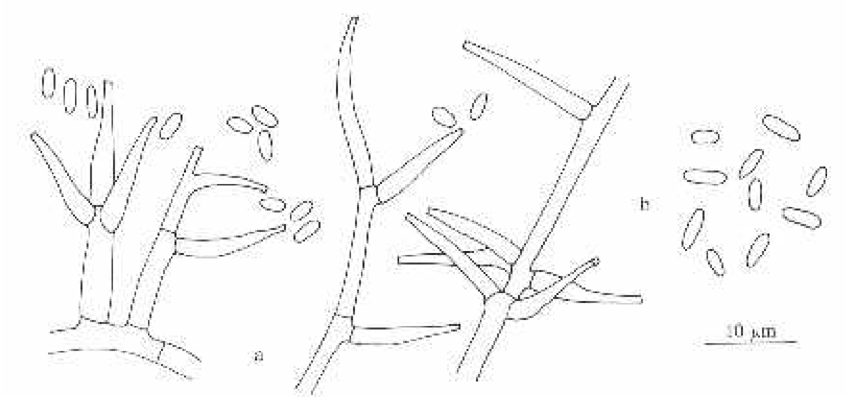


Fig. 4-3. *Lecanicillium lecanii*. a. IMI 317441, b. Conidia of IMI 331550, both 8 days on PCA.

Distribution mainly tropical: W. Indies, Dominican Republic, Peru, Jamaica, USA, Sri Lanka, Indonesia, Turkey (see also ZARE & GAMS 2003 d).

STRAINS EXAMINED (for type specimens see Gams 1971):
IMI 079606, ex *Pulvinaria floccifera* (Coccidae), Turkey, 1960, O. Ozbas.
IMI 255033, ex *Coccus viridis*, Sri Lanka, 1981, Kanagaratnam.
IMI 288986, ex scale insect (Coccidae), USA, 1984, R.T. Mimillan.
IMI 304807 = CBS 101247, together with teleomorph *T. confragosa*, ex *Coccus viridis*, W. Indies, 1985, C. Prior.
IMI 304817, ex scale insect (Coccidae), W. Indies, 1986, C. Prior, single-ascospore isolate of *T. confragosa*.
IMI 317441, ex *Coccus viridis*, Jamaica, 1987, C. Prior.
IMI 331550 = CBS 102067, together with teleomorph *T. confragosa*, ex *Coccus viridis*, Indonesia, Sulawesi, H.C. Evans, **ex-neotype**.
IMI 331588 = CBS 101246, together with teleomorph *T. confragosa*, ex forest scale (Coccidae), Dominican Republic, 1984, H.C. Evans, single-ascospore isolate.
I 97-1029a = CBS 101283, ex *Coccus viridis*, Peru, 1997, H.C. Evans.
I 97-1032, ex *Coccus viridis* (Coccidae), Peru, 1997, H.C. Evans, single-ascospore isolate of *T. confragosa*.

Ten strains of this species were examined that accurately match the descriptions given by ZIMMERMANN (1899), VIÉGAS (1939), and PETCH (1925) for isolates obtained from *Coccus viridis* in Indonesia and Brazil, respectively. A dried culture of IMI 331550 = CBS 102067, a single-ascospore isolate of *Torrubiella confragosa*, isolated by H.C. EVANS from *Coccus viridis* in Indonesia, that perfectly fits the original description, was designated by GAMS & ZARE (2001 – part 3) as **neotype** and deposited in IMI.

BAŁAZY (1973) provided a key to the numerous species formerly distinguished by PETCH and others around this taxon. He proposed *Cephalosporium* subgenus *Proverticillium* for strains with verticillate conidiophores, with the type *Cephalosporium lecanii* Zimmerm., whereas he retained strains with predominantly single phialides in *Cephalosporium* subgenus '*Eu-Cephalosporium*'. GAMS (1971) examined a large number of specimens and isolates obtained from insects and various other sources. He listed some 20 names as synonyms for *V. lecanii*, being aware that the adopted species concept might be too broad. Several clusters were noticed that could hardly be distinguished morphologically. Among these numerous synonyms, we assume that mainly those referring to fungi found on scale insects and having relatively small conidia and strongly tapering phialides might belong to this species, while most of the others are more likely to refer to *L. muscarium*. An accurate identification would only be possible after molecular examination of these specimens. PETCH (1925) reported *Verticillium lecanii* as a common species in Sri Lanka on *Coccus viridis*, especially on coffee. EVANS & HYWEL-JONES (1997) listed nineteen species of soft scales on which *Verticillium lecanii* had been reported and almost certainly these belong to *L. lecanii* since the species appears to be specific to soft scale insects, Coccidae or Lecaniidae.

Until recently, the teleomorph of *V. lecanii* was considered to be unknown (GAMS 1971, BRADY 1979). MAINS (1949) described the anamorph of *T. confragosa* as a species of *Hirsutella*. He described short synnemata on the host with 14–20 µm long, aculeate phialides that taper from 1.5 µm to a pointed tip in the fungus that was originally found on scale insects in Rio Grande do Sul, Brazil. EVANS & SAMSON (1982) linked *T. confragosa* with *V. lecanii*, based on material collected on soft scale insects (*Saissetia* sp.) on various plant hosts in the Galápagos Islands, and, after an examination of MAINS's type material, synonymized *Hirsutella confragosa*. Subsequent *in vitro* studies have confirmed this association (EVANS & SAMSON 1984, YUN *et al*., 1991). Earlier, PARKIN (1906) recorded the presence of *Torrubiella* perithecia on insects in Sri Lanka parasitized by *V. lecanii,* whilst JOHNSTON (1918) gave a brief description in Spanish of a new species, *Torrubiella lecanii*, associated with *V. lecanii* on the soft scale *Saissetia hemispherica* in Cuba. Thus, although *T. lecanii* probably is the earliest recorded name of the teleomorph of *V. lecanii*, it has not been possible to confirm the species since the type cannot be located. Moreover, the illustration (JOHNSTON 1918, p. 83) shows an ascus containing numerous small, ovoid to cylindrical spores. As stated by MAINS (1949) and EVANS & SAMSON (1982), the filiform ascospores do not break into part-spores. Thus *H. confragosa* is regarded as a confirmed synonym of *L. lecanii* and the connection is firmly established by the proposed neotypification. Five out of the 11 strains listed have been associated with *T. confragosa*.

2. ***Lecanicillium muscarium*** (Petch) Zare & W. Gams, Nova Hedwigia 73: 13-15, 2001. Figs 4-4, 4-5

≡ *Cephalosporium muscarium* Petch, Naturalist 1931: 102, Apr. 1931 (basionym).
= *Cephalosporium aphidicola* Petch, Trans. Br. Mycol. Soc. 16: 71, Sept. 1931.
?= *Oospora necans* Sacc. & Trotter, Ann. Mycol. 3: 514, 1905.
?= *Cephalosporium lefroyi* Horne, Gard. Chron. 57: 139, 1915; Trans. Brit. Mycol. Soc. 5: 241, 1915.
= *Cephalosporium thripidum* Petch, Trans. Br. Mycol. Soc. 16: 234, 1932.
?= *Cephalosporium subclavatum* Petch, Trans. Br. Mycol. Soc. 25: 262, 1942.
= *Verticillium hemileiae* Bouriquet & Bassino, Rev. Mycol. 31: 323, 1966 [Bouriquet, Rech. Systém. sur les Maladies des Plantes Cultivées à Madagascar. p. 66–73, Paris 1939; Encycl. Mycol. 12: 66–73, 1947 ('1946') (nom. inval., Art. 36)].

Colonies reaching (14–)25–30 mm diam. after 10 days, rather compact, white, with cream-coloured to pale yellow (rarely yellow) or uncoloured reverse. Phialides produced directly on prostrate hyphae or on secondary branches. Secondary branches less frequent than in *L. lecanii*. Phialides generally longer than those of *L. lecanii* and less tapering, measuring (15–)20–35 × 1.0–1.7 µm. Conidia produced in globose heads, ellipsoidal to subcylindrical, more irregular in size and shape, longer and narrower than in *L. lecanii*, measuring (2–)2.5–5.5(–6) × 1–1.5(–1.8) µm. Octahedral crystals commonly present. Temperature optimum: (21–)24(–27)°C, depending on the strain (14–21 mm diam.). No growth at 33°C.

Distribution mainly temperate: UK, Italy, etc. (ZARE & GAMS 2003 f).

STRAINS EXAMINED (for specimens see Gams 1971):
IMI 068689 = CBS 143.62 = ATCC 28300 = MUCL 9713, ex *Trialeurodes vaporariorum* (Homoptera), UK, 1957, N.W. Hussey, dried culture in herb. CBS (**epitype**, designated in Nova Hedwigia 73: 15, 2001).
IMI 179173, ex *Brachycaudus helichrysi* (Aphididae), UK, 1973, R.A. Hall.
IMI 235048 = CBS 101375, ex *Cecidophyopsis ribis* (Acari, Arachnida), UK, 1979, R.A. Hall.
IMI 246427, ex peat, UK, 1980, C. Mathews.
IMI 268316, ex *Thrips tabaci* (Thysanoptera), UK, 1982, N. Hall.
IMI 268317, ex *Thrips tabaci*, UK, 1982, R. Pickford.
IMI 270376, ex contaminated pesticide solution, UK, 1982, A. Machon.
IMI 272211, ex *Pteridium aquilinum* (Pteridophyta), UK, 1982, G. Cuff.
IMI 282532, ex *Cossus cossus* (Lepidoptera), Italy, 1984, A. Kovacs.
IMI 290953a, ex water from domestic supply, England, 1985.
IMI 293226, ex laboratory glyphosate solution, UK, 1985, D. Park.
ZGB, ZGE, and ZG39, ex *Zyginidia pullula*, Italy, 1982, P. Piatti.
Isolates 'Vertalec', 957, T2, C30, K19, A1, T16, ex insects, without details of origin.
19.79 = CBS 102071, ex *Trialeurodes vaporariorum*, England, 11979 (Mycotal).
CBS 413.70C = LCP 1052, ex *Hemileia vastatrix*, New Caledonia, J. Nicot.

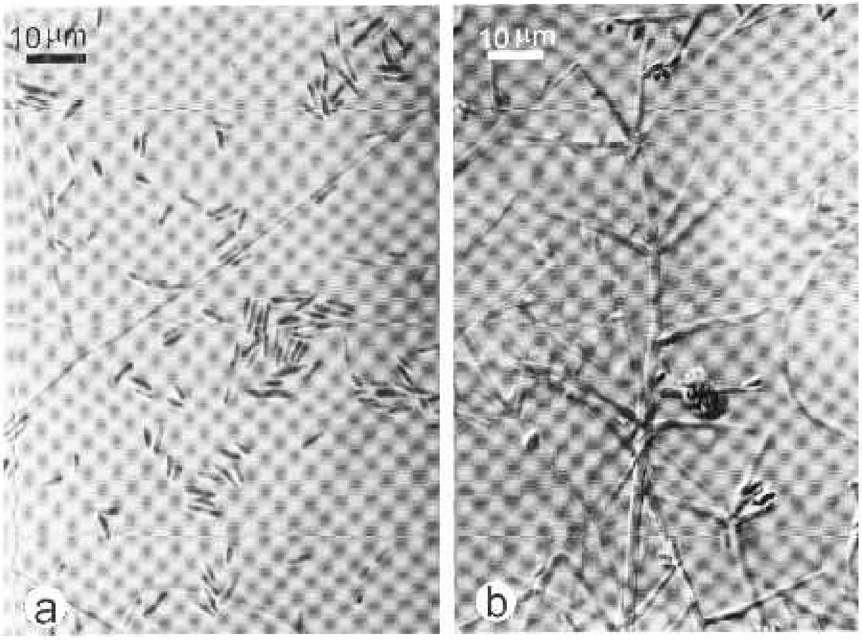


Fig. 4-4. *Lecanicillium muscarium*. a. Conidia of IMI 270376. b. Conidiophore of IMI 290953a. DIC.

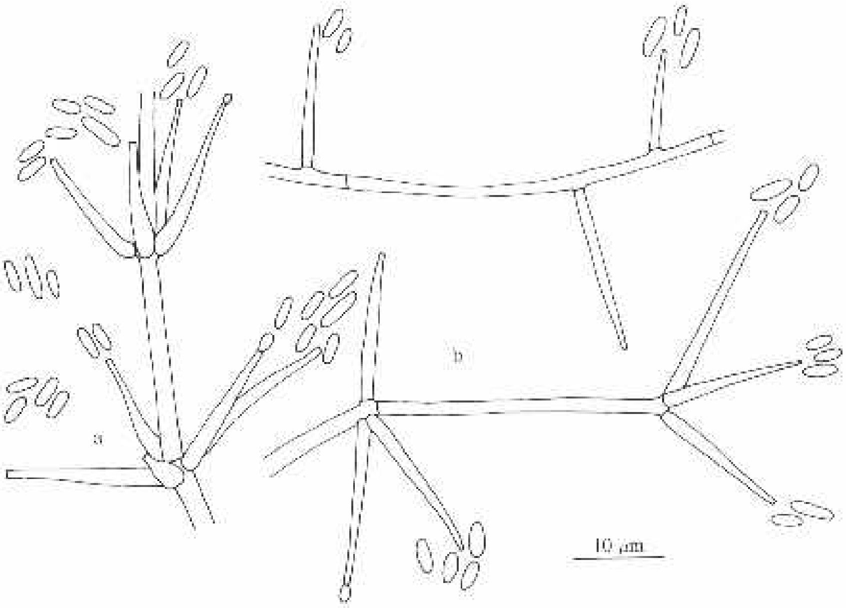


Fig. 4-5. *Lecanicillium muscarium*. a. IMI 179173, 8 days on PCA, b. CBS 102071, 9 days on MEA.

In view of the extant herbarium type specimen (in K, see GAMS 1971), a living culture, IMI 068689 = CBS 143.62, isolated by N.W. Hussey, is dried to serve as **epitype** for this taxon (herb. CBS). Among the synonyms listed, *Cephalosporium muscarium* is not the oldest, but it is the oldest well-documented name available for this fungus. We therefore chose deliberately for reactivating this name by epitypification because it is well-documented by PETCH's (1931) description and his specimens. This name predates the equally well-documented *C. aphidicola* by a few months. In the cladograms of ZARE *et al*. (2000 – part 1) and GAMS & ZARE (2001 – part 3), this species is referred to as *C. aphidicola* and *L. aphidicola*, respectively.

The very similar *L. lecanii* can be distinguished from *L. muscarium* on the basis of its shorter, broader and more homogeneous conidia, shorter, broader and more aculeate phialides, more frequent secondary branches, and deep yellow colour of the colony reverse (see also ZARE & GAMS 2003 d, f). The two taxa are distinct in ITS sequences (Fig. 4-1) and RFLP patterns (Table 4-I). *Lecanicillium longisporum* is easily distinguished from *L. muscarium* by its longer and broader conidia (see also ZARE & GAMS 2003 e).

Two strains of *V. lecanii s. lato* have been developed as biological control agents of glasshouse pests under the names Mycotal and Vertalec (HALL & BURGES 1979, HALL 1981, 1982, QUINLAN 1988). Strain 19.79 (Mycotal), here classified as *L. muscarium*, is used for the control of whitefly, *Trialeurodes vaporariorum* Westwood, and strain 1.72 (Vertalec), classified as *L. longisporum*, for the control of aphids such as the chrysanthemum stem aphid, *Macrosiphoniella sanborni* Gillette. A mixture of these two strains has been used as part of a biological control programme for pests of chrysanthemum (HELYER *et al*. 1992). In bioassay experiments, CHANDLER *et al.* (1993) noticed differences in the host-mortality ratios of the fungal strains and regarded strain 19.79 as less host-specific than strain 1.72. MILLER & GUBLER (1988) introduced '*V. lecanii*' as a biological control agent for *Sphaerotheca macularis* f. sp. *fragariae*, causal agent of powdery mildew on strawberry. JACKSON & HEALE (1985) found that 16 isolates of '*V. lecanii*', originating from insect and non-insect hosts and from soil, exhibited a wide range in conidial volumes, from 2.18 to 18.25 μm^3 as recorded by

Coulter counter analysis, and they all were haploid. All these observations probably relate to *Lecanicillium muscarium*. *Lecanicillium muscarium* has not yet been connected to a teleomorph. It has been isolated from a range of substrata, mainly insects and fungi, while *L. lecanii* has been isolated mainly from soft scale insects.

3. ***Lecanicillium longisporum*** (Petch) Zare & W.Gams, Nova Hedwigia 73: 16, 2001.

Figs 4-6, 4-7

≡ *Cephalosporium longisporum* Petch, Trans. Br. Mycol. Soc. 10: 166, 1925 (basionym) [non *Verticillium longisporum* (Stark) Karapappa *et al.*].
?= *Acrostalagmus aphidum* Oudem., Nederl. Kruidk. Arch. 3(2): 759, 1902 [no type found in L, only a drawing which does not allow a definite conclusion].
= *Cephalosporium dipterigenum* Petch, Naturalist 1931: 102.

Colonies reaching 10–30 mm diam. in 10 days on PDA, rather high, white, then becoming sulphur-yellow, with cream-coloured to pale yellow reverse. Phialides rather long, 20–40 × 1.2–2.7 µm, tapering towards the apex (sub-aculeate), often produced singly on prostrate hyphae or up to 5–6 at each node, or on secondarily produced phialides. Conidia produced in globose heads, 5.0–10.5 × 1.5–2.5 µm, ellipsoidal to oblong-oval, mostly 1-celled, rarely 1-septate. Octahedral or prismatic crystals commonly present. Temperature optimum: 21–24°C (15–20 mm diam.). No growth at 33°C.

Distribution: UK, Sri Lanka, Peru (ZARE & GAMS 2003 e).

STRAINS EXAMINED:
IMI 021167 = ATCC 46578 = CBS 126.27 = MUCL 9702, ex *Icerya purchasi* (Coccidae), Sri Lanka, 1924, T. Petch, **ex-type**.
IMI 115197 = CBS 317.70b, ex *Myzus persicae* (Aphididae), UK, 1965, Q. McGarvie.
IMI 179172, ex *Macrosiphoniella sanborni* (Aphididae), UK, 1973, R.A. Hall.
1.72 = ATCC 46578 = CBS 102072 (Vertalec), ex *Macrosiphoniella sanborni*, England, D. Chandler, 1972.
I 97-1028, ex *Citrus* aphid, Peru, 1997, H.C. Evans.

GAMS (1971) synonymized *Cephalosporium longisporum* with *V. lecanii*, while SUKAPURE & THIRUMALACHAR (1966) considered them distinct based on conidial size. The taxa are also distinct in ITS sequences (see Fig. 4-1) and LSU+SSU sequences of rDNA (SUNG *et al.* 2001 – part 2). No consistent differences could be found in the RFLP patterns of the ITS, but those of the β-tubulin gene and mtDNA also differentiate the two taxa. Among the available names we chose the oldest that is sufficiently documented by herbarium material. *Acrostalagmus aphidum* was described as having conidia 7–14 × 2.3 µm. In OUDEMANS's herbarium (L) only a somewhat artistic drawing showing large globose conidial heads is preserved but no specimen.

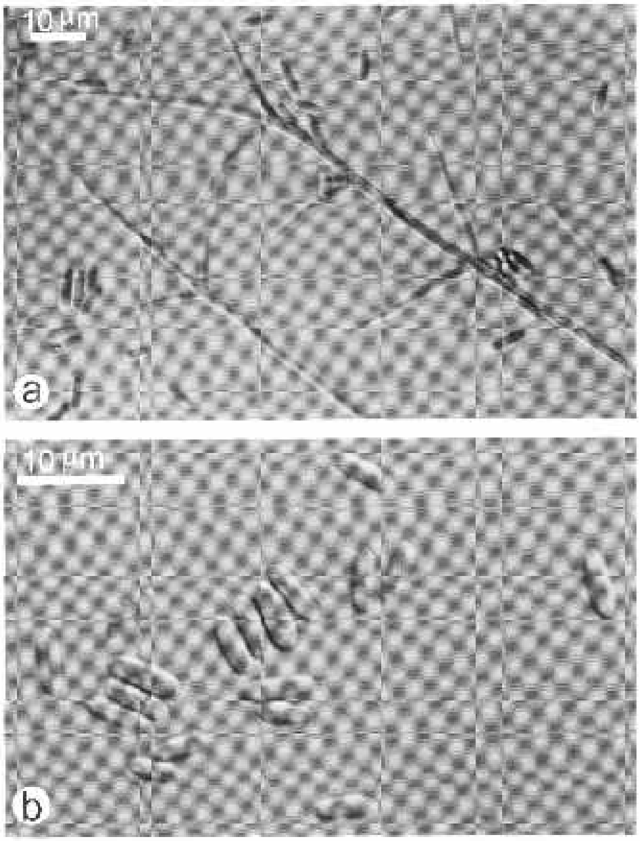


Fig. 4-6. *Lecanicillium longisporum*. a, b. Conidiophores and conidia. a, b. 197–1028, DIC.

The original strain of the commercial product Vertalec (1.72), isolated from the aphid *Macrosiphoniella sanborni* (CHANDLER *et al*. 1993), belongs to this species. Another strain also received as Vertalec ('Vertalec' see under *L. muscarium*) with small conidia is here identified as *L. muscarium*. This may indicate that Vertalec was first developed from strain 1.72, but later the strain was changed or mixed with other strains (see HELYER *et al*. 1992), or in other countries different strains are being used under the same name.

Strain I 97-1028, isolated from a *Citrus* aphid by H.C. EVANS in Peru in 1997, produced conidia of various sizes (5–8 × 1.5–2.5 µm), generally smaller than the average, but deviates only slightly in the RFLP of the β-tubulin gene and mtDNA (see also ZARE & GAMS 2003 e).

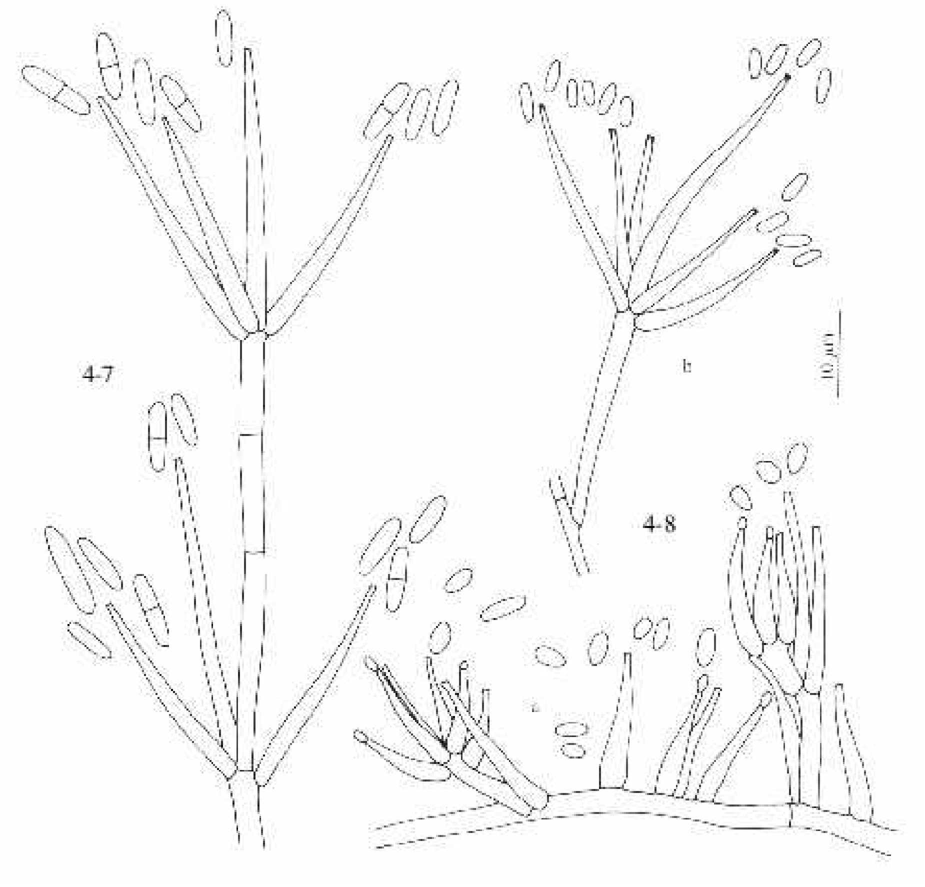


Fig. 4-7. *Lecanicillium longisporum*. CBS 317.70B, 14 days on PCA.
Fig. 4-8. *Lecanicillium nodulosum*. a, CBS 387.85, 30 days on MEA; b, IMI 338014, 14 days on PCA.

4. **Lecanicillium nodulosum** (Petch) Zare & W. Gams, Nova Hedwigia 73: 18, 2001. Figs 4-8, 4-9

≡ *Cephalosporium nodulosum* Petch, Trans. Br. Mycol. Soc. 23: 144, 1939 (basionym).

Colonies reaching 12–24 mm diam. in 10 days, white, with cream-coloured reverse. Aerial hyphae stout, 4 µm wide, diminishing to 2 µm and inflated at intervals into 3 µm wide swellings. These swellings may give rise to up to 6 phialides or to a hypha, or a mixture of both. Phialides simple, subulate, 10–20 × 1.5 µm. Conidia produced in heads of about 10 µm diam., hyaline, oval, 2.5–4.5 × 1.2–1.5 µm. Octahedral crystals present. Temperature optimum: 21–24°C (7–12 mm diam.). No growth at 33°C; in CBS 745.73 no growth at 30°C.

Distribution: Papua-New Guinea, Mexico, Ghana, France and the Netherlands.

STRAINS EXAMINED:
[Type material in Herb. Missouri Bot. Garden; fraction in BPI examined by Gams 1971].
CBS 745.73, ex Lepidopteran pupa, Ghana, 1973, H.C. Evans, **epitype**, dried culture in herb. CBS, designated in Nova Hedwigia 73: 19, 2001.
CBS 387.85, ex Varroa mite, Netherlands, CBS 1985, G.S. de Hoog.
IMI 338014, ex scale insect (Coccidae) Mexico, 1988.
CBS 192.97, ex decaying wood and *Datronia mollis*, France, 1996, W. Gams.

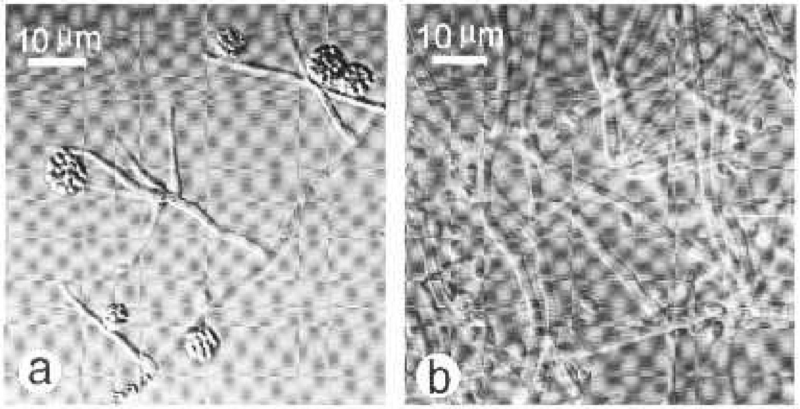


Fig. 4-9. *Lecanicillium nodulosum*. Conidiophores and conidia. a. IMI 338014, b. CBS 192.97, DIC.

This species was originally collected by T. PETCH in New Guinea from a Pyralid moth (PETCH 1939). Although GAMS (1971) merged this species with *Verticillium lecanii*, it can be distinguished from this species and *L. muscarium* by its swellings (nodules) and very short conidia. This species produced two unique types of ITS-RFLPs; one for strain IMI 338014 and the other for CBS 192.97, CBS 745.73 and CBS 387.85. The four strains had four different mtDNA-RFLP patterns. A similar fungus is *Hirsutella liberiana* Mains (MAINS 1949) which has a tendency to form synnemata on PDA (CBS 722.73 A and B, CBS 102068).

5. ***Lecanicillium attenuatum*** Zare & W. Gams, Nova Hedwigia 73: 19, 2001.

Figs 4-10, 4-11

Colonies reaching 16 mm diam. in 10 days on PDA, high, white, reverse yellowish white. Phialides produced on prostrate hyphae or on secondary branches up to 3–5 per node, measuring 9–15.5 × 1–2 µm. One conidium is usually produced at the end of the phialides, but sometimes up to 4 in dry clusters. Conidia cylindrical with attenuate base, occasionally 2-celled, 4.5–6.5 × 1.5–2.0 µm, homogeneous in size and shape. Octahedral crystals present. Temperature optimum: 24(–27)°C (12–15 mm diam.). No growth at 33°C.

Distribution: USA, Poland.

STRAINS EXAMINED:
CBS 170.76, ex caterpillar of *Carpocapsa pomonella*, Poland, 1975, S. Bałazy, **ex-type** (Type: herb. CBS).
CBS 402.78, ex leaf litter of *Acer saccharum*, USA, 1978, G.A. Kuter.

This species is close to *L. lecanii*, *L. muscarium* and *L. longisporum* on the basis of conidial shape, but it is distinct from them by the production of few, basally apiculate conidia on each phialide, and by its ITS (Fig. 4-1) and SSU+LSU sequences (SUNG *et al.* 2001 – part 2, as *Verticillium* sp. next to *L. longisporum*). CBS 402.78 is indistinguishable from CBS 170.76 on morphological grounds and ITS and β-tubulin RFLPs, but it differed slightly in its mtDNA-RFLP.

Fig. 4-10. *Lecanicillium attenuatum*. Conidia. CBS 402.78, DIC.

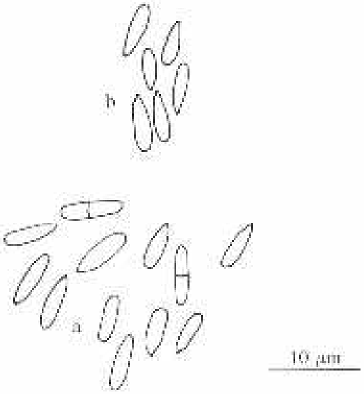


Fig. 4-11. *Lecanicillium attenuatum*. Conidia, a. CBS 170.76, b. CBS 402.78.

6. ***Cordyceps militaris*** (L. : Fr.) Link, Handb. Erk. nutzbr. häufigsten Gewächse, Berlin 3: 347, 1833.

Anamorph: *Lecanicillium* sp. (*Cephalosporium militare* Y. Kobay., Sci. Rep. Tokyo Bunrika Daig., Sect. B, 5: 113, 1941, sine diagn.).

Introducing a formal binomial for the anamorph seems unnecessary as this fungus is normally encountered as a teleomorph.

For description of the anamorph see GAMS (1971).

SEQUENCES OBTAINED FROM GENBANK:
AF153264.1 (based on EFCC-C738, deposited by J.E. Park, G.Y. Kim, M.G. Ha and J.D. Lee in Korea, unpublished) was included in sequence comparison (Fig. 4-1).
ITS sequences of AF199591.1 (strain KACC 500171), AF199590.1 (strain KACC 500161), AF153265.1, AF153266.1 showed 98-100% homology with AF153264.1 (deposited by the same authors).

7. ***Lecanicillium psalliotae*** (Treschow) Zare & W. Gams, Nova Hedwigia 73:21, 2001. Figs 4-12, 4-13

≡ *Verticillium psalliotae* Treschow, Dansk Bot. Ark. 11(1): 7, 1941 (basionym).
= *Verticillium saksenae* Kushwaha, Curr. Sci. 49: 948, 1980 [published as '*saksenii*'].

Colonies reaching 24–47 mm diam. in 10 days on PDA, high, white, with red, reddish cream to cream-coloured reverse. Red to purple pigment usually diffusing into the agar, particularly on PDA. Phialides rather long, aculeate, arising from undifferentiated prostrate conidiophores, solitary or more often 3–4(–6) in whorls on each node, measuring 25–35 × 1.0–1.5 µm. Conidia typically solitary or few fasciculate at right angles to the phialide tip, commonly falcate; in fascicles the first-formed conidium usually falcate and subsequent ones oval to ellipsoidal; falcate macroconidia curved, usually with sharply pointed ends, 1-celled, exceptionally 2-celled, 5–10 × 1.2–1.7 µm; subsequently formed microconidia, if present, oval or ellipsoidal, 2.7–3.7 × 1–1.5 µm. Octahedral crystals present. Temperature optimum: 21–24(–27)°C (24–38 mm diam., depending on the strain). No growth at 33°C (with exception of CBS 194.79, which showed optimal growth at 27°C).

Distribution: USA, Cuba, Ghana, Spain, UK, India (ZARE & GAMS 2003 g).

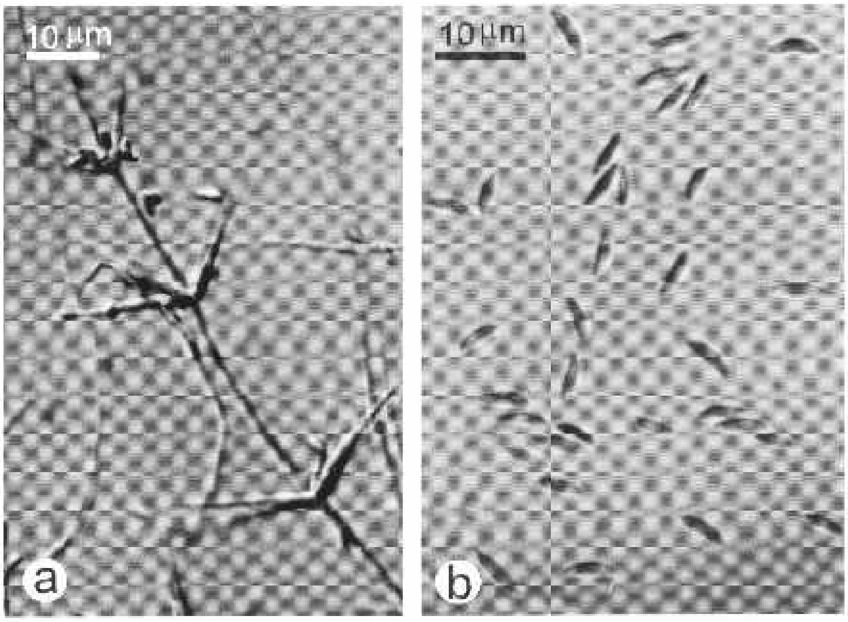
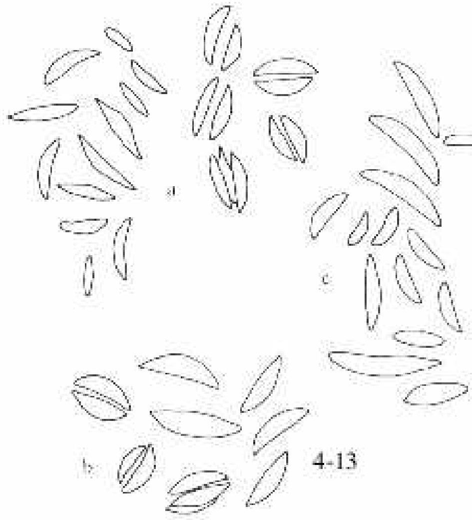
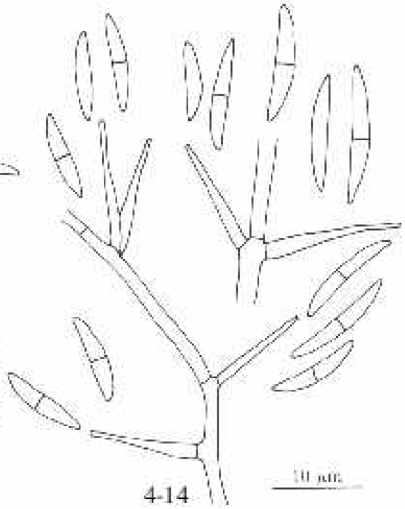


Fig. 4-12. *Lecanicillium psalliotae*. Conidiophores and conidia. a. CBS 367.86, b. CBS 505.48, DIC.



4-13



4-14

Fig. 4-13 a–b. *Lecanicillium psalliotae*. Conidia. a. CBS 505.48, b. CBS 194.79, 8 days on PCA. c. *Lecanicillium* sp. 1, conidia of CBS 716.95, 14 days on PCA.
Fig. 4-14. Type material of *Verticillium deflectans* with septate conidia.

STRAINS EXAMINED:
CBS 505.48 = IMI 092096 = MUCL 9800, ex casing soil in mushroom culture, England, 1947, J.C. La Touche, **ex-neotype**.
IMI 163640 = CBS 101270, ex soil, UK, 1972, J.E. Appleton.
CBS 194.79, ex *Agaricus bitorquis*, England, 1979, J.T. Fletcher.
IMI 179841 = CBS 532.81 = ITCC 1890, ex forest soil, India, 1981, S.C. Agrawal, **ex-type** of *Verticillium saksenae*.
CBS 367.86 = INIFAT C86/63-2, ex *Puccinia graminis*, Cuba, 1986, R. F. Castañeda (rather short, less pointed conidia).
CBS 100171 and 100172, ex nymph of *Ixodes* (tick), USA, 1997, G.J. Samuels.
Somewhat deviating strains:
CBS 724.73, ex Cicadellidae on leaf of *Theobroma cacao*, Ghana, 1973, H.C. Evans (1–2 slender, gently curved macroconidia on each phialide).
CBS 171.97 = INIFAT C96/144, ex dead insect, Spain, 1996, R.F. Castañeda (slender, gently curved macroconidia in heads, slow-growing).

Strain CBS 505.48 = IMI 092016, isolated from casing soil of *Agaricus bisporus* in England in 1947 by J.C. La Touche, comes closest to TRESCHOW's description (with mostly solitary conidia, red pigment, and association with *A. bisporus*). The dried culture of IMI 092016 was therefore designated in Nova Hedwigia 73: 23, 2001 as **neotype** (ZARE & GAMS 2001a).

TRESCHOW (1941) described the production of single falcate macroconidia on each phialide in a transverse position. He also reported the presence of irregular generally 2-celled chlamydospores, an observation not confirmed by any author since. DAYAL & BARRON (1970) redescribed the species with two types of hyaline conidia (falcate and oval). In the case of production of solitary conidia, the conidium is very stable on the phialide and still attached even after several weeks. GAMS (1971) characterized this species rather broadly by the presence of fusiform, slightly curved conidia with a wide range of conidial sizes, 3.0–9.5 × 1–2 µm. This concept requires correction.

A diffusing pigment, normally produced after 10 days, gives the agar medium a pinkish red or purplish red appearance; the pigment was characterized as a dibenzoquinone, named oosporein, by WAINWRIGHT *et al*. (1986). This compound was found to be a weak mycotoxin. In one strain (CBS 171.97), macroconidia were larger (10.5–16 × 1.7–2.7 µm); nevertheless, this deviating strain, which did not produce red pigment in the agar, is still placed under this species. Except for its *Msp* I digestion patterns of the ITS region and its mtDNA RFLPs, it matches with the rest of the strains examined. Its temperature optimum was at 21°C, and it had the slowest growth of any of the strains examined (21 mm diam.).

CBS 532.81, the ex-type strain of *Verticillium saksenae*, was indistinguishable from *V. psalliotae* on morphological grounds and also in its ITS and β-tubulin gene RFLPs, but had slightly different mtDNA RFLPs and also differed in 11 positions of its ITS sequences (8 in ITS-1, 2 in ITS-2, and 1 in 5.8S). Red coloration of the agar was absent. Conidia were produced in fascicles at right angles to the phialide tip, not solitarily, contrary to what was reported by KUSHWAHA (1980). Usually the first-formed conidia are falcate, 1-celled, rarely 1-septate, 5.5–8 × 1.3–2.5 µm, and the subsequent ones oval to short-ellipsoidal to subglobose, 2.5–3.5 × 1.2–1.5 µm. KUSHWAHA (1980) found the species originally on feathers and reported the production of keratinase by this strain and mentioned that the fungus utilized autoclaved feathers and human nails as substrates. When we grew this strain and several other species of *Lecanicillium* on autoclaved feathers placed on water agar, all showed equally luxurious growth on this substrate without causing noticeable decrease in stability of the feathers within 15 days. This criterion therefore does not help to distinguish species.Consistently 1-septate conidia are described for *Verticillium deflectans* (Bres.) de Hoog, van Oorschot & W. Gams (Fig. 4-14, DE HOOG & VAN OORSCHOT 1985), of which no living strain is available.

CBS 367.86 is noteworthy as a tropical parasite of rust fungi which is confirmed as a member of the *L. psalliotae* aggregate, while similar fungi from the same origin were otherwise identified as the unrelated *V. epiphytum*. The two tropical strains CBS 724.73 and CBS 171.97 had slightly different ITS (*Msp* I) and mtDNA RFLPs, but their β-tubulin RFLPs were identical with those of the rest of the strains examined. Therefore they are also classified under this taxon (see also ZARE & GAMS 2003 g).

8. *Lecanicillium* sp. 1 Figs 4-13c, 4-15

Colonies reaching 24–28 mm diam. in 10 days, cottony, high, white, with cream-coloured to yellowish cream-coloured reverse, without discoloration of the agar. Phialides arising from prostrate hyphae, solitary or up to 5 per node, measuring 18–30 × 1.3–1.5 µm. Macro- and microconidia produced in globose to ellipsoidal heads; macroconidia 0(–1)-septate, varying in size and shape, with pointed to rounded ends, measuring 5.5–10 × 1.5–3 µm; microconidia ellipsoidal to fusiform, sometimes rounded at one end and pointed at the other, measuring 3.5–5 × 1.3–1.5 µm. Octahedral crystals present.

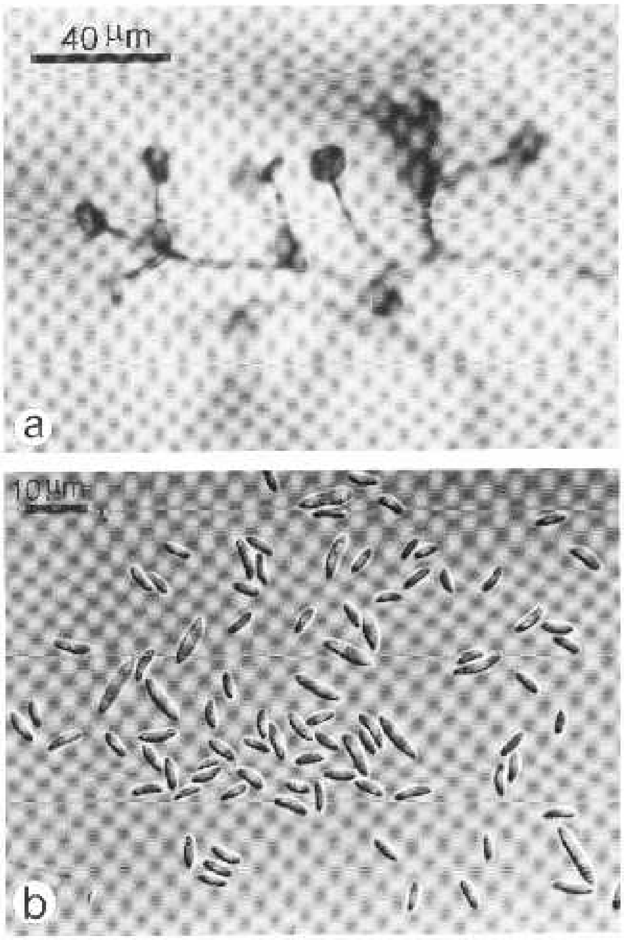


Fig. 4-15. *Lecanicillium* sp. 1. a. Conidiophore and conidial heads. b. Conidia. CBS 716.95, DIC.

STRAINS EXAMINED:
CBS 639.85, ex rhizosphere of *Pseudotsuga menziesii*, Germany, 1985, H. Schnhar.
CBS 716.95, ex myxomycete, Netherlands, 1995, W. Gams.
CBS 100890, contaminant in mushroom spawn, USA, 1988, J. Kremser.

This species is distinct from *L. psalliotae* in its RFLP patterns. It can be distinguished from *L. psalliotae* by its broader conidia, which are somewhat rounded at one or both ends. Because of limited material available and the difficult morphological distinction, we refrain from a formal description at this point.

Strain CBS 100890 deviates from the other two in having 1-celled, long (9.5–13.5 × 1.5–1.7 µm) macroconidia, straight with pointed ends; microconidia ellipsoidal, 3.5–4.0 × 1.0 µm.

9. ***Lecanicillium dimorphum*** (J.D. Chen) Zare & W. Gams, Nova Hedwigia 73: 24, 2001. Figs 4-16, 4-17

≡ *Aphanocladium dimorphum* J.D. Chen, Acta Mycol. Sin. 4: 230, 1985 (basionym).

Colonies reaching 24–42 mm diam. in 10 days on PDA, high, white, with cream to brownish cream or, exceptionally, red reverse. None of the available strains produced any diffusing pigment. Conidiogenous cells of two kinds: (1) aculeate, persistent phialides and (2) short, inflated, caducous aphanophialides, with intermediates frequently observed as secondarily branching phialides (Fig. 4-17). Phialides produced on prostrate hyphae, solitary or up to 4–5 in terminal whorls, tapering gradually towards the apex, measuring 14–30 × 1.0–1.5 µm. Conidia on true phialides generally held together in fascicles at right angles to the phialide tip. Macroconidia falcate with sharply pointed ends, usually evenly curved, with more or less pointed ends, measuring 6–11 × 1.5–2.5 µm. Aphanophialides 5–12 × 0.7–1.5 µm, usually collapsing soon, forming small and hardly visible denticles producing only solitary conidia. Macro- and microconidia can be formed on phialides and only single microconidia are produced on aphanophialides; microconidia oval to ellipsoidal, 2.5–4.5 × 1.0–1.5 µm. Octahedral crystals commonly produced. Temperature optimum: 24–27°C (according to CHEN *et al.* 1985, 26°C) (27–32 mm diam.). No growth at 33°C (ZARE & GAMS 2003 c).

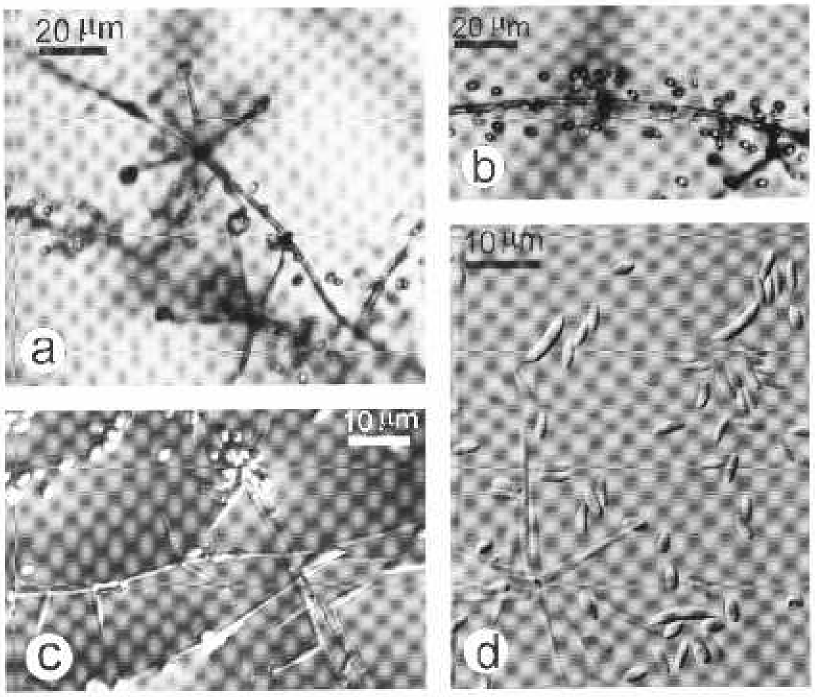


Fig. 4-16. *Lecanicillium dimorphum*. a–c. Phialides and aphanophialides. d. Conidia. a–c. CBS 573.77, DIC.

STRAINS EXAMINED:
CBS 345.37 = ATCC 22608, ex *Puccinia coronata*, Germany, 1937, F.H. van Beyma.
CBS 504.48 = MUCL 9782, ex *Agaricus bisporus*, England, 1947, J.C. La Touche.
CBS 463.70 = ATCC 22098, ex *Agaricus bisporus*, Israel, R. Kenneth.
CBS 573.77, ex leaf litter of *Acer saccharum*, USA, 1977, G.A. Kuter.
CBS 154.82, ex sandy soil, Netherlands, 1981.
CBS 363.86 = AS 3.4483 = NRRL 26542 = IAM 14706, ex *Agaricus bisporus*, China, J.D. Chen, **ex-type**.

This species is close to *L. psalliotae* and *L. aphanocladii*. Strains of this species were erroneously identified as *Verticillium psalliotae* by GAMS (1971) and GAMS *et al.* (1998). The species differs from *L. psalliotae* by possessing aphanophialides, and from *L. aphanocladii* by its macroconidia. *Lecanicillium dimorphum* seems to link *L. psalliotae* and *L. aphanocladii* as it produces two kinds of conidia characteristic of either species; in colony features and growth rate is very close to both of them (see also ZARE & GAMS

2003 b, c, g). According to ITS sequences, it also takes an intermediate position between these two species.

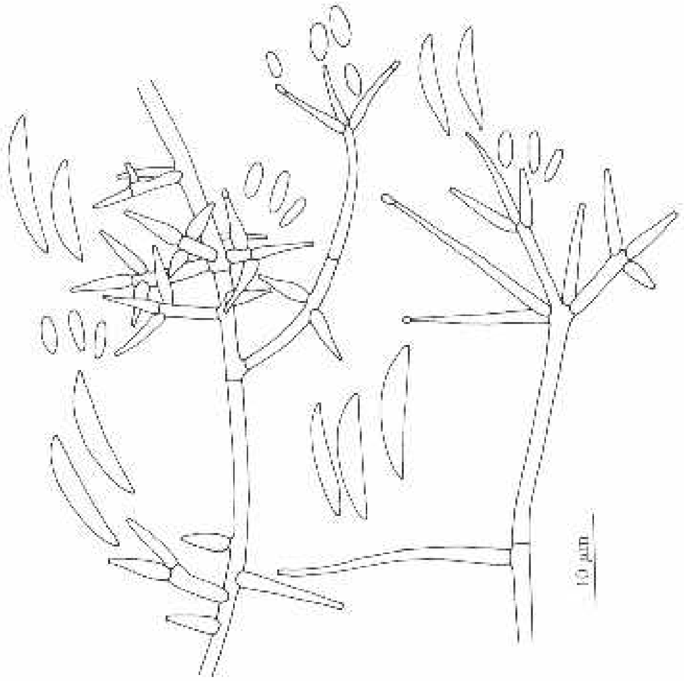


Fig. 4-17. *Lecanicillium dimorphum*. a. Conidiophores with secondarily produced phialides and conidia of CBS 154.82, 14 days on MEA.

10. ***Lecanicillium aphanocladii*** Zare & W. Gams, Nova Hedwigia 73: 27, 2001.

Figs 4-18, 4-19

≡ *Acremonium aranearum* Petch, Trans. Br. Mycol. Soc. 16: 242, 1932 (replaced synonym) [non *Cephalosporium aranearum* Petch ≡ *Lecanicillium aranearum* (Petch) Zare & W. Gams].

≡ *Aphanocladium aranearum* (Petch) W. Gams, *Cephalosporium*-artige Schimmelpilze, p. 198, 1971.

= *Aphanocladium aranearum* var. *sinense* C.D. Chen, Acta Mycol. Sin. 3: 96–101, 1984.

?= *Acremonium fimicolum* Massee & Salm., Ann. Bot. 16: 79, 1902.

?= *Sporotrichum roseolum* Oudem. & Beijerinck, Ned. Kruidk. Arch., Ser. 3, 2: 910, 1903.

Colonies reaching 30–50 mm diam. after 10 days on PDA, very high, white, with red, reddish white to cream-coloured reverse. The diffusing red pigment is the same as that in *L. psalliotae*. Aphanophialides produced singly, in pairs, verticillate, or in dense irregular clusters on prostrate hyphae, flask-shaped in the beginning, tapering into a thread-like neck, but soon collapsing and becoming reduced to very fine denticles with solitary conidia still remaining at the apex. Aphanophialides varying in size from very small denticles to larger structures, measuring 4.5–11 × 1.0–1.8 µm. Conidia solitary, oval to sub-globose, measuring 2.7–4 × 1.5–2.2 µm. Octahedral crystals present. Temperature optimum: 24–27°C (28–36 mm diam.). No growth at 33°C (ZARE & GAMS 2003 b).

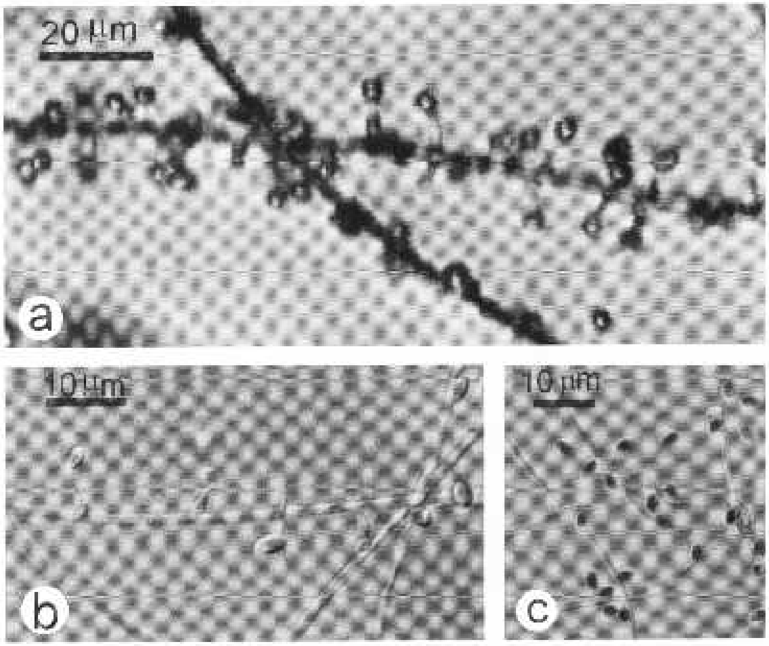


Fig. 4-18. *Lecanicillium aphanocladii*. a, b. Aphanophialides of CBS 165.45, in situ and mounted, c. conidia of CBS 797.84. b, c. DIC.

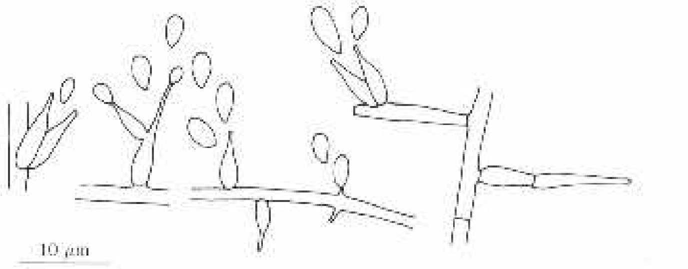


Fig. 4-19. *Lecanicillium aphanocladii*. a. More or less collapsed aphanophialides of CBS 451.93, 4 days on PCA.

STRAINS EXAMINED (for specimens see GAMS 1971):
CBS 165.45 = MUCL 9794, ex *Agaricus bisporus*, Netherlands, 1945, P.J. Bels.
IMI 96000b = CBS 101286, ex *Agaricus bisporus*, UK, 1962, E.W. Mason.
CBS 581.66, ex leaf litter of *Acacia karroo*, S. Africa, 1966, M.C. Papendorf.
CBS 274.76, ex *Abelmoschus esculentus*, Bulgaria, 1976, E. Mirkova.
CBS 376.77, ex *Agaricus bitorquis*, Netherlands, 1977, Fransman.
CBS 458.82 = NRRL 28027, ex *Sphaerotheca fuliginea*, Netherlands, T. Hijwegen.
CBS 774.83, ex *Agaricus bitorquis*, Netherlands, CBS 1983, Rutjens.
CBS 797.84 = AS 3.4419, ex *Agaricus bisporus*, China, 1980, J.D. Chen, **ex-epitype** designated in Nova Hedwigia 73: 29, 2001, **ex-type** of *A. aranearum* var. *sinense*. The type specimen (HMAS 43734) and a living culture (AS 3.4419) of *A. aranearum* var. *sinense*, and hence the epitype of *L. aphanocladii*, are preserved at the Institute of Microbiology, Academia Sinica, Beijing, China (CHEN *et al*. 1984).
CBS 798.84 = AS 3.4421, ex *Agaricus bisporus*, China, 1980, J.D. Chen.
CBS 451.93 = NRRL 26544, ex skin and egg of *Trialeurodes vaporariorum*, Italy, 1993, F. Ciccarese.

GAMS (1971) identified many isolates of this species as *Aphanocladium album*. The type and other authentic herbarium specimens of *A. aranearum* could not be sharply delimited from such cultures, but showed very dense aphanophialides. Additional abundant material seen subsequently showed that the criterion of more or less dense aphanophialides does not hold to distinguish species. Molecular work (GAMS *et al.* 1998, this study) subsequently made it clear that isolates of *A. album* from the original substratum, slime moulds, were phylogenetically quite unrelated to others obtained from diverse substrata. The generic name *Aphanocladium* is tied to *A. album*, whose phylogenetic position is not yet sufficiently resolved. Sequences of the small and large subunits of rDNA suggest its classification basal to the Nectriaceae (SUNG *et al*. 2001 – part 2). The main character found to differentiate *L. aphanocladii* from *A. album* is the growth rate, as *A. album* has much slower and thinner colonies and often undulate aerial fertile hyphae (see also ZARE & GAMS 2003 b).

As shown here, the fast-growing isolates identified as *Aphanocladium* belong to *Lecanicillium*, in the close vicinity of *L. psalliotae*. To distinguish them from *A. album sensu stricto*, ZARE *et al.* (2000 – part 1) identified these isolates as *Aphanocladium aranearum*, a species that must be renamed *Lecanicillium aphanocladii* in order to avoid homonymy with *L. aranearum* (Petch) Zare & W. Gams. The identity of *Acremonium fimicolum* and *Sporotrichum roseolum* with the present species is more than unlikely according to GAMS (1971). Therefore none of these names is reactivated by neotypification.

Lecanicillium aphanocladii has mainly been reported from fungi, particularly the genus *Agaricus*, but *A. album s. stricto* is so far only known from myxomycetes. The majority of *L. aphanocladii* (seven out of 10 isolates examined in this study) produced red pigments in the agar, while none of the *A. album* strains (CBS 401.70, CBS 892.72) produced such pigments.

Aphanocladium aranearum var. *sinense* C.D. Chen (CHEN *et al*. 1984) was supposed to deviate from the type variety by a structure called prophialides, i.e. occasionally produced conidiogenous cells with a short stalk cell. The occasional presence of short stalks bearing the conidiogenous cells was not regarded by GAMS (GAMS *et al*. 1998) as a significant difference, nor do the molecular results support a segregation.

11. ***Lecanicillium tenuipes*** (Petch) Zare & W. Gams, Nova Hedwigia 73: 29, 2001.

≡ *Sporotrichum aranearum* Cavara, Fungi Longob. exsicc. 5: fasc. 240, 1895.
≡ *Acremonium tenuipes* Petch, Trans. Br. Mycol. Soc. 21: 64, 1937 [non *Acremonium aranearum* Petch]
≡ *Verticillium tenuipes* (Petch) W. Gams, Cephalosporium-artige Schimmelpilze: 176, 1971.
≡ *Engyodontium aranearum* (Cavara) W. Gams, de Hoog & Samson, Persoonia 12: 138, 1984.
?= *Cephalosporium falcatum* Petch, Trans. Br. Mycol. Soc. 11: 259, 1926
≡ *Verticillium falcatum* (Petch) W. Gams, Cephalosporium-artige Schimmelpilze: 187, 1971.

Description and discussion in GAMS *et al*. (1984).

Temperature optimum 21–24°C (diam. 19 mm). No growth at 30°C.

STRAIN EXAMINED:
CBS 309.85, ex spider, Spain, E. Descals, 1985.

Other strains documented in GAMS *et al*. (1984). A specimen collected by T. PETCH on a spider in Holthouse Wood 1931 and preserved under *A. tenuipes* in K, contains a different fungus probably belonging to *Acrodontium*.

In order to avoid homonymy with *L. aranearum*, the next younger available epithet, *tenuipes*, must be chosen, which was introduced for the same species, as PETCH (1937) also had to avoid homonymy. The remaining species of *Engyodontium* still require a molecular analysis, in order to assess their proper affinities. Other species of *Engyodontium* (including the type species, *E. parvisporum* (Petch) de Hoog) are probably closer to *Beauveria*. *Engyodontium* sp. (Fig. 8 in GAMS *et al.* 1984) is very similar to *Microhilum oncoperae* H.-Y. Yip & A.C. Rath.

12. ***Lecanicillium aranearum*** (Petch) Zare & W. Gams, Nova Hedwigia 73: 30, 2001. Figs 4-20, 4-21

≡ *Cephalosporium aranearum* Petch, Trans. Br. Mycol. Soc. 16: 226, 1932 (basionym).
≡ *Verticillium aranearum* (Petch) W. Gams, *Cephalosporium*-artige Schimmelpilze, p. 186, 1971.
≡ *Engyodontium arachnophilum* H. C. Evans & Samson, Persoonia 12: 143, 1984 [non *E. aranearum* (Cavara) W. Gams *et al.*].

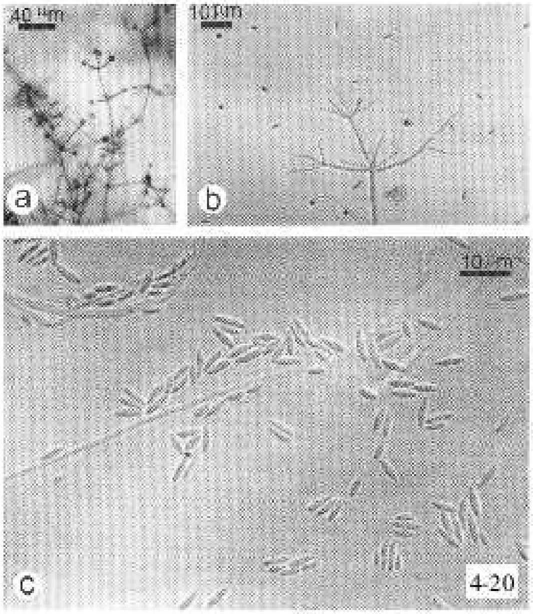
Teleomorph: ***Torrubiella alba*** Petch, Trans. Br. Mycol. Soc. 16: 226, 1932.

Colonies reaching 40 mm diam. in 10 days on PDA, rather thin, white, with yellowish cream reverse, slow-growing. No pigment diffusing into the agar. Phialides 20–30 × 1.2–1.5 µm, tapering towards the apex. Conidia slightly pointed at one or both ends, straight or curved, usually asymmetrically narrowed or subacute at the ends, measuring 5–8 × 0.7–1.5 µm. Octahedral crystals present. Temperature optimum: 21–24°C (23 mm diam.). No growth at 30°C.

Distribution: Ghana, India.

STRAIN EXAMINED:
CBS 726.73A, ex Araneida, Ghana, 1973, H.C. Evans.

Because only one living strain of this species, CBS 726.73A, is available, and we cannot judge the variation of the species, we refrain from designating an epitype for it. GAMS (1971) documented several specimens of this species collected by PETCH.



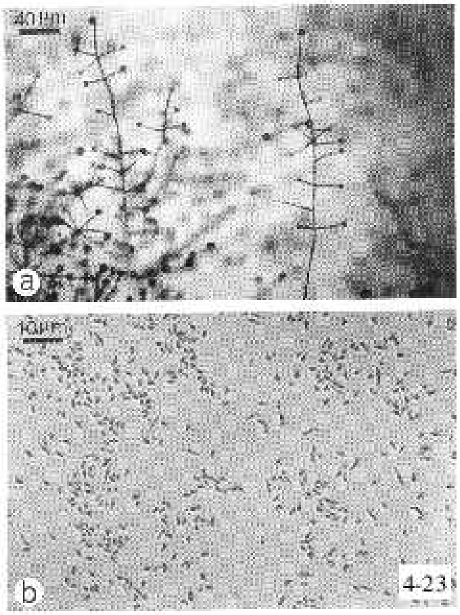


Fig. 4-20. *Lecanicillium aranearum*. a, b. Conidiophores, c. conidia. CBS 726.73a, DIC.
Fig. 4-23. *Lecanicillium evansii*. a. Conidiophores, b. conidia. CBS 726.73b, DIC.

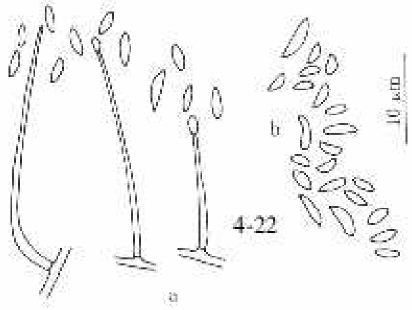


Fig. 4-21. *Lecanicillium aranearum*. Conidia of CBS 726.73A, 14 days on PCA.
Fig. 4-22. *Lecanicillium evansii*. a. Phialides and conidia of CBS 621.82, 11 days on OA, b. conidia of CBS 726.73B, 14 days on PCA.

This species can be distinguished from *L. psalliotae* by its small conidia and from *L. evansii* by slightly less curved conidia. Evans & Samson (in GAMS *et al*. 1984) described a polyblastic mode of conidiogenesis for this species, which they therefore classified under *Engyodontium*. Such conidiogenesis was seen by GAMS (1971, unpubl.) in a specimen R 571, but not in the others of this species, including the lectotype specimen R 214, designated by GAMS (1971). This complex will require further studies to resolve the conflicting views.

13. ***Lecanicillium evansii*** W. Gams & Zare, Nova Hedwigia 73: 32, 2001.

Figs 4-22, 4-23

Colonies reaching 17–25 mm diam. in 10 days, white, thin, with creamy, brownish cream to brown reverse, without discoloration of the agar. Phialides solitary or up to 3–4 per node, 20–45 × 1.0–1.2 µm at the tip. Conidia in globose to ellipsoidal heads and of two types; macroconidia 1-celled, 4.5–7.5 × 0.8–1.2 µm, slightly falcate with pointed ends; microconidia ellipsoidal with pointed ends, sometimes slightly curved, 2.0–3.0 × 0.8–1.2 µm. Octahedral crystals present. Temperature optimum: 21–24°C (12–19 mm diam.). No growth at 30°C.

Distribution: Ghana, Germany, Belgium and Denmark.

STRAINS EXAMINED:
CBS 726.73B, ex Coccidae, Ghana, 1973, H.C. Evans, **ex-type** (Type: herb. CBS).
CBS 621.82, ex *Marasmiellus ramealis*, Belgium, 1982, W. Gams.
CBS 713.88, ex *Sistotrema brinkmannii*, Germany, 1988, H. Grosse-Brauckmann.
CBS 268.89, ex needle litter of *Pinus sylvestris*, Germany, Berlin, 1989, G. Kraepelin.
CBS 100550, ex compost of *Miscanthus* and pig slurry, Denmark, 1998, C. Jakobsen.

Lecanicillium evansii is close to *L. aranearum*, but differs from it by the presence of microconidia and more strongly curved conidia. The distinction is supported by ITS sequences (Fig. 4-1) and RFLPs of the ITS region, the β-tubulin gene and mtDNA. All the strains had identical RFLP patterns of ITS and β-tubulin gene, but strain CBS 726.73B differed from the rest in three out of five mtDNA bands. This species has smaller conidia than *L. psalliotae*. The equally similar *S. lamellicola* has straight and very narrow macroconidia and a brown colony reverse.

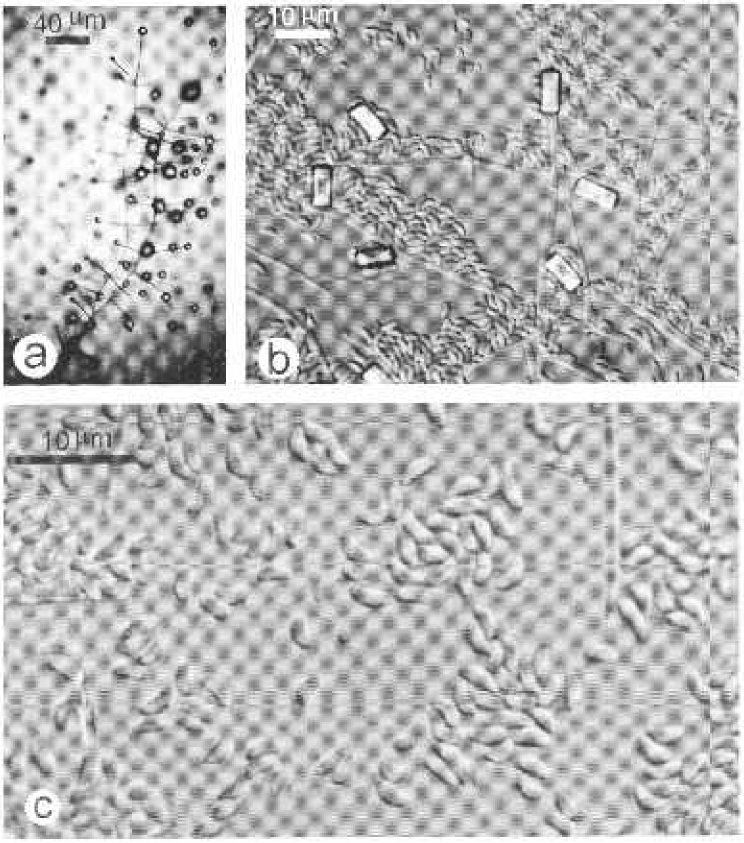


Fig. 4-24. *Lecanicillium* sp. 2. a. Conidiophores, b. crystals, c. conidia. CBS 352.77, DIC.

14. *Lecanicillium* sp. 2 — Fig. 4-24

Colonies reaching 27 mm diam. in 10 days, compact, yellowish white, with centrally brown and marginally cream-coloured reverse, without discoloration of the agar. Phialides solitary or up to 4 per node, 25–35 × 1 µm at the tip. Conidia short, curved, with moderately pointed ends, produced in globose heads, measuring 2.3–3.5 × 0.7–1.2 µm. Prismatic crystals present.

STRAIN EXAMINED:
CBS 352.77, substrate unknown, Turkey, 1977, T. Nemli.

This isolate is close to *L. aranearum* and *L. evansii*, but it is readily distinguished by its short and curved conidia. Until more material becomes available, we refrain from the formal description of a new taxon.

15. ***Lecanicillium antillanum*** (R.F. Castañeda & G. Arnold) Zare & W. Gams, Nova Hedwigia 73: 34, 2001. Figs 4-25, 4-26

≡ *Verticillium antillanum* R.F. Castañeda & G. Arnold, Feddes Repert. Spec. Nov. Reg. Veg. 98: 411, 1987 (basionym).

Colonies reaching 18 mm diam. in 10 days on PDA, with irregular margin, white, with cream-coloured reverse, without pigment diffusing into the agar. Phialides developing on prostrate hyphae, singly or up to 6 in verticils, subulate, measuring 18–31 × 1 µm (at the tip). Conidia solitary and of two types; primary conidia fusiform, sigmoidally curved with very narrow and pointed ends, 1-celled, hyaline, measuring 11–18 × 0.8–1.5 µm; secondary conidia ellipsoidal, 3–4 × 0.8–1.2 µm. Octahedral crystals present. Temperature optimum: 21–24°C (18–20 mm diam.). No growth at 33°C.

STRAIN EXAMINED:
CBS 350.85 = INIFAT C84/96.1, ex agaric, Cuba, 1985, R.F. Castañeda, **ex-type**.

This species resembles *L. acerosum* in conidial size, but the latter is easily distinguished by its straight conidia. The two species also have totally different RFLP patterns of the ITS region, β-tubulin gene and mtDNA.

16. ***Lecanicillium fusisporum*** (W. Gams) Zare & W. Gams, Nova Hedwigia 73: 34, 2001. Figs 4-27, 4-28

≡ *Verticillium fusisporum* W. Gams, *Cephalosporium*-artige Schimmelpilze, p. 182, 1971 (basionym).

Colonies reaching 24–29 mm diam. in 10 days, high, white, with red reverse and pigment diffusing into the agar, rather fast-growing. Phialides produced solitarily on undifferentiated

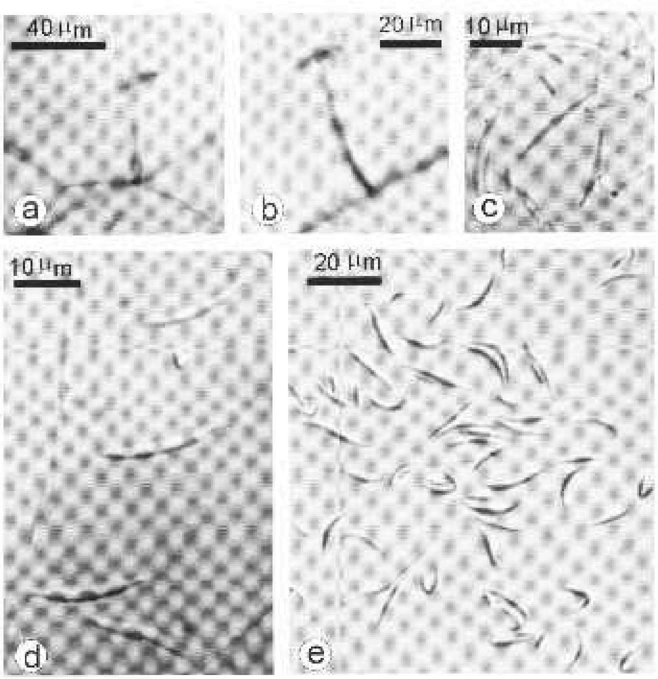


Fig. 4-25. *Lecanicillium antillanum*. a, b. Conidial heads, c–e. conidia. CBS 350.85, DIC.

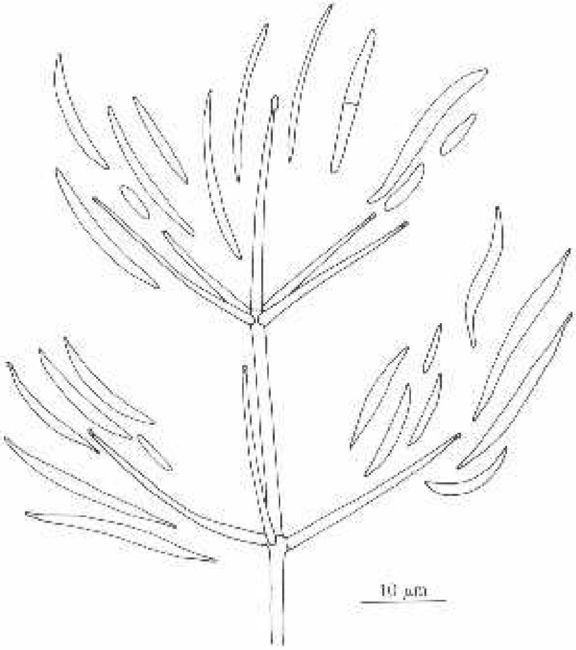


Fig. 4-26. *Lecanicillium antillanum*. Conidiophore and conidia, CBS 350.85, 21 days on PCA.

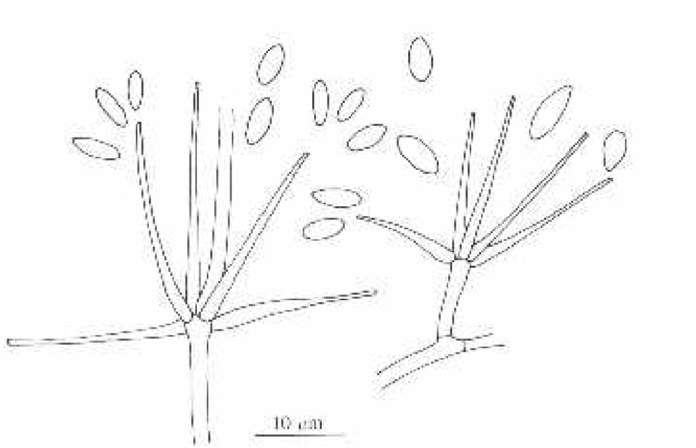


Fig. 4-27. *Lecanicillium fusisporum*. a. Conidiophores and conidia of CBS 164.70, 14 days on OA.

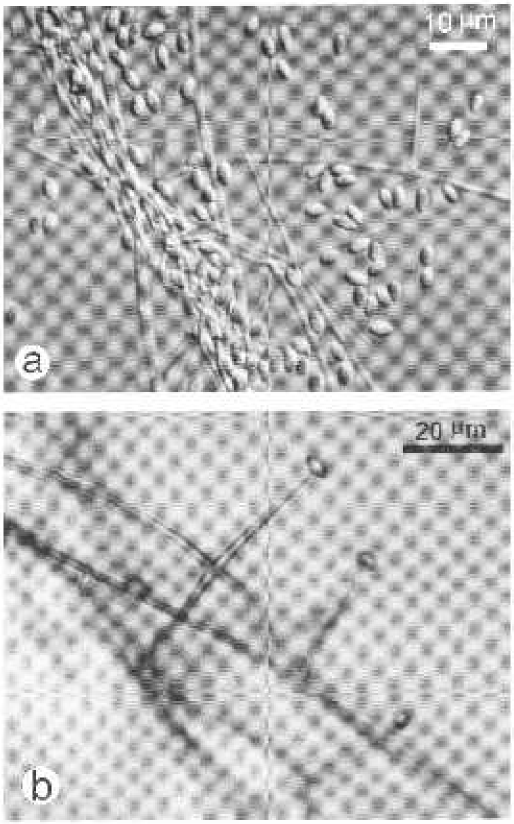


Fig. 4-28. *Lecanicillium fusisporum*. a. Conidia. b. Phialides and conidial heads. a, b. CBS 164.70, DIC.

prostrate hyphae or up to 5 in whorls, 16–26 × 1.0–1.5 µm, producing solitary or very few in fascicles at right angles to the phialide tip. Conidia uniformly fusiform, straight and rather broad, measuring 3–5 × 1.5–2.0 µm. Octahedral crystals present. Temperature optimum: 21°C (CBS 162.70) or 24°C (CBS 163.70) (23–24 mm diam.). No growth at 30°C.

STRAINS EXAMINED:
CBS 162.70 = MUCL 1193, ex leaf litter of *Fagus sylvatica*, Belgium, 1960, G.L. Hennebert.
CBS 163.70 , ex soil under *Pinus resinosa*, Canada, 1967, C.G. Bhatt.
CBS 164.70, ex *Coltricia perennis*, Netherlands, 1968, W. Gams, **ex-type**.

Lecanicillium fusisporum is closest to *L. psalliotae*. It is distinguished by its straight, rather broad conidia and the lack of microconidia. The three strains examined had unique, identical RFLP patterns of ITS region, β-tubulin gene and mtDNA. ITS sequences (Fig. 4-1) and SSU + LSU sequences of rDNA (SUNG *et al.* 2001 – part 2) support its distinctness from other species of *Lecanicillium*.

Lecanicillium fusisporum has been reported to be pathogenic to insects by EKBOM & AHMAN (1980) and STEENBERG & HUMBER (1999); figures in the latter publication suggest that the authors did not work with the correct taxon as they reported macro- in addition to microconidia.

17. ***Lecanicillium acerosum*** W. Gams, H.C. Evans & Zare, Nova Hedwigia 73: 37, 2001.

Figs 4-29, 4-30

Colonies reaching 50 mm diam. in 10 days, thin, white, with yellow reverse, without discoloration of the agar. Phialides arising from prostrate hyphae, solitary or up to 4–5 at each node, gradually tapering towards the apex, measuring 30–32 × 1.8–2.2 µm. The first-formed conidium is a macroconidium positioned at a right angle to the phialide tip, and subsequent ones are microconidia. Macroconidia 1-celled, fusiform, straight to slightly falcate with acute ends, measuring 15–20 × 1.6–2.2 µm, microconidia fusiform with acute ends, 4.5–7.5 × 1.0–1.5 µm. Octahedral crystals present. Temperature optimum: 24°C (45 mm diam.), maximum 30°C. No growth at 33°C.

STRAIN EXAMINED:
CBS 418.81 = ODA 727, ex *Crinipellis perniciosa* on *Theobroma cacao*, Brazil: Amazon, 1981, H.C. Evans, **ex-type** (Type: herb. CBS).

Lecanicillium acerosum resembles *L. antillanum*, but it has straight conidia compared with sigmoidally curved conidia of *L. antillanum*. This species is so conspicuous that its distinctness is raised beyond all doubt.

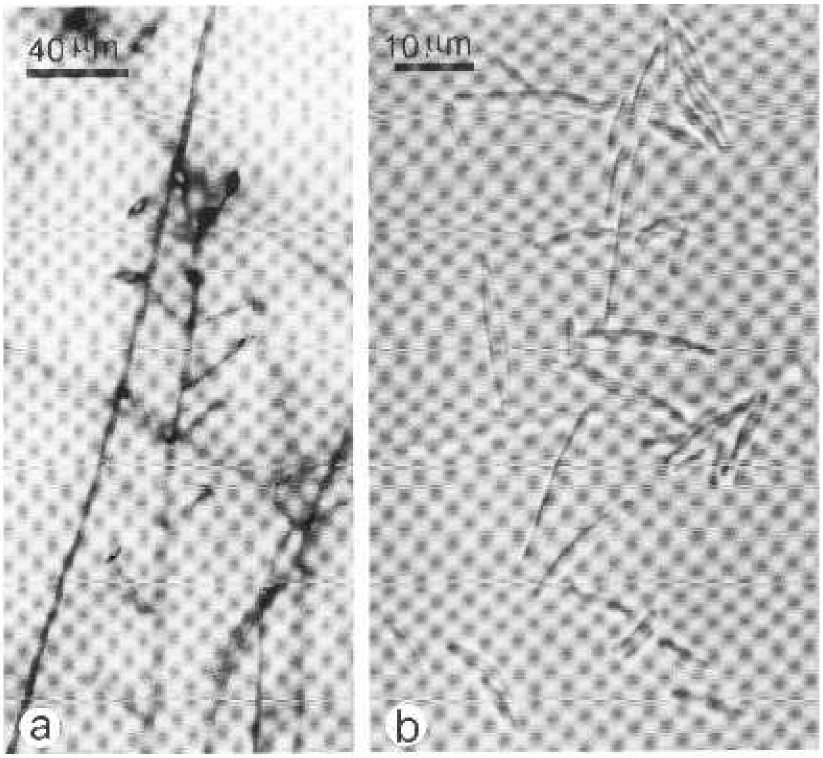


Fig. 4-29. *Lecanicillium acerosum*. a–c. Conidiophores and conidia, CBS 418.81, DIC.

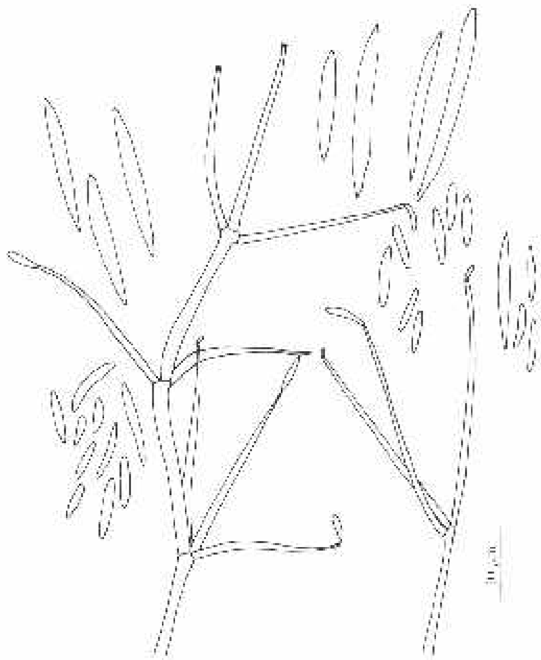


Fig. 30. *Lecanicillium acerosum*. Conidiophores and conidia, CBS 418.81, 14 days on PCA.

SIMPLICILLIUM W. Gams & Zare, Nova Hedwigia 73: 38, 2001.

Similar to *Lecanicillium*, but with mostly solitary phialides arising from aerial hyphae, usually prostrate and little differentiated from the subtending hyphae. Phialides discrete, aculeate and narrow, with a very narrow tip in which collarette and periclinal wall thickening are not visible. Conidia adhering in globose slimy heads or imbricate chains, short-ellipsoidal to subglobose or obclavate, not cyanophilic. Colonies rather fast-growing, reaching (10–)24–34 mm diam. in 10 d on PDA or MEA. Crystals commonly present in the agar. Fungicolous and on various other substrata.

Type species: *Simplicillium lanosoniveum* (F.H. van Beyma) Zare & W. Gams

Etymology: Lat. *simplex* = simple and suffix *-cillium* from *Verticillium*, referring to the unbranched conidiophores.

18. ***Simplicillium lanosoniveum*** (F.H. van Beyma) Zare & W. Gams, Nova Hedwigia, 73: 39, 2001. Fig. 4-31

≡ *Cephalosporium lanosoniveum* van Beyma, Antonie van Leeuwenhoek 8: 121, 1942 (basionym).
= *Cephalosporium salviniae* R.T. Jones & L. Frederick, Mycopath. Mycol. appl. 43: 195, 1971 [published as '*salvinae*'].

Teleomorph: *Torrubiella* sp.

Colonies reaching 24–34 mm diam. in 10 days on PDA, high, white; with cream-coloured, brownish cream to pale yellow reverse. Phialides always solitary and rather long and narrow, 15–35 × 0.7–1.5 µm. Conidia in small globose heads at the apex of the phialides. Conidia very small, 1.5–3 × 0.7–1.3 µm, but rather variable in size and shape, from subglobose, oval or ellipsoidal to subcylindrical. Octahedral crystals present. Phialides exclusively solitary, sometimes appearing to form opposite pairs, but under higher magnification it is clear that the two phialides are not actually quite opposite. Temperature optimum: (21–)24(–27)°C, depending on the strain (27–34(–38) mm diam.). No growth at 33°C.

Distribution: Venezuela, Peru, Colombia, USA, Jamaica, Sri Lanka, Austria, the Netherlands and the Philippines.

STRAINS EXAMINED:
CBS 123.42, ex hair of *Cibotium schiedei* in greenhouse, Netherlands, 1942, F.H. van Beyma, **ex-type**.
IMI 255034 = CBS 101895, ex *Coccus viridis*, Sri Lanka, 1981, P. Kanagaratnam.
IMI 303103b, ex *Hemileia vastatrix*, Colombia, 1986 (deviating by longer conidia).
CBS 531.72 = ATCC 22503 = AU 1147, ex *Salvinia rotundifolia*, USA, **ex-type** of *C. salviniae*.
CBS 704.86, ex *Hemileia vastatrix*, Venezuela, 1986, B. Eskes, forming immature ascomata of *Torrubiella* sp., *in vitro*.
IMI 317424 = CBS 101282, ex *Hemileia vastatrix*, Jamaica, 1987, C. Prior (conidia subglobose).
IMI 317442 = CBS 101267, ex *Hemileia vastatrix*, Jamaica, 1987, C. Prior, associated with teleomorph, *Torrubiella* sp.
IMI 317451 = CBS 101241, ex *Hemileia vastatrix*, Jamaica, 1987, C. Prior, single-ascospore isolate of teleomorph, *Torrubiella* sp.
IMI 331542 = IIBC I855 = CBS 101245, ex pupa of *Penthocrates styx* (Lepidoptera), Philippines, H.C. Evans, 1985, single-ascospore isolate from teleomorph, *Torrubiella* sp.
CBS 405.89, from unknown substratum, Austria, Graz, 1989, I. Panzenböck.
I 97-1029b, ex *Hemileia vastatrix* (coffee rust), Peru, 1997, H.C. Evans.

Simplicillium lanosoniveum somewhat resembles *L. lecanii* and *L. muscarium*, but it can be distinguished from them by its short-ellipsoidal and smaller conidia and complete lack of verticillate branches and its somewhat faster growth rate. *Simplicillium obclavatum* is distinct in producing obclavate conidia in imbricate chains.

One strain, IMI 303103b with longer conidia, 3.2–4.0 × 0.8 µm, nearly cylindrical in shape, and two strains (IMI 255034, IMI 317424) with very small, 1.5–2.0 × 1.0–1.7 µm, almost globose conidia deviate morphologically from the type. In RFLP studies, the isolates examined evinced three ITS subgroups, four β-tubulin types, and six mtDNA types. But these differences were considered insignificant, especially with the ITS and β-tubulin gene RFLPs, where minor polymorphisms were generated by one or two enzymes only. Therefore, considering the morphological similarities and a strong homogeneity in the ITS sequences of the two strains (Fig. 4-1) with the highest degree of RFLP distinction, all these subgroups are classified under *S. lanosoniveum*.

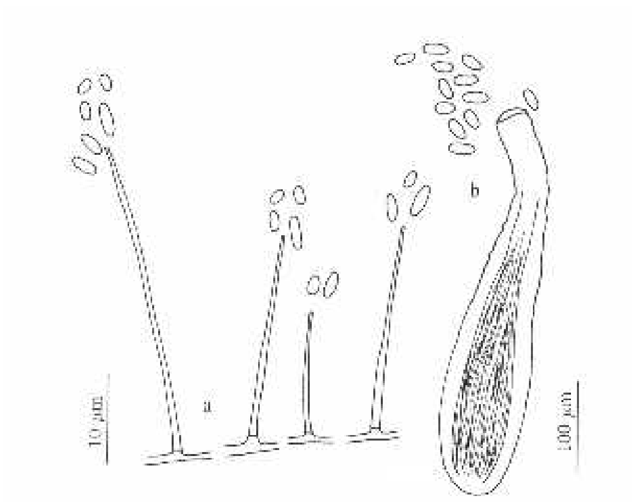


Fig. 4-31. *Simplicillium lanosoniveum*. a. Phialides and conidia of CBS 123.42, 5 days on CMA, b. conidia and immature ascoma of CBS 704.86, 14 days on PCA.

This species has been found mainly associated with rusts, in particular the coffee rust, *Hemileia vastatrix*. Four strains were received as single-ascospore isolates of a Torrubiella sp. Another strain, CBS 704.86, produced immature ascomata in culture. But the material is not yet sufficient to formally describe the teleomorph taxon in *Torrubiella*.

JONES & FREDERICK (1971) reported the observation of terminal and intercalary chlamydospores in the ex-type strain (CBS 531.72) of *Cephalosporium salviniae* R.T. Jones & L. Frederick. We examined this strain carefully and found no chlamydospores. This species is therefore considered as a synonym for *S. lanosoniveum*; its RFLP pattern of the ITS region matches that of the majority of the strains, including the ex-type strain, CBS 123.42; mitochondrial DNA and β-tubulin patterns fall in the range of variation of other isolates.

19. ***Simplicillium obclavatum*** (W. Gams) Zare & W. Gams, Nova Hedwigia 73: 41, 2001. Fig. 4-32

≡ *Acremonium obclavatum* W. Gams, Persoonia 12: 135–147, 1984 (basionym).

Colonies reaching 30–32 mm diam. in 10 days on PDA, high, white, with brownish cream reverse, rather high. Phialides always solitary, long and slender, 30–52 × 0.8–1.2 µm. Conidia produced obliquely, forming short imbricate chains, obclavate to ellipsoidal, 2.5–3.5 × 1–2 µm. Octahedral crystals present. Temperature optimum: 27–30°C (25–29 mm diam.). Good growth at 33°C.

Distribution: India, Saudi Arabia.

STRAINS EXAMINED:
CBS 311.74 = IMI 185383, ex air above sugarcane field, India, Kamal, **ex-type**.
CBS 250.76, ex soil, India, S.C. Agrawal.
CBS 586.81, ex tannin-bearing bark, Tamil Nadu, India, C. K. Rao.
CBS 510.82, ex rust pustules on *Arachis hypogaea*, Tamil Nadu, India, B. P. R. Vittal.
CBS 101713, ex human nail, Saudi Arabia, A.A. Al-Khanfur.

GAMS *et al*. (1984) placed this species in *Acremonium* section *Albolanosa*, being aware of its distant relation with other sections of *Acremonium*. The remaining members of this section have been transferred to *Neotyphodium* (GLENN *et al.* 1996), a genus accommodating anamorphs of *Epichloë* that is not suitable for *S. obclavatum* and

S. lanosoniveum. *Simplicillium obclavatum* has the highest cardinal temperatures among the taxa studied here.

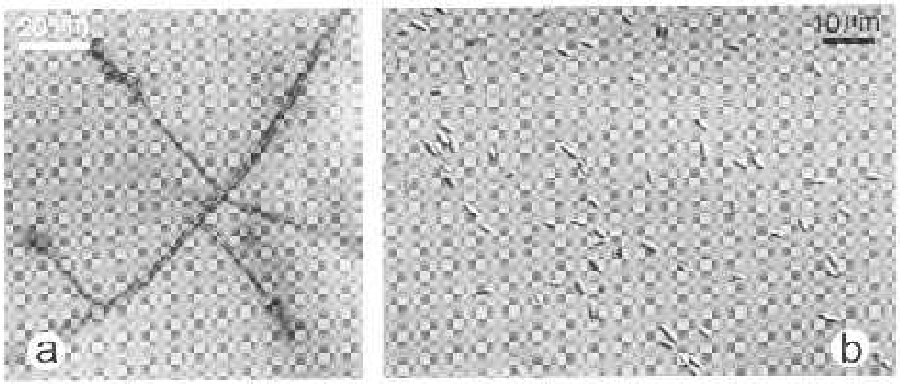


Fig. 4-32. *Simplicillium obclavatum*. a. Conidiophores with imbricate conidial chains, b. conidia. CBS 250.76, DIC.

20. **Simplicillium lamellicola** (F.E.W. Smith) Zare & W. Gams, Nova Hedwigia: 73: 42, 2001. Fig. 4-33

≡ *Cephalosporium lamellicola* F.E.W. Smith, Trans. Br. Mycol. Soc. 10: 93, 1924 [as '*lamellaecola*'].

≡ *Verticillium lamellicola* (F.E.W. Smith) W. Gams, *Cephalosporium*-artige Schimmelpilze, p. 183, 1971.

Colonies reaching 10–30 mm diam. in 10 days, thin but compact, white, reverse light- to dark-brown, usually with yellow pigment diffusing into the agar, rather slow-growing. Phialides produced on prostrate and very narrow hyphae (0.7–1.2 µm wide), solitary or up to 4 in whorls, very delicate and narrow, measuring 15–50 × 0.7–1.0 µm. Conidial heads varying from subglobose to ellipsoidal with pointed ends, then positioned at a right angle to the phialide tip. Usually the first-formed conidium is a macroconidium and the subsequent ones are microconidia. Macroconidia narrowly spindle-shaped, mostly straight to slightly curved, 1-celled, measuring 4.5–9.0 × 0.8–1.2 µm; microconidia oval to ellipsoidal, sometimes with slightly pointed ends, 2.0–3.0 × 0.7–1.2 µm. Octahedral crystals usually abundant. Temperature optimum: 21–24°C (12–19 mm diam.), with CBS 150.82 exceeding all other strains in growth rate with 30 mm diam. No growth at 33°C.

Distribution: Probably worldwide, with records from Germany, Austria, Denmark, the Netherlands, England, Scotland, Indonesia.

STRAINS EXAMINED:
CBS 116.25, ex *Agaricus bisporus*, UK, 1925, F.E.V. Smith, **ex-type**.
CBS 138.37 = ATCC 22602 = MUCL 9740, ex *Puccinia glumarum*, Germany, 1937, K. Hassebrauk.
IMI 093439, Leo Pharmaceuticals, Denmark, 1962.
CBS 302.70A, ex *Polyporus varius*, Denmark, 1965, W. Gams.
CBS 302.70B, ex *Hypoxylon deustum*, Austria, 1970, W. Gams.
CBS 302.70G = IMI 103732, ex mouldy hay, England, 1960, M.E. Lacey.
CBS 412.70A, ex *Agaricus bisporus*, Netherlands, 1970, W. Gams.
CBS 596.71 = IMI 051463 = IAM 14701, ex spore of *Pteridium aquilinum*, Scotland, S.A. Hutchinson.
IMI 204793, ex mushrooms, UK, 1976, S.E. Ogilvy.
IMI 234410, ex *Azolla* sp., UK, Royal Bot. Gardens Kew, 1979, M. Coode.
CBS 150.82, ex *Agaricus bitorquis*, Netherlands, 1982, A. van Zaayen.
IMI 339421, ex tissue of a dog, 1990, C.K. Campbell.

This taxon is rather homogeneous in molecular features; morphologically it is variable, particularly in the proportion of macro- and microconidia and the shape of the macroconidia. SMITH (1924) reported the production of only one type of conidia, which were 1-celled, but a certain dimorphism is quite commonly observed in all isolates available. This species can be distinguished from *Lecanicillium psalliotae* by its smaller and less curved macroconidia and absence of red pigment diffusing into the agar. Besides, in *L. psalliotae* colonies are usually much higher and never brown in reverse. Strain CBS 302.70G differs from the rest of the strains in two out of five mtDNA bands.

The species was originally described as causing ‘gill mildew’ on cultivated *Agaricus bisporus*, a disease that does not seem to be of any importance. GAMS & VAN ZAAYEN (1982) found it to cause occasionally small brown spots on *Agaricus bitorquis*. GAMS (1988) reported occurrence of this species as a parasite on cysts of *Heterodera glycines* and eggs of *Meloidogyne arenaria*. *Simplicillium lamellicola* is also one of the many parasites of rust fungi.

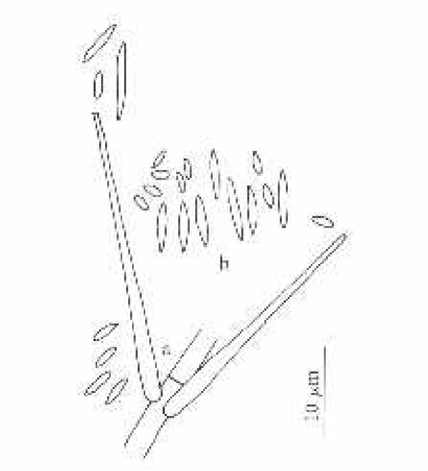


Fig. 4-33. *Simplicillium lamellicola*. a. Conidiophore and conidia of IMI 234410, 8 days on PCA. b. conidia of IMI 204793.

21. *Simplicillium wallacei* H.C. Evans, Nova Hedwigia 73: 43, 2001.

Figs 4-34c, d, 4-35, 4-36a

Colonies high, white, reverse cream-coloured to creamish brown, reaching 35–38 mm diam. after 10 days on PCA; older colonies with a distinctive earthy odour. Phialides produced on narrow, aerial hyphae (1.7–2.2 µm wide), solitary or up to 3–4 in a whorl, delicate, aculeate, narrow, (14–)17–25(–29) × 0.7–1.2 µm. Conidia adhering in ellipsoidal to subglobose heads and of two types, with the first formed macroconidium forming a right angle with the phialide tip. Macroconidia aseptate, guttulate, fusiform to falcate, often with acute ends, (7.0–)8.5–10.5(–12.5) × 1.0–1.5 µm; microconidia aseptate, ellipsoidal to slightly falcate, (3.0–)4.0–5.5(–6.5) × 0.7–1.2 µm. Immature perithecia may occur in older cultures.

MATERIAL EXAMINED:
IMI 331549, ex lepidopteran larva on palm leaf, Dumoga Bone forest, Sulawesi, Indonesia, Mar. 1985, H.C. Evans (holotype in herb. IMI; living culture CBS 101237).

Etymology: This species is named in honour of Alfred Russell Wallace, the great Victorian naturalist, and after Wallacea, the unique biogeographical zone from where the type specimen was collected.

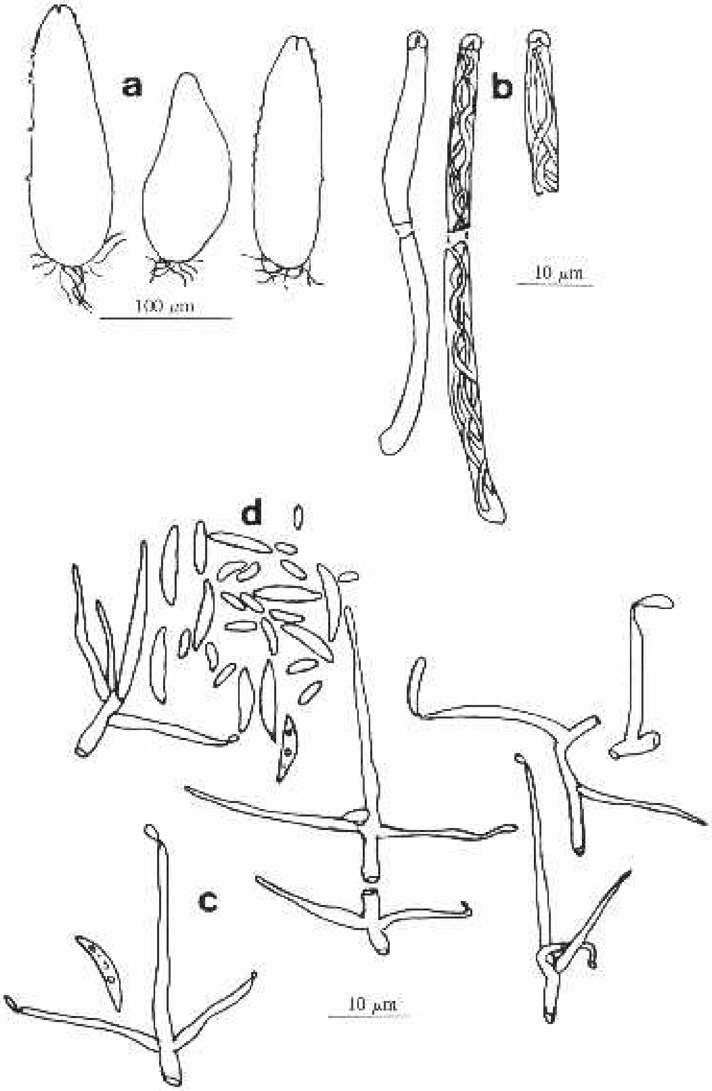


Fig. 4-34. *Torrubiella wallacei*, IMI 331549, ex host. a. Perithecia; b. asci and ascospores. *Simplicillium wallacei*, ex PCA 7 days. c. phialides; d. macro- and microconidia.

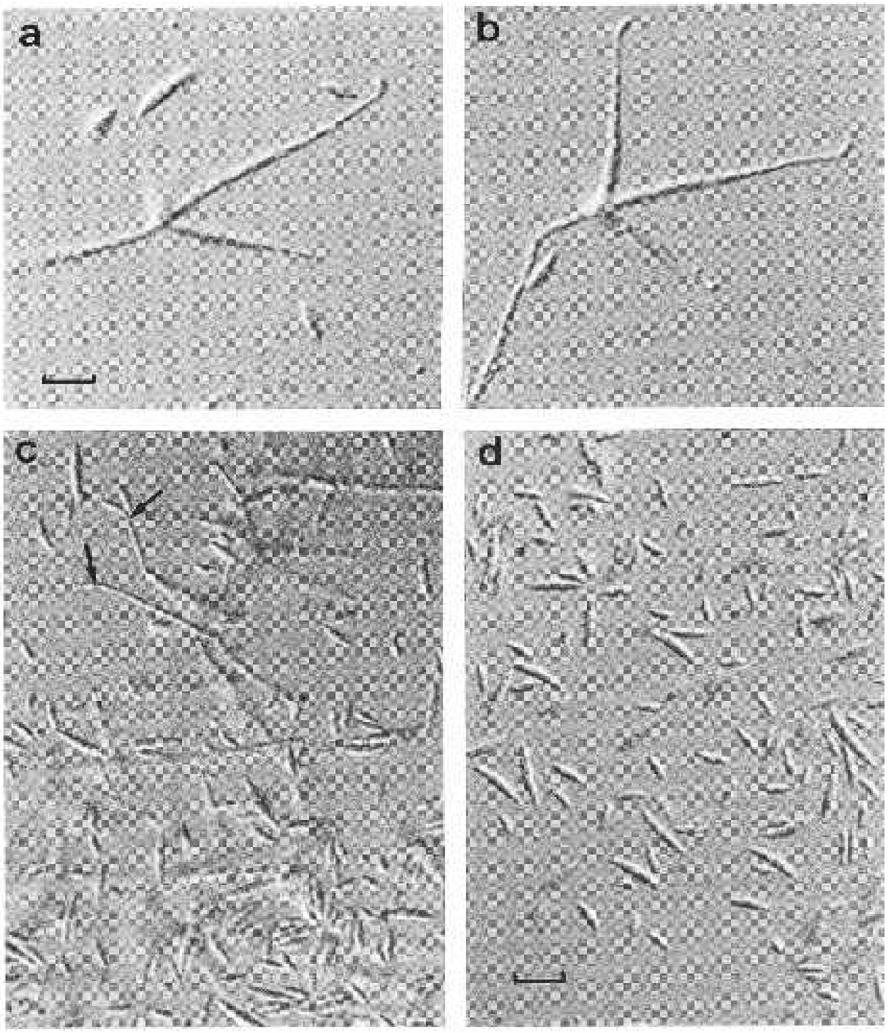


Fig. 4-35. *Simplicillium wallacei*, IMI 331549, 7 days on PCA. a. and b. Phialidesin verticils, bar = 6 µm; c. and d. macro- and microconidia, with solitary phialides (arrow); bar = 10 µm.

Strain IMI 331549 was grouped initially within *Simplicillium lamellicola* since this species is morphologically heterogeneous. However, based on the following criteria, it is now considered that separation at the species level is justified: entomogenous and tropical habit; presence of a teleomorph; larger conidia, and differing RFLP patterns of the ITS region and mtDNA. Earlier, YUN *et al.* (1991) included this isolate in their chemotaxonomic study of verticillioid fungi and concluded that it probably represented an undescribed taxon.

The type specimen consists of a white pupal case (4 mm long) with the remains of the larval stage above, attached to a palm leaf. A thin, white mycelium (1.7–2.5 µm) is present on the larva which bears typical phialides and dimorphic conidia. Perithecia are confined to the upper part of the pupal case. The association was confirmed by ascospore isolations.

Teleomorph: ***Torrubiella wallacei*** H.C. Evans, Nova Hedwigia 73: 46, 2001.

Figs 4-34a & b, 4-36b–e

Perithecia produced directly on a white mycelium that sparsely covers the pupal host; hyaline, delicate, smooth, very thin-walled, obclavate to naviculate, 150–320 × (45–)60–90 µm, with a prominent ostiole. Asci 8–spored, with a prominent cap, narrowly cylindrical with an inflated base, 80–110 × (2.5–) 3–4 µm. Ascospores hyaline, filiform, spirally twisted in the ascus, approximately the same length as the ascus and less than 1.0 µm wide, septa indistinct, not breaking into part spores.

Holotype IMI 331549 on lepidopteran pupa, Dumoga Bone, Sulawesi, Indonesia, 1985, H.C. Evans (in herb. IMI).

This is the second species of *Torrubiella* associated with a *Simplicillium* anamorph. An anamorph close to *S. obclavatum* is associated with a *Cordyceps* (H.C. EVANS, unpubl.), which will be studied further. The teleomorph of *S. lanosoniveum* appears to be similar but it cannot yet be formally described until more material becomes available to serve as type. The teleomorphs of *Simplicillium* differ from the more robust, strongly pigmented and thick-walled *T. confragosa*-type teleomorphs in having hyaline or lightly pigmented, delicate, thin-walled perithecia.

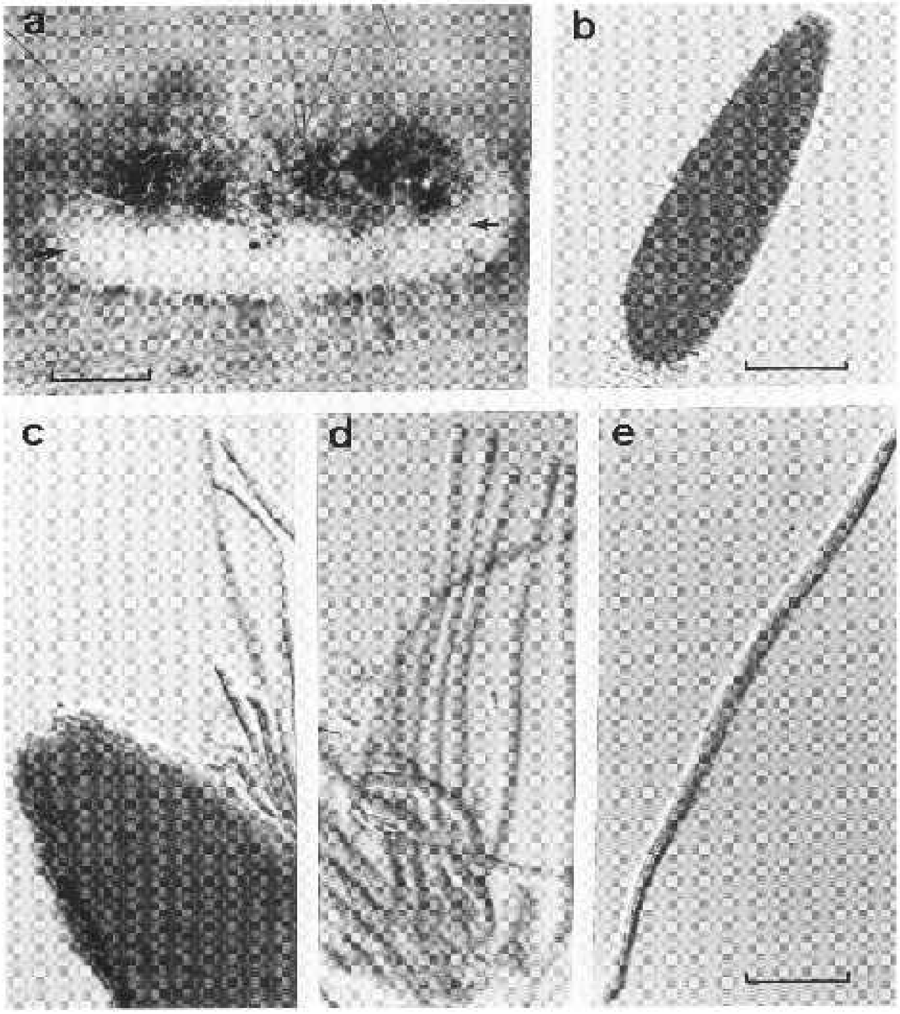


Fig. 4-36. *Torrubiella wallacei*, IMI 331549. a. Lepidopteran host attached to palm leaf, consisting of larval remains on white pupal case, white delicate *Simplicillium* anamorph on larva and hyaline perithecia on pupa (arrows), bar = 1 mm; b. perithecium from host, bar = 40 µm; c. asci emerging from perithecial squash; d. immature asci with prominent caps; e. mature ascus showing apical cap and inflated base, with narrow ascospores; bar = 15 µm for c–e.

PART FIVE:
THE GENUS *POCHONIA*, WITH NOTES ON *ROTIFEROPHTHORA*

The species of this genus mainly parasitize cyst nematodes (except *P. microbactro-spora*, which was isolated from a rotifer), *P. chlamydosporia* var. *chlamydosporia* and its teleomorph also grow on slug eggs. Most species produce dictyochlamydospores or at least some irregularly swollen vegetative hyphae. However, dictyochlamydospores may be totally absent in some strains, while they occur also in the other discrete genera *Haptocillium* (GAMS 1988, SUNG *et al*. 2001 – part 2, GAMS & ZARE 2001 – part 3, ZARE & GAMS 2001b – part 6) and *Rotiferophthora* G.L. Barron (BARRON 1991).

The term dictyochlamydospore is used by all authors working on these fungi to designate a unique feature with a stalked, hyaline, thick-walled, multicellular resting stage, that can be formed either in large numbers in the aerial mycelium or submerged in the agar. In the latter case, they are more or less scanty and often showing a roughened surface. But the theme of dictyochlamydospores is variable; the shape need not be subglobose or isodiametric with cells slightly bulging out. They can be more irregular in shape and less differentiated, and stalks may be absent. Many species of *Rotiferophthora* have flattened dictyochlamydospores. *Pochonia bulbillosa* has a strong tendency to form intercalary swollen hyphal cells rather than well-differentiated dictyochlamydospores. In some isolates the capacity to form dictyochlamydospores is lost due to degeneration; in some others they have never been seen at all. GAMS (1988) gave a full treatment of four species and two varieties of the genus including ecological observations, which is not repeated *in extenso* here.

A reconstruction of the phylogeny of the species distinguished is given in Fig. 5-1. A systematic representation of RFLP patterns is shown in Table 5-I.

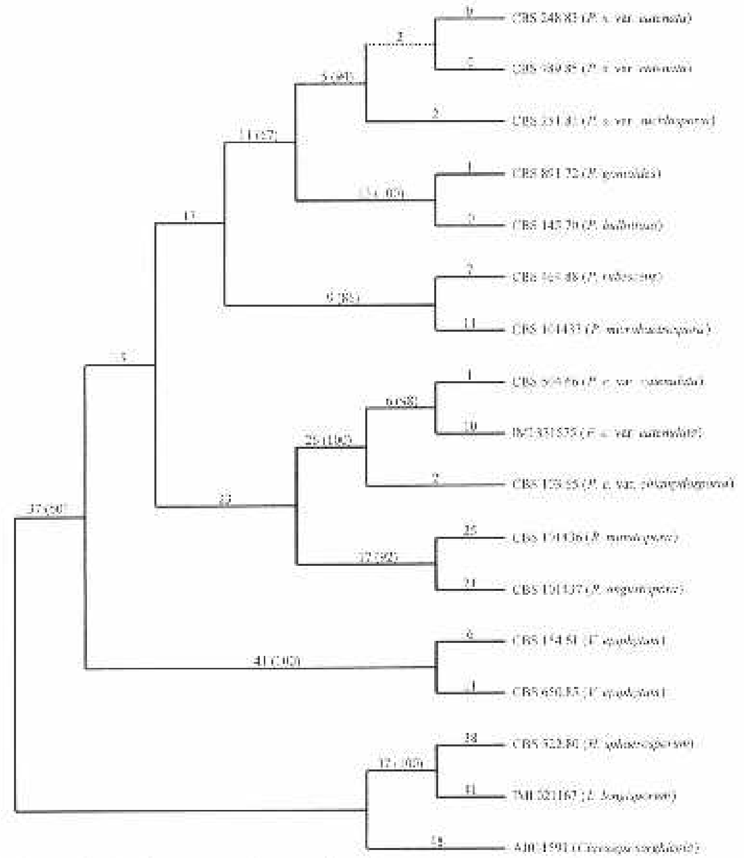


Fig. 5-1. One of the three equally parsimonious trees recovered using sequences of ITS region and 5.8S gene. Dashed lines indicate branches that collapsed in the strict consensus tree. Plain numbers above lines indicate branch lengths, and numbers in brackets show the Jackknife support values. CI = 0.79, HI = 0.20, RI = 0.72, RC = 0.57, tree length = 483. *L.* = *Lecanicillium*, *P.* = *Pochonia*, *R.* = *Rotiferophthora*, *V.* = *Verticillium*, *H.* = *Haptocillium.*

POCHONIA Batista & O.M. Fonseca, Publ. Inst. Micol. Recife 462: 4, 1965.

= *Diheterospora* Kamyschko [Bot. Mater. Otd. Sporov. Rast. Bot. Inst. Komarova Akad. Nauk S.S.S.R. (Not. syst. Sect. Crypt. Inst. bot. Akad. Sci. USSR) 15: 137. 1962 (nom. inval., Art. 37.1)] ex G.L. Barron & Onions, Canad. J. Bot. 44: 861. 1966.

Type species *Pochonia humicola* Batista & O.M. Fonseca [= *P. chlamydosporia* (Goddard) Zare & W. Gams].

Teleomorph: *Cordyceps*.

Table 5-1. RFLP patterns of the ITS region, mtDNA and β-tubulin gene in species of *Pochonia* (*P.*), *Rotiferophthora* (*R.*) and residula species of *Verticillium* (*V.*). Symbols for different patterns in the second column: ITS patterns = Capital letters, mtDNA = Arabic figures, β-tubulin gene = Roman figures.

Taxa and Accession codes	Pattern	RFLPs of ITS region (fragment size in bp)			RFLPs of mtDNA (fragment size in kbp)	RFLPs of the β-tubulin gene (fragment size in bp)			
		Hha I	*Hinf* I	*Hae* III	*Hae* III	*Alu* I	*Cfo* I	*Hinf* I	*Hae* III
P. chlamydosporia var. *chlamydosporia*, CBS 103.65, IMI 156157 IMI 359253, IMI 321551	A, 1, I	260, 220, 150	270, 220, 90, 50	330, 170, 90, 50	7.1, 5.8, 5.1, 3.2, 3	340, 120, 80	460	360, 180	240, 180, 140
P. c. var. *chlamydosporia*, IMI 331547, CBS 429.64	A, 2, I'	260, 220, 150	270, 220, 90, 50	330, 170, 90, 50	7.4, 4.6, 3.7, 3.2, 3, 1.6	340, 120, 80	460	540	240, 180, 140
P. c. var. *chlamydosporia*, CBS 391.66, CBS 600.88	A, 3, I'	260, 220, 150	270, 220, 90, 50	330, 170, 90, 50	8, 6.2, 3.8, 3.1, 3.3, 2.2	340, 120, 80	460	540	240, 180, 140
P. c. var. *chlamydosporia*, IMI 113169	A, 4, I	260, 220, 150	270, 220, 90, 50	230, 170, 90, 70	9.2, 6.1, 5.2, 3.9, 3.2, 3, 2	340, 120, 80	460	360, 180	240, 180, 140
P. cf. *c. chlamydosporia*, CBS 293.95	A, 5, I''	260, 220, 150	270, 220, 90, 50	190, 170, 90, 50	7.6, 6.7, 4.3, 2.8, 1.7	340, 120, 80	320, 160	540	360, 180
P. c. var. *catenulata*, IMI 060556	A, 6, I''	260, 220, 150	270, 180, 90, 50	190, 170, 90, 50	8.7, 5.4, 3.5, 2.8, 2.4, 2.2, 1.8	340, 120, 80	320, 160	540	360, 180
P. c. var. *catenulata*, CBS 504.66	A, 7, I''	260, 220, 150	270, 180, 90, 50	190, 170, 90, 50	6.5, 4.5, 3.2, 3, 2.4, 1.6	340, 120, 80	320, 160	540	360, 180
P. c. var. *catenulata*, CBS 397.69, IMI 076472	A, 8, I'	260, 220, 150	270, 220, 90, 50	330, 170, 90, 50	7.4, 4.6, 3.7, 3.2, 3, 1.6	340, 120, 80	460	540	240, 180, 140
P. c. var. *catenulata*, IMI 113172	A, 9, I	260, 220, 150	270, 220, 90, 50	330, 170, 90, 50	7.2, 5.8, 5.4, 3.3	340, 120, 80	460	360, 180	240, 180, 140
P. c. var. *catenulata*, (*Cordyceps* sp.) IMI 336013, IMI 331575	A, 10, I''	260, 220, 150	270, 220, 90, 50	330, 170, 90, 50	7.4, 4.5, 3.7, 3.4, 3, 2.5, 1.6	340, 120, 80	320, 160	540	360, 180
P. c. var. *catenulata*, IMI 060555, CBS 406.86	A, 11, I	260, 220, 150	270, 220, 90, 50	330, 170, 90, 50	9.4, 5.4, 3, 2.4, 1.6	340, 120, 80	460	540	240, 180, 140
P. suchlasporia var. *suchlasporia*, CBS 251.83	B, 12, II	260, 190, 150	320, 240, 90	300, 150, 90, 50	8.2, 5, 3, 3.6, 3.8, 2.8	340, 120, 80	280, 200	320, 220	240, 180, 140
P. s. var. *suchlasporia*, CBS 816.83	B, 13, II	260, 190, 150	320, 240, 90	300, 150, 90, 50	5, 6, 4.1, 4, 3.6, 2.8	340, 120, 80	280, 200	320, 220	240, 180, 140
P. s. var. *catenata*, IMI 113078	B, 14, II	260, 190, 150	320, 240, 90	300, 150, 90, 50	7, 6, 4.1, 2.6	340, 120, 80	280, 200	320, 220	240, 180, 140
P. s. var. *catenata*, CBS 383.70a, CBS 416.95	B, 15, II	260, 190, 150	320, 240, 90	300, 150, 90, 50	6.8, 7, 4.6, 2.6	340, 120, 80	280, 200	320, 220	240, 180, 140
P. s. var. *catenata*, CBS 780.85, CBS 495.80	B, 16, II	260, 190, 150	320, 240, 90	300, 150, 90, 50	8, 6.5, 4.4, 2.5	340, 120, 80	280, 200	320, 220	240, 180, 140

Taxa and Accession codes	Pattern	RFLPs of ITS region (fragment size in bp)			RFLPs of mtDNA (fragment size in kbp)	RFLPs of the β-tubulin gene (fragment size in bp)			
		Msp I	*Hinf* I	*Hae* III	*Hae* III	*Alu* I	*Cfo* I	*Msp* I	*Hae* III
P. chlamydosporia var. *catenulata*, CBS 248.83, CBS 817.87	**B, 17, II**	290, 190, 150	320, 240, 90	330, 150, 90, 50	9, 6.2, 5.4, 3.4, 2.8	340, 120, 80	280, 200	320, 220	240, 180, 140
P. rubescens, CBS 122.50a, CBS 357.73	**C, 23, II**	290, 220, 150	300, 160, 90, 80	330, 150, 90, 50	9, 7, 5.2, 4.6, 2.8, 2.6	340, 120, 80	280, 200	320, 220	240, 180, 140
P. rubescens, CBS 161.88	**C, 24, II**	290, 220, 150	300, 160, 90, 80	330, 150, 90, 50	9, 7, 5, 4.6, 2.8	340, 120, 80	280, 200	320, 220	240, 180, 140
P. rubescens, CBS 105.70	**C, 25, II**	290, 220, 150	300, 160, 90, 80	330, 150, 90, 50	8, 7, 4.6, 2.8	340, 120, 80	280, 200	320, 220	240, 180, 140
P. suchlasporia, IMI 293698	**C, 26, II**	290, 220, 150	300, 160, 90, 80	330, 150, 90, 50	12.5, 7, 4.6, 4.2, 2.8, 2.0	340, 120, 80	280, 200	320, 220	240, 180, 140
P. gonioides, CBS 891.72	**D, 18, III**	260, 190, 150	320, 240, 90	280, 150, 90, 80	7.5, 4.3, 4.1, 4, 3.8, 2.6, 1.8	340, 120, 80	280, 200	540	240, 180, 140
P. gonioides, CBS 611.69	**D, 19, III**	260, 190, 150	320, 240, 90	280, 150, 90, 60	10.5, 7.5, 6, 5.5, 4.1, 4, 2.6	340, 120, 80	280, 200	540	240, 180, 140
P. bulbillosa, CBS 145.70, CBS 578.78, CBS 426.81	**D, 20, III**	260, 190, 150	320, 240, 90	280, 150, 90, 60	8, 5.5, 4.7, 2.8	340, 120, 80	280, 200	540	240, 180, 140
P. bulbillosa, CBS 247.68	**D', 21, II**	260, 190, 150	320, 240, 90	370, 150, 90	7.1, 6.3, 5, 4.4, 3.85, 1.7, 1.4	340, 120, 80	280, 200	320, 220	240, 180, 140
R. [illegible]spora, CBS 101433	**E, 22, -**	290, 200, 150	310, 150, 90, 70	340, 150, 100	7.5, 4, 3.5, 3, 2.3, 1.8				
R. angustispora, CBS 101437	**F, 27, IV**	270, 220, 150, 60	280, 270, 90, 50	270, 150, 120, 90	9.5, 8.7, 6.5, 6, 1.8	500	380, 200	380, 170	
R. [illegible], CBS 101436	**G, 28, V**	250, 200, 150, 50	260, 250, 90, 80	270, 150, 90, 60	6.2, 7, 4.5, 4.2, 4.1, 3.9, 2.6, 2.5, 1.2, 1.1	540	280, 200	540	210, 180, 140
V. leptobactrum, CBS 460.88	**H, 29, -**	270, 180, 140	290, 220, 90	430, 90, 80	4.5, 4.1, 3.9, 2.5, 2.2, 1.4, 1.2	540			
V. epiphytum, CBS 384.81	**I, 30, VI**	480, 150	250, 250, 90, 50	550, 90	8.5, 4.8, 4.2, 3.5, 2.4	420, 120	300, 200	350, 180	380, 160
V. epiphytum, CBS 154.61, IMI 346543, IMI 256186	**I, 31, VII**	480, 150	250, 250, 90, 50	550, 90	8, 6.2, 4.2, 2.0, 2.5, 2.6	420, 120	300, 200	540	400, 140
V. epiphytum, CBS 651.85	**I, 32, VII**	480, 150	250, 250, 90, 50	550, 90	8.3, 4.4, 2.6	420, 120	300, 200	540	400, 140
V. epiphytum, IMI 356015	**I, 33, VII**	480, 150	250, 250, 90, 50	550, 90	8.6, 5, 4.4, 4.2, 3.5, 1.4	420, 120	300, 200	540	400, 140

Colonies rather fast-growing, reaching 15-40 mm diam. in 10 days. Conidiophores usually prostrate and little differentiated from the vegetative hyphae, but sometimes erect and differentiated. Conidiogenous cells phialides, aculeate, i.e. tapering to a narrow tip, in which collarette and periclinal wall thickening are hardly visible, verticillate or solitary. Conidia adhering in globose heads or chains, subglobose, ellipsoidal to rod-shaped, isodiametric-polyhedric, or falcate with blunt ends. Dictyochlamydospores produced on the surface of the colony or submerged in the agar, three-dimensional, pluricellular, thick-walled structures, usually formed on short stalks. Crystals mostly absent.

Species accommodated in *Pochonia* mainly parasitize nematode cysts and all of them produce dictyochlamydospores or at least some irregularly swollen hyphae. The species can be rather easily distinguished from each other on the basis of conidial shape and the position and abundance of dictyochlamydospores. The relative affinity of the taxa distinguished by GAMS (1988) is confirmed here by molecular studies.

KEY TO THE SPECIES OF *POCHONIA*

1. At least part of the conidia crescent-shaped or falcate .... 7. *P. bulbillosa*
[If chlamydospores 1-celled and moderately thick-walled, parasites of epiphyllous fungi in the tropics, see *V. epiphytum* at the end of this paper]
1´. Conidia not crescent-shaped or falcate **2**

2. Conidia isodiametric-polyhedric; dictyochlamydospores present, usually on the agar surface .... 6. *P. gonioides*
2´. Conidia rod-shaped, smooth, with truncate ends, 2.0–2.5 × 0.8–1.0 µm; dictyochlamydospores sparse, submerged in the agar .... 8. *P. microbactrospora*
2´´. Conidia of other shapes, oval, subglobose to subcylindrical, smooth; dictyochlamydospores above or in the agar.. **3**

3. Dictyochlamydospores, at least in fresh isolates, abundant, particularly in the aerial mycelium; conidiophores typically prostrate **4**
3´. Dictyochlamydospores, if present, mostly submerged in the agar; conidiophores prostrate or erect **5**

4. Conidia only in heads, never in chains ....1. *P. chlamydosporia* var. *chlamydosporia*
4´. Conidia mostly in chains; some heads may be present .... 2. *P. chlamydosporia* var. *catenulata*

5. Colony reverse developing red shades on PDA; conidiophores prostrate, verticillate; dictyochlamydospores scanty or absent..5. *P. rubescens*
5´. Colony reverse yellow to cream (not red) on PDA; conidiophores partly erect, richly verticillate; dictyochlamydospores partly submerged in the agar.................. **6**

6. Conidia only in heads, never in chains ....3. *P. suchlasporia* var. *suchlasporia*
6´. Conidia mostly in chains, some heads may be present ....
....4. *P. suchlasporia* var. *catenata*

1. ***Pochonia chlamydosporia*** (Goddard) Zare & W. Gams, var. ***chlamydosporia***

Nova Hedwigia 72: 334, 2001. Figs 5-3–5-5, 5-6 a, b

≡ *Verticillium chlamydosporium* Goddard, Bot. Gazette 56: 275, 1913 (basionym).
≡ *Diheterospora chlamydosporia* (Goddard) G.L. Barron & Onions, Canad. J. Bot. 44: 866, 1966.
= *Stemphyliopsis ovorum* Petch, Trans. Br. Mycol. Soc. 23: 146, 1939.
= *Diheterospora heterospora* Kamyschko, Bot. Mater. Otd. Sporov. Rast. Bot. Inst. Komarova Akad. Nauk S.S.S.R. (Not. Syst. Sect. Crypt. Inst. Bot. Acad. Sci. USSR) 15: 138, 1962 (generic nom. inval., Art. 37.1).
= *Pochonia humicola* Batista & O.M. Fonseca, Publ. Inst. Micol. Recife 462: 5, 1965.
= *Dictyoarthrinopsis kelleyi* Dominik & Majchrowicz, Mycopathol. Mycol. Appl. 28: 210, 1966.

Teleomorph: *Cordyceps chlamydosporia* H.C. Evans (see below)

Colonies reaching 20–38 mm diam. in 10 days, white, later becoming cream-coloured, appearing powdery with age due to the production of dictyochlamydospores; in fresh isolates appearing finely granular due to abundant dictyochlamydospores produced on the surface of the colony; reverse cream-coloured, pale yellow to orange. Conidiophores prostrate aerial hyphae, producing solitary phialides or 2–3 phialides per node along the conidiophore or terminal whorls of 4–5 phialides. Phialides slender subulate, 12–26 × 1.0–1.5 µm. Conidia formed in globose heads, subglobose to ovoid or ellipsoidal, smooth-walled, (1.8–) 2.5–4.5 × (1.0–)1.2–2.2 µm. Dictyochlamydospores produced in the aerial mycelium, stalked, measuring (10.5–)15–25 (–30) × 14–25 µm, becoming thick-walled with age. Young dictyochlamydospores staining blue when mounted in lactic acid-cotton blue, later often turning reddish or remaining unstained. Crystals absent. Temperature optimum: 24–27(–30)°C (23–40 mm diam., depending on the strain). No growth at 33°C. GAMS (1988) reported a minimum temperature for growth of both varieties near 10°C (ZARE & GAMS 2003 h).

STRAINS EXAMINED:

CBS 429.64 = MUCL 9880, ex soil, Brazil, 1964, Oliveira da Silva, **ex-type** of *P. humicola*.
CBS 103.65 = ATCC 16289, ex soil under *Brassica napus* (rape seed), Germany, 1965, W.Gams, **ex-neotype** (designated by GAMS 1988).
IMI 113169, ex soil, Canada, 1965, J.W. Carmichael.
CBS 594.66, ex soil, Africa, T. Dominik & I. Majchrowicz, **ex-type** of *Dictyoarthrinopsis kelleyi*.
IMI 156157, ex root of *Piper betle*, India, 1971, P.D. Wangikar.
CBS 600.88, ex *Heterodera avenae*, Sweden, 1982, C. Dackman.
IMI 321351, ex cyst nematode on *Solanum tuberosum*, 1988, D.H. Crump.
IMI 359233, ex gall of *Meloidogyne*, Chile, 1993, B.A. Latorne.
CBS 292.95, ex soil under *Araucaria*, São Paulo, Brazil, 1995, L. Pfenning, deviating by absence of dictyochlamydospores.

GAMS (1988) subsumed the former species *Verticillium catenulatum* (Kamyschko ex G.L. Barron & Onions) W. Gams as a variety of this species, creating var. *chlamydosporium* for the type variety. The varietal ranking of similar taxa differing only in conidial arrangement seems to be supported by our molecular observations (Fig. 5-1). RFLP patterns of the ITS region were identical, those of mtDNA were variable, those of the β-tubulin gene were less variable, but these variations were not correlated with the varieties distinguished.

Strain CBS 292.95, though it failed to produce any dictyochlamydospores, had ITS and β-tubulin RFLP patterns identical to the rest of the strains placed under this taxon.

Some authors considered *V. chlamydosporium* to be a complex showing continuous variation in morphology and physiology (IRVING & KERRY 1986; KERRY *et al*. 1986), but with the segregation of *V. suchlasporium* by GAMS (1988) the species was rendered more homogeneous. ARORA *et al.* (1996) found some variation in RFLP patterns generated with ITS and IGS amplicons and in ERIC-RAPD patterns among 28 isolates identified as *V. chlamydosporium.* Unfortunately, except that of *P. rubescens* (CBS 646.88), no ex-type strains or other comparable isolates of the taxa presented here were included in that study.

The varieties of *Pochonia suchlasporia* are distinguished by their taller, mainly erect and more densely verticillate conidiophores and scanty dictyochlamydospores which are mostly buried in the agar (see also ZARE & GAMS 2003 h, i).

Biochemical and molecular studies by CARDER *et al*. (1993) did not confirm the distinction between *P. suchlasporia* and *P. chlamydosporia* and their varieties. These authors partly examined the same strains as those used by ZARE *et al.* (2000 – part 1). Our results do not support their conclusions but rather confirm the classification proposed by GAMS (1988).

Teleomorph: ***Cordyceps chlamydosporia*** H.C. Evans, Nova Hedwigia 73: 59, 2001.

Figs 5-2, 5-4, 5-5

Mycelium white to pale yellow, densely covering the egg mass. Stromata single, unbranched, white to pale orange, narrow cylindrical, 12–16 × 0.5–1.25 mm, widening towards the tip. Fertile area terminal, 2.0–3.5 mm in length, white to pale orange. Perithecia crowded, initially immersed in the mycelium, becoming erumpent and superficial; smooth, pale yellow, occasionally brownish orange, broadly flask-shaped, (500–)600–650 × 250–300 µm, with a prominent ostiole and a long neck region, 150–200 × 80–120 µm, lined with periphyses. Asci 8-spored, cylindrical, (160–)200–500 × (2–)3–4(–4.5) µm, with a prominent cap. Ascospores hyaline, filiform, two fascicles of four somewhat distended in the ascus, septa indistinct, not fragmenting into part-spores, 150–350 × 1.0 µm.

Holotype IMI 380125, on mollusc eggs in forest litter, Pichilingue, Los Ríos Province, Ecuador, Apr. 1974, H.C. Evans (herb. IMI).

MATERIAL EXAMINED (paratypes):
IMI 380126, on mollusc eggs in forest soil, Pichilingue, Los Ríos Province, Ecuador, May 1974, H.C. Evans.
IMI 380127 (380128), on mollusc eggs in forest log, Guamá, Pará State, Brazil, Sep. 1977 (Jan. 1980), H.C. Evans.
IMI 380129, on mollusc eggs in forest soil, Ouro Preto do Oeste, Rondonia State, Brazil, May 1985, H.C. Evans (culture deposited as IMI 331547 = CBS 101244).
IMI 380130, on mollusc eggs in forest soil, Rio Napo, Napo Province, Ecuador, July 1987, T. Læssøe.

The type specimen comprises three egg clutches, up to 7.0 × 4.5 mm, two bearing *Cordyceps* stromata and the other producing five branched synnemata, 14–18 × 0.5–0.8 mm, white to pale tan, compact at the base, becoming broader towards the tip due to a loose mass of aerial hyphae bearing phialides and pale yellow dictyochlamydospores, imparting a yellow powdery appearance to the synnemata. The holotype, with abundant dictyochlamydospores, not only on the egg masses but also on well-defined synnemata, provided circumstantial evidence of the teleomorph–anamorph connection. However, it was not until typical cultures were obtained from the ascospores of a specimen from Brazil (IMI 331547 = CBS 101244) that the association was confirmed.

The perithecia of *C. chlamydosporia* are buried initially but become semierumpent and later superficial as they mature; possibly due to the transition from the anamorph (synnemata) to the teleomorph (stromata). The latter in IMI 380127 and 380130 are well developed, with little evidence of the anamorph remaining, and up to 4 cm in length. The holotype and several paratypes possess perithecia with a distinct palisade of cylindrical cells composing the outer layer of the perithecial neck, whilst this is ill-defined in others. In IMI 380126, the old stromata bear yellow to reddish brown perithecia. Nevertheless, this variation is considered to be acceptable within such geographically-separated populations, stretching from coastal Ecuador to the lower Amazon in Brazil.

The last major revision of the genus *Cordyceps* was undertaken by MAINS (1958), for North American species only, in which he recognized four subgenera, based primarily on the presence or absence of an apical cap in the ascus and the degree of immersion of the perithecia on the stroma. Those species with superficial or free perithecia were assigned to the subgenus *Racemella*, and all six described species possess filiform ascospores which either do not form partspores or separate only tardily. *Cordyceps chlamydosporia* falls readily into this grouping, together with *C. atewensis* Samson, H.C. Evans & Hoekstra, described on an homopteran insect from Ghana (SAMSON *et al*. 1982). The germinating ascospores of this species were reported to produce a *Hirsutella* stage and a remnant palisade of hirsutella-like conidiogenous cells occurs on the stromata of both IMI 380128 and 380130. It is possible that several synanamorphs exist in the life-cycle and that the strategy in a forest habitat with dispersed host populations would be for local spread by rain-splashed slime-spores, with long-distance dispersal being provided by the forcibly-discharged, aerial ascospores, and the dictyochlamydospores serving for long-term survival. Their abundant production on well-defined synnemata, particularly in the type, suggests that they are also actively-dispersed propagules.

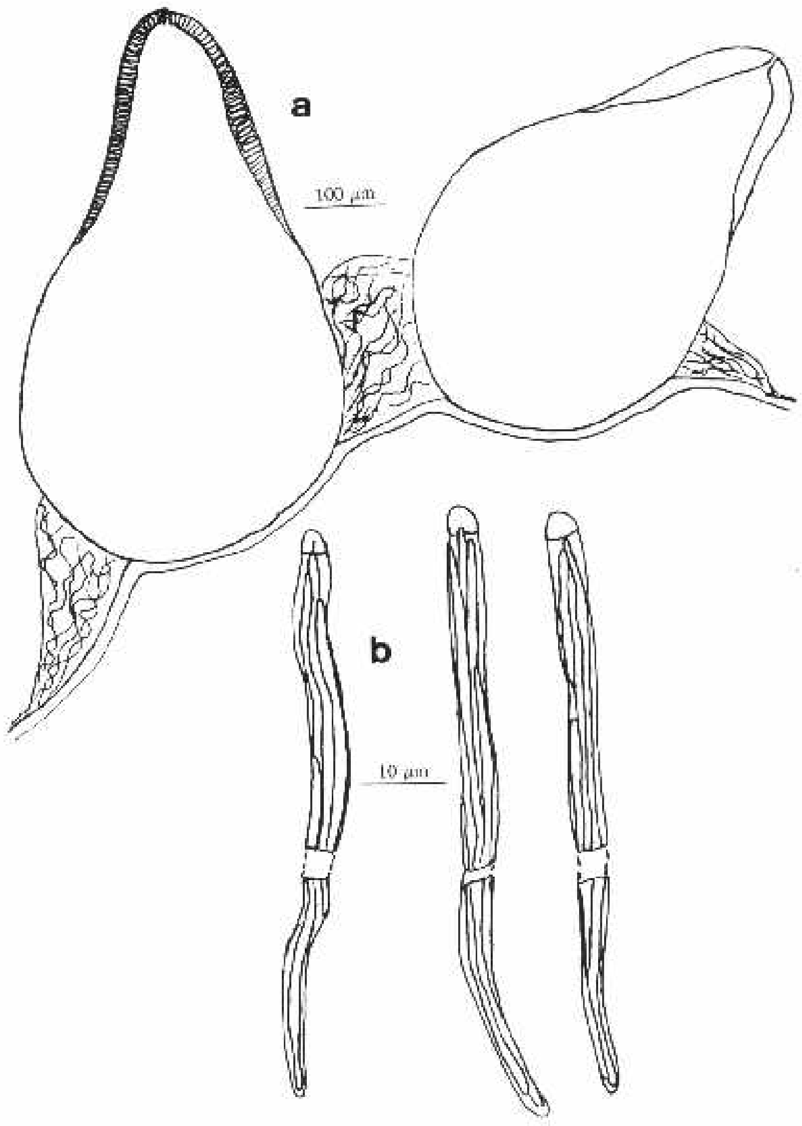


Fig. 5-2. *Cordyceps chlamydosporia*, holotype IMI 380125. a. Perithecia on stroma; b. asci and ascospores.

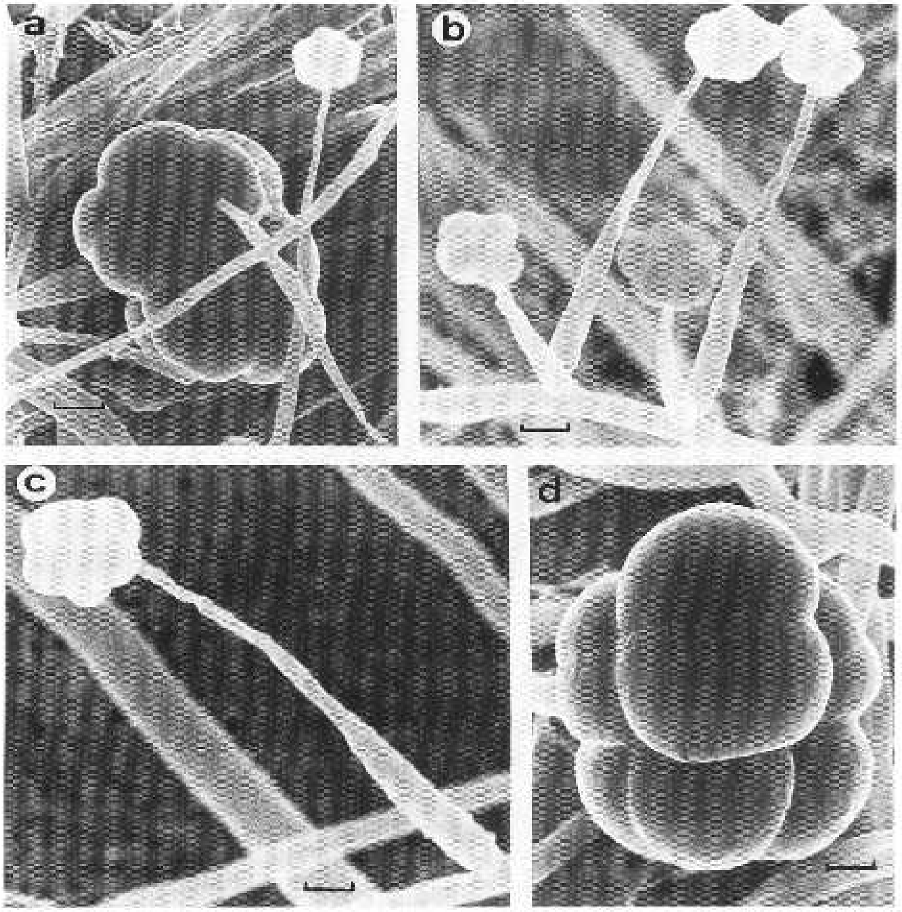


Fig. 5-3. *Pochonia chlamydosporia* var. *chlamydosporia*, IMI 331547, SEM of spore structures, 10 days on PCA. a. Phialides and dictyochlamydospores, bar = 3 µm; b. phialides and conidial heads, bar = 2 µm; c. details of solitary phialide and conidial head, bar = 1.5 µm; d. dictyochlamydospore; bar = 2 µm.

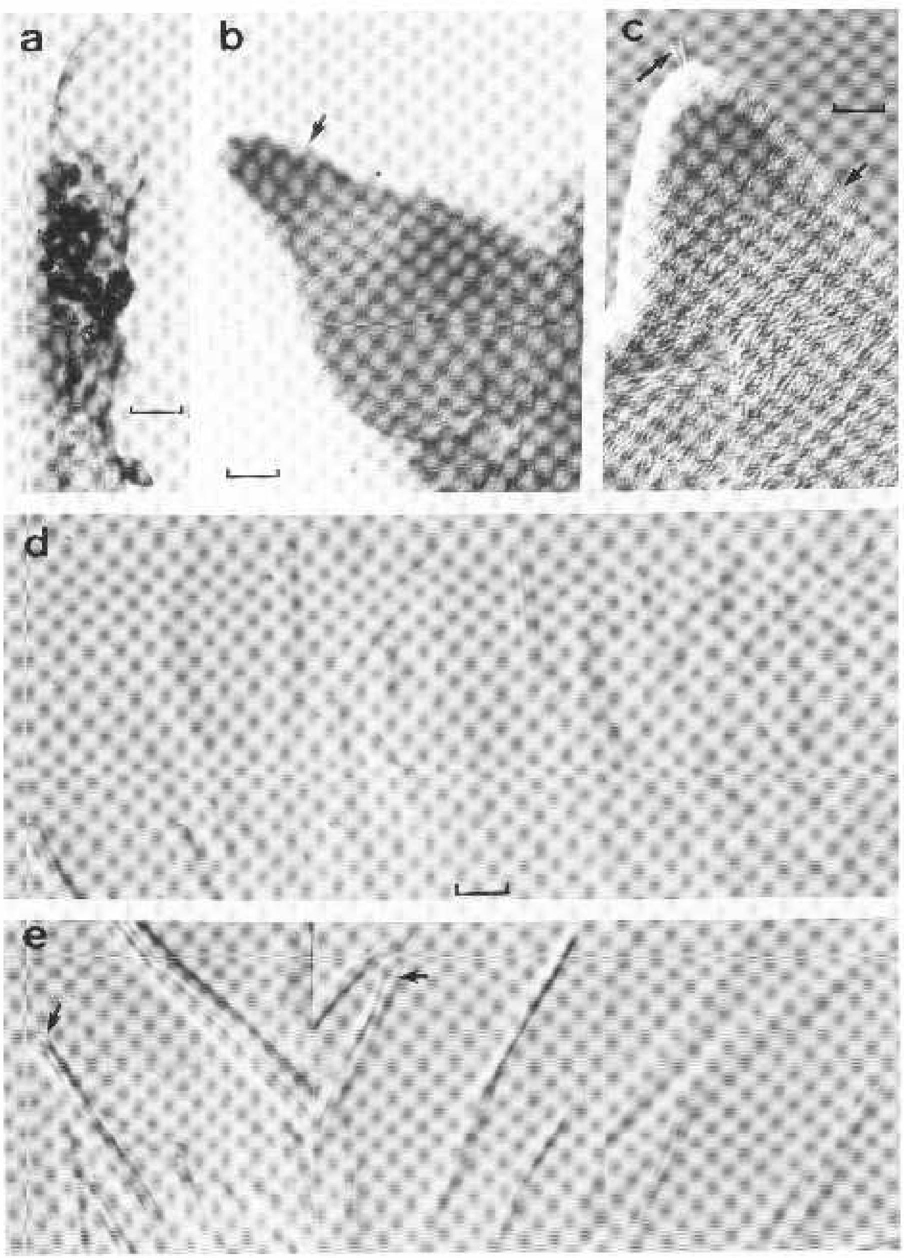


Fig. 5-4. *Cordyceps chlamydosporia*, holotype IMI 380125. a. Apical region of stroma with mature, erumpent perithecia, bar = 1 mm; b. perithecium superficially embedded in stromatal tissue, showing palisade of cylindrical cells in neck region (arrow), bar = 60 µm; c. perithecial neck, showing palisade layer (short arrow) and asci emerging from ostiole (long arrow), bar = 120 µm; d. immature asci with prominent caps; e. mature asci showing ascospores pushing into the cap region (arrow); bar = 7 µm for d and e.

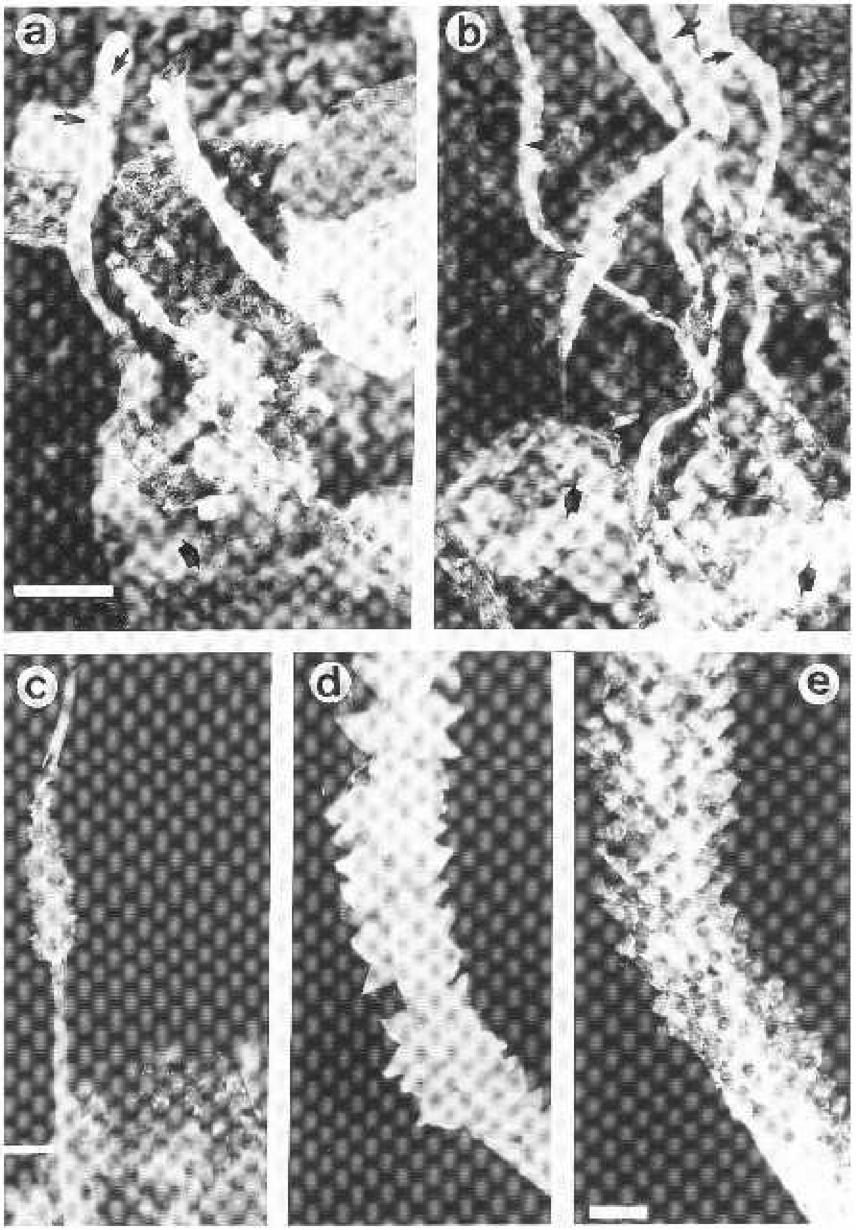


Fig. 5-5. *Cordyceps chlamydosporia*, macromorphology. a. Holotype, IMI 380125 *in situ*, showing young stroma with semi-erumpent perithecia (small arrows), arising from mollusc egg sac (large arrow), bar = 30 mm for a and b; b. holotype *in situ*, showing synnemata arising from two mollusc egg sacs (large arrows), the upper region is surrounded by a loose mycelial covering producing phialides and powdery dictyochamydospores (small arrows); c. upper region of mature stroma with pale (yellow) perithecia, bar = 1.5 mm; d. IMI 380127, showing erumpent, pale (yellow) perithecia on mature stroma; e. IMI 380126, showing dark (reddish brown) perithecia on old stroma; bar = 0.5 mm for d and e).

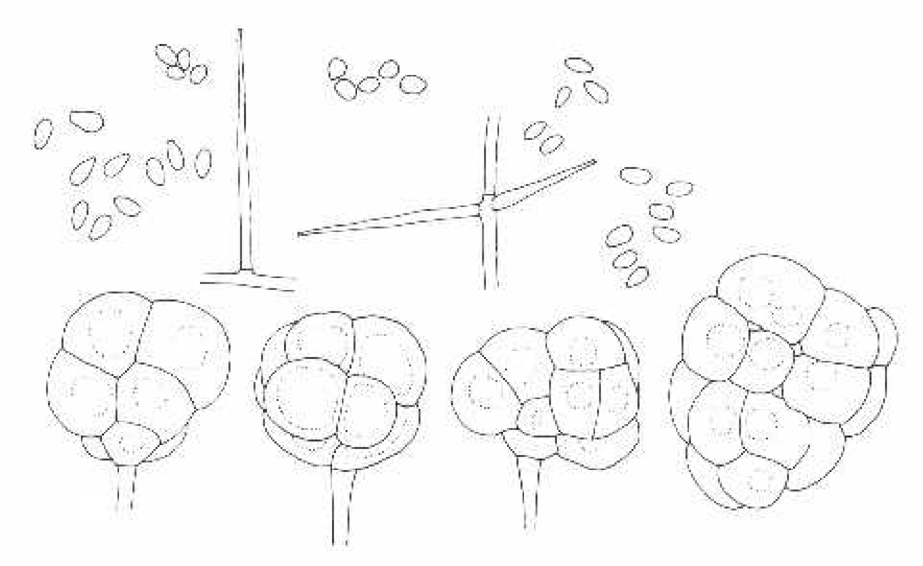


Fig. 5-6a. *Pochonia chlamydosporia* var. *chlamydosporia*. Phialides, conidia and dictyochlamydospores of CBS 361.64 on PCA.

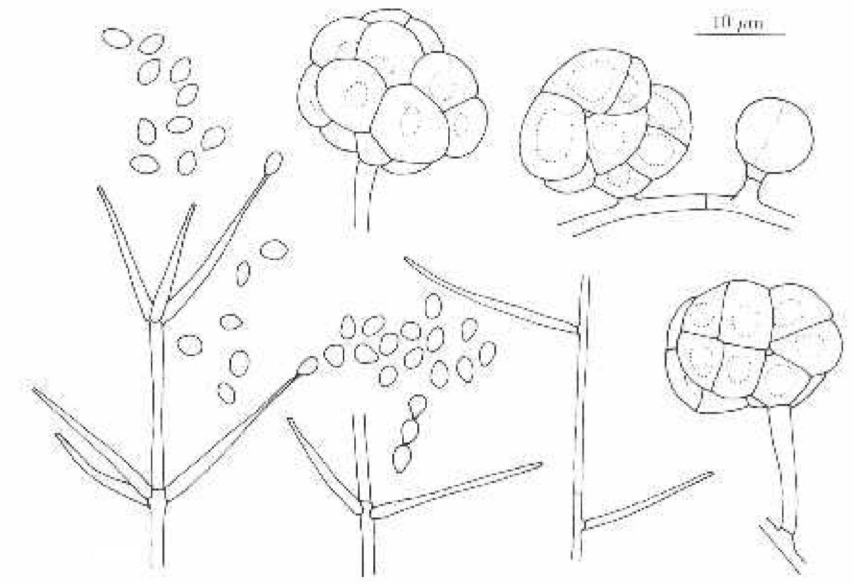


Fig. 5-6b. *Pochonia chlamydosporia* var. *catenulata*, phialides and dictyochlamydospores of CBS 504.66 on PCA.

Thus, many of the records of this fungus are from soil, but there is no doubt that *P. chlamydosporia* is a primary pathogen of mollusc eggs, as well as of cyst nematodes (KERRY 1995). Whether or not distinct ecotypes or pathotypes of this extremely wide-ranging species exist, remains to be proven. However, given the increasing importance of mollusc pests in agriculture, especially of alien or non-indigenous species (COWIE 2001), the potential of *P. chlamydosporia* as a biocontrol agent warrants further investigation. PETCH (1939) reported this fungus (as *Stemphyliopsis ovorum*) as a problem in breeding units of the giant African snail (*Achatina fulica* Bouw., Achatinidae) in Sri Lanka in the 1920s, which was then being spread around the tropics as a potential food source. Ironically, this snail has since become a major pest wherever it was introduced, and unfortunately, ill-conceived, non-scientific and, therefore, ecologically disastrous attempts have been made to control it through the importation of predatory snails (COWIE 2001).

Pochonia chlamydosporia has been reported as a parasite of cysts and eggs of *Heterodera* (but only exceptionally of *Globodera*) species (WILLCOX & TRIBE 1974, BURSNALL & TRIBE 1974, KERRY & CRUMP 1977, TRIBE 1977, MORGAN-JONES *et al*. 1981, GINTIS *et al*. 1983) and occasionally of *Meloidogyne* species (GODOY *et al*. 1982, MORGAN-JONES *et al*. 1983). It is a potential biocontrol agent of *Heterodera* cyst nematodes (DE LEIJ & KERRY 1991, DE LEIJ 1992, GAMS & ZARE 2003). The species is also listed as ovicidal to eggs of *Ascaris lumbricoides*, penetrating the eggs and destroying the embryo (FASSATIOVÁ & LYSEK 1982); it was already reported from snail eggs (GAMS 1971). The teleomorph specimens and strain IMI 331547 examined in this study were obtained from slug eggs (Mollusca). More data on the distribution of the species are given by DACKMAN & NORDBRING-HERTZ (1985).

2. ***Pochonia chlamydosporia*** var. ***catenulata*** (Kamyschko ex G.L. Barron & Onions) Zare & W. Gams, Nova Hedwigia 72: 334, 2001. Fig. 5-6 c, d

≡ *Diheterospora catenulata* Kamyschko, [Bot. Mater. Otd. Sporov. Rast. Bot. Inst. Komarova Akad. Nauk S.S.S.R. (Not. Syst. Sect. Crypt. Inst. Bot. Acad. Sci. USSR) 15: 140, 1962, nom. gener. inval., Art. 37.1] ex G.L. Barron & Onions, Canad. J. Bot. 44: 868, 1966 (basionym).

≡ *Verticillium catenulatum* (Kamyschko ex G.L. Barron & Onions) W. Gams, *Cephalosporium*-artige Schimmelpilze, p. 190, 1971.

≡ *Verticillium chlamydosporium* var. *catenulatum* (Kamyschko ex G.L. Barron & Onions) W. Gams, Netherlands J. Pl. Pathol. 94: 134, 1988.

Teleomorph: *Cordyceps* sp., similar to *C. chlamydosporia*. It differs from *C. chlamydosporia* by perithecia which are never erumpent but always buried in a more organized stroma. Because of insufficient material, we cannot yet formally describe this new species.

Colonies similar to *P. chlamydosporia* var. *chlamydosporia*, reaching 22–40 mm diam. in 10 days. Phialides arising from prostrate hyphae, solitary or up to 5 per node. Conidia produced in chains, partly also in heads, globose to subglobose, with slightly apiculate base, measuring (1.5–)2.0–3.5 × 1.5–3.0 µm. Dictyochlamydospores as in *P. c.* var. *chlamydosporia*. Crystals absent. Temperature optimum: 24–27°C (27–36 mm diam.). Very little growth at 33°C (ZARE & GAMS 2003 h).

STRAINS EXAMINED:
IMI 113172, ex hair bait buried in garden soil, UK, 1956, P.M. Stockdale.
IMI 076422, ex soil, Trinidad, 1959, I.D. Firman.
IMI 080555, ex soil, Australia, 1960, J.W. Carmichael & G.L. Barron.
IMI 080556, ex soil, Australia, 1960, Durie & Frey.
CBS 504.66 = IMI 113164, ATCC 16683, OAC 10250, ex soil, Canada, 1963, G.L. Barron, **ex-type**.
CBS 397.69 = MUCL 15018, ex soil, Canada, 1969, G.C. Bhatt.
IMI 331575 and 338017, ex beetle larva (Coleoptera), Ecuador, 1974, H.C. Evans, single-ascospore isolates of the *Cordyceps* teleomorph.
CBS 496.96, ex leaf litter, Cuba, 1996, R.F. Castañeda.

Dictyochlamydospores are indistinguishable in the two varieties. Strain IMI 080556 consistently produced smaller dictyochlamydospores, 14.5 × 10.5 µm. Strains IMI 331575 and IMI 338017, single-ascospore isolates of a *Cordyceps* sp., are indistinguishable from other strains studied under this taxon.

The difference in arrangement of the conidia in chains or heads is again found to be of limited significance, and strains showing either of the arrangements are therefore only distinguished at varietal rank. Conidia in *P. c.* var. *catenulata* are generally more globose to subglobose, while those of var. *chlamydosporia* are more ellipsoidal. RFLPs of ITS region, β-tubulin gene and mtDNA do not consistently separate the two varieties, but parsimony analysis using sequences from ITS regions distinguished them (Fig. 5-1, also part 1).

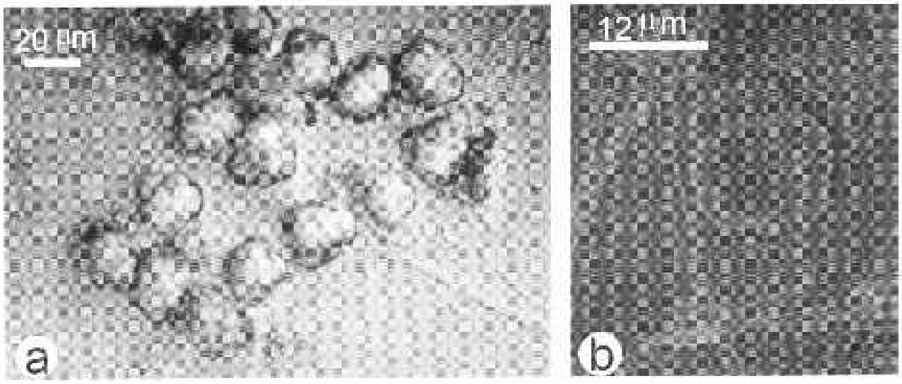


Fig. 5-7. *Pochonia chlamydosporia* var. *catenulata*. a. Dictyochlamydospores, b. conidial chains. a. IMI 331575, b. IMI 338017. DIC.

3. ***Pochonia suchlasporia*** (W. Gams & Dackman) Zare & W. Gams, var. ***suchlasporia***, Nova Hedwigia 73: 67, 2001. Fig. 5-8

≡ *Verticillium suchlasporium* W. Gams & Dackman, Netherlands J. Pl. Pathol. 94: 136, 1988 (basionym).

Colonies reaching 10–12 mm diam. in 10 days, high, woolly, white to yellow, reverse yellow to brownish cream, rather slow-growing. Conidiophores mostly erect but also prostrate, rather thick-walled, up to 4–5 µm wide at the base, tapering apically to 1.5–2.5 µm, bearing up to 3 whorls of 3–4 phialides. Phialides 15–30 × 1.2–2.0 µm, sometimes swollen near the base to 2.5 µm. Conidia in dry heads, subglobose, chromophilic, measuring 2.3–4 × 1.5–2.5 µm. Dictyochlamydospores usually scanty, submerged in the agar, rarely near the surface, surrounded by a slimy layer, 15–25 × 10–20 µm. Crystals absent. Temperature optimum: 18–21°C (17–18 mm diam.). No growth at 27°C (ZARE & GAMS 2003 i).

STRAINS EXAMINED:
CBS 251.83, ex eggs of *Heterodera avenae*, Sweden, 1983, C. Dackman, **ex-type**.
CBS 816.83, ex eggs of *H. avenae*, Denmark, 1983, M. Juhl. More strains documented in GAMS (1988).

GAMS (1988) noticed another, uncommon form of sporulation in which phialides give rise to a few elongate conidia measuring 5–12 × 1.5–2 µm (Fig. 5-8 c).

Pochonia suchlasporia is rather widely separated from *P. chlamydosporia* in molecular analyses (ZARE *et al*. 2000, SUNG *et al*. 2001), but it forms a tight cluster with the remaining species of the genus in its LSU + SSU sequences, with

98% bootstrap support (SUNG *et al*. 2001). The RFLP patterns of the ITS region and the β-tubulin gene are identical in all isolates of both varieties of either species but different between the species.

Like in *P. chlamydosporia*, both varieties of *P. suchlasporia* had identical RFLP patterns of the ITS region and the β-tubulin gene; the more variable patterns of mtDNA did not consistently separate the two varieties; but parsimony analysis using sequences from the ITS regions distinguished them (Figs 1-1 and 5-1).

The two varieties of *P. suchlasporia* form a significant portion of egg and cyst parasites of *Heterodera* species in Sweden (DACKMAN & NORDBRING-HERTZ 1985), Denmark (JUHL 1982) and the Netherlands (G.J. BOLLEN & F. ZOON, pers. comm.). They are at an ecological advantage over *P. chlamydosporia* because of the lower cardinal temperatures (GAMS 1988).

4. ***Pochonia suchlasporia*** var. ***catenata*** (W. Gams & Dackman) Zare & W. Gams, Nova Hedwigia 73: 69, 2001. Fig. 5-9

≡ *Verticillium suchlasporium* var. *catenatum* W. Gams & Dackman, Netherlands J. Pl. Pathol. 94: 140, 1988 (basionym).

Colonies, conidiophores and phialides as in *P. s.* var. *suchlasporia*. Conidia subglobose to ovoid, chromophilic, measuring 2–3.7 × 1.7–2.3 µm, cohering in chains or partly in dry heads. Dictyochlamydospores scanty, 16–28 × 14–25 µm, submerged in the agar. Crystals absent. Temperature optimum: 21°C (14–21 mm diam.). No or very little growth at 27°C (ZARE & GAMS 2003 i).

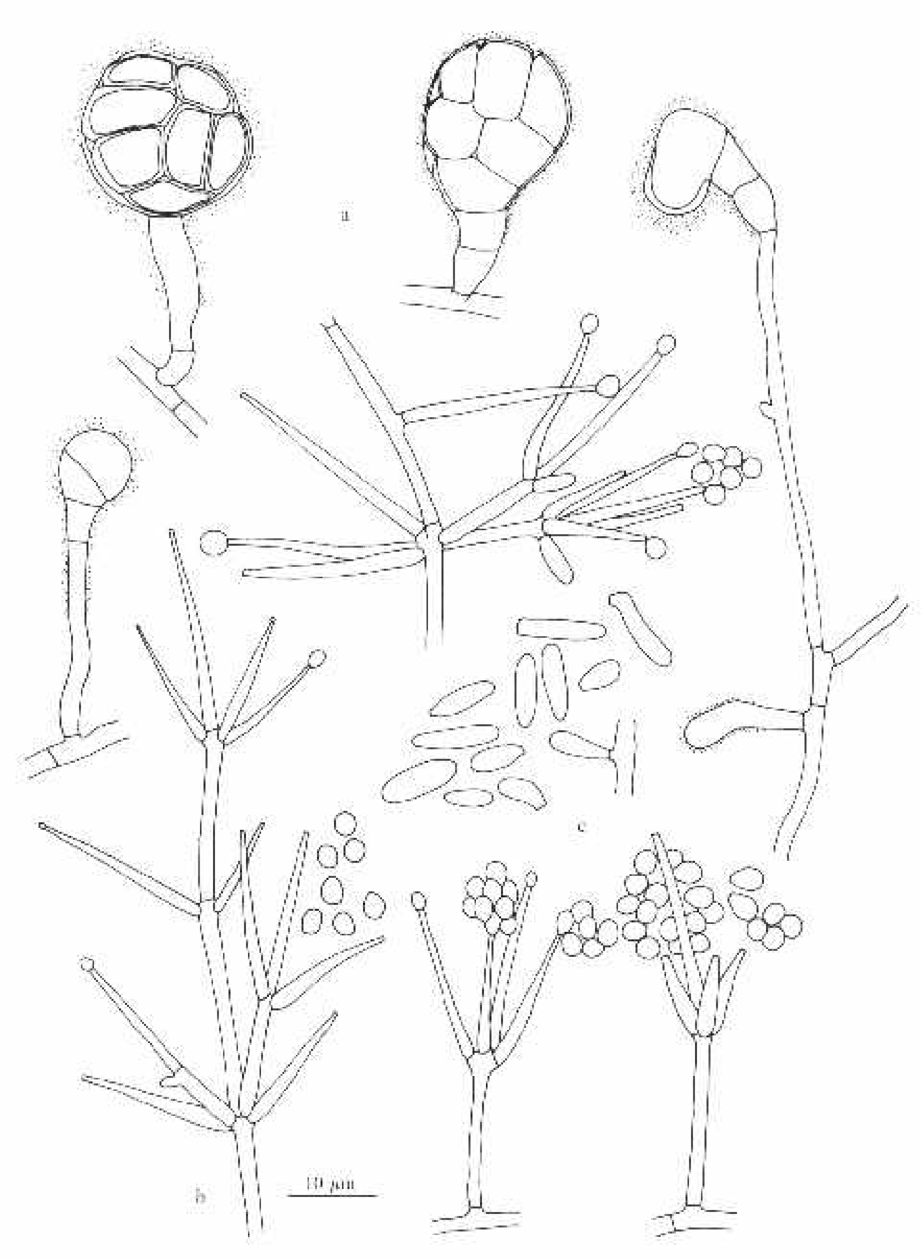


Fig. 5-8. *Pochonia suchlasporia* var. *suchlasporia*. a. Dictyochlamydospores of CBS 228.82B on SEA, b. conidiophore and conidia of CBS 228.82C on OA, c. conidiophore, conidia and elongate submerged conidia of CBS 228.82B on PCA.

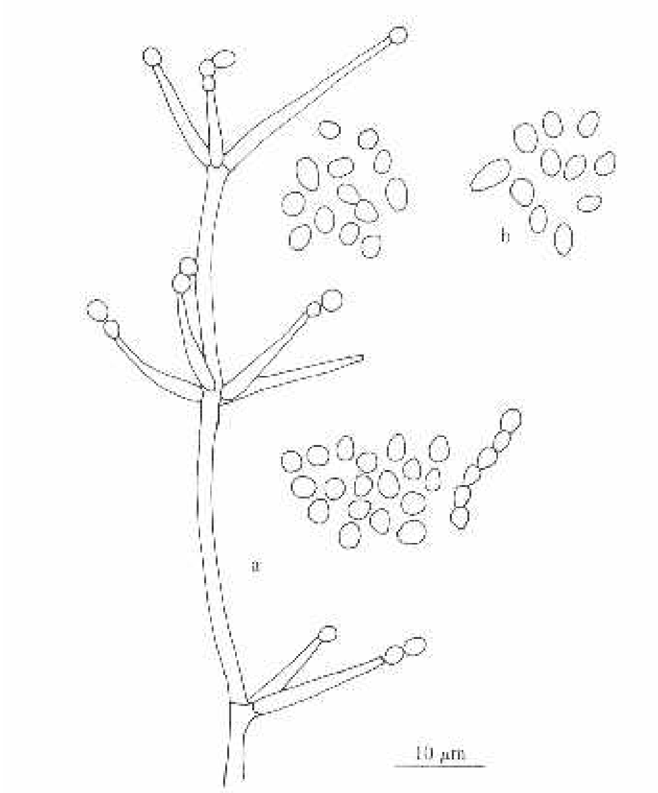


Fig. 5-9. *Pochonia suchlasporia* var. *catenata*. a. conidiophore and conidia of CBS 876.85 on MEA, b. conidia of CBS 248.83.

STRAINS EXAMINED:
IMI 113078 = CBS 101265, ex millipede droppings, UK, 1965, T.F. Hering.
CBS 383.70a, ex garden soil, Netherlands, 1970, H.A. van der Aa.
CBS 248.83, ex eggs of *Heterodera avenae*, Sweden, 1983, C. Dackman, **ex-type**.
CBS 817.83, ex eggs of *H. avenae*, Sweden, C. Dackman.
CBS 789.85, ex eggs of *Lymantria dispar* (gipsy moth), USA, G.C. Carroll.
CBS 495.90, ex soil, Canada, G. Thorn.
CBS 416.95, ex worm on dead leaf of *Roystonia regia*, Cuba, 1994, R.F. Castañeda.

No dictyochlamydospores were observed in strains IMI 113078, CBS 817.83 and CBS 789.85, but these strains otherwise conform well with the taxon, and have RFLP patterns identical with those of dictyochlamydosporic isolates.

5. ***Pochonia rubescens*** Zare, W. Gams & López-Llorca, Nova Hedwigia 73: 69, 2001.

Fig. 5-10

Colonies rather fast-growing, reaching 25–29 mm diam. in 10 days, thin, white, reverse red (at least on PDA), with a reddish pigment diffusing into the agar after 10 days. Conidiophores usually prostrate, rarely erect but not thick-walled. Phialides solitary or up to 2–4 per node, 18–25 × 0.7–1.0 µm. Conidia formed in dry heads, globose to subglobose, smooth-walled, measuring 2.5–3.5 × 2.0–3.0 µm. Dictyochlamydospores scanty, scattered in chains or loosely held together forming irregular shapes, usually deeply submerged in the agar, sometimes totally absent or very difficult to detect. Crystals absent. Temperature optimum: 24°C (30–32 mm diam.). No growth at 33°C (see also ZARE & GAMS 2003 i).

STRAINS EXAMINED:
CBS 352.70, ex soil from *Thuja-Abies* bog, USA, 1967, W.F. Whittingham.
CBS 405.70, ex agricultural soil, Netherlands, 1968, J. W. Veenbaas-Rijks.
CBS 425.80a, ex cysts of *Heterodera schachtii*, 1980, G.J. Bollen.
IMI 293909 = CBS 101238, ex eggs of *Heterodera avenae*, UK, 1985, J.M. Duncan.
CBS 464.88 = ATCC 76547, ex eggs of *Heterodera avenae*, UK, Scotland, 1988, L. López-Llorca, **ex-type** (Holotype: herb. CBS).

Conidia of this species resemble those of *P. suchlasporia*. *Pochonia rubescens* is distinct mainly by its red pigment on the colony reverse and also the reddish yellow pigment diffusing into the agar. Dictyochlamydospores are very rare in *P. rubescens*; they appear as single cells or loosely compacted cell aggregates composed of irregular shapes or scattered chains; sometimes they are totally absent (see ZARE & GAMS 2003 i).

Five strains represent this taxon, three of which were isolated either from cysts of *Heterodera schachtii* or eggs of the cereal cyst nematode, *H. avenae*. The other two were obtained from soil. The ex-type strain was reported as an egg parasite on *H. avenae* (LÓPEZ-LLORCA & DUNCAN 1988, LÓPEZ-LLORCA 1990). GAMS (1988) placed this strain under *V. suchlasporium*. The species has a distinct ITS RFLP pattern, that of the β-tubulin gene is the same as in *P. suchlasporia* and mtDNA is too variable to draw any conclusion.

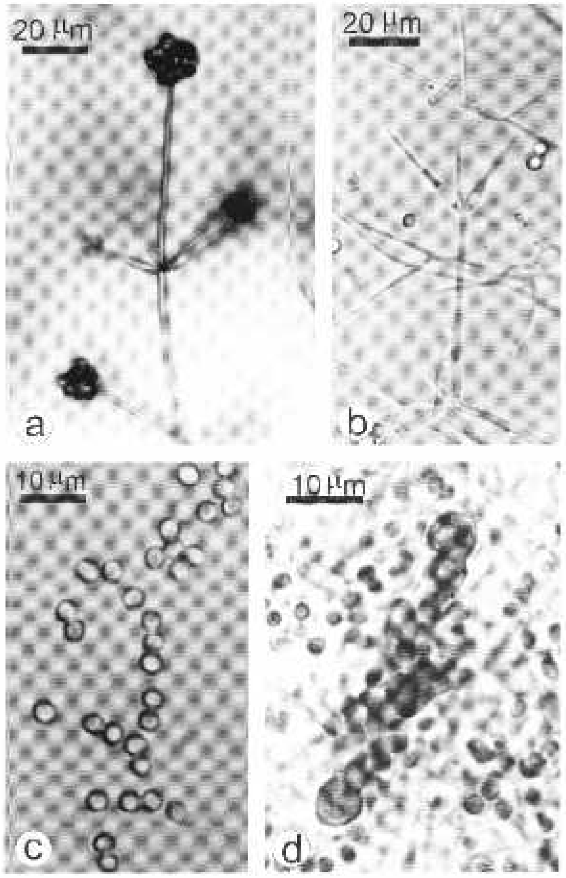


Fig. 5-10. *Pochonia rubescens*. a,b. Conidiophores, c. conidia, d. submerged dictyochlamydospores. a, b, c. CBS 352.70; d. CBS 425.80A; a–d. DIC, d. stained in cotton blue.

The characteristic red pigment, most abundantly produced on acidic media, was also noted by LÓPEZ-LLORCA *et al*. (1994) and LÓPEZ-LLORCA & OLIVARES-BERNABEU (1998) in strain CBS 464.88. These authors extracted the pigment in chloroform/methanol and proved its inhibitory effect (mycotoxicity) on the growth of *Cladosporium cucumerinum*. They also showed that the extract had nematicidal effect on the potato root cyst nematode, *Globodera rostochiensis*.

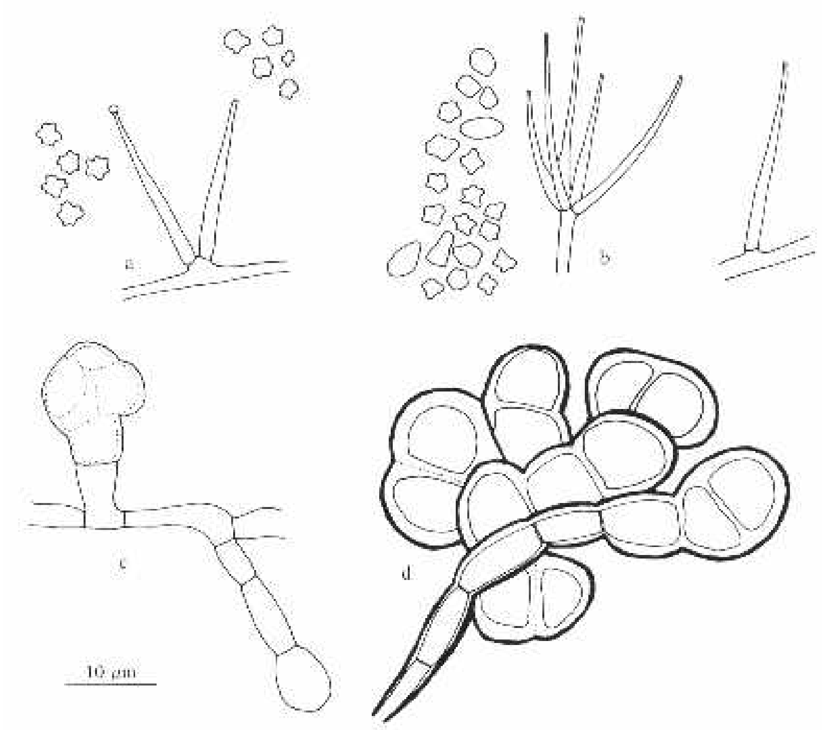


Fig. 5-11. *Pochonia gonioides*. a, b. Conidiophores and conidia of CBS 611.89 and 241.80 on PCA and MEA, c. young and d. old dictyochlamydospores of CBS 241.80 on SEA.

6. ***Pochonia gonioides*** (Drechsler) Zare & W. Gams, Nova Hedwigia 73: 72, 2001.

Fig. 5-11

≡ *Acrostalagmus gonioides* Drechsler, J. Washington Acad. Sci. 32: 347, 1942 (basionym).
≡ *Verticillium gonioides* (Drechsler) W. Gams & Stalpers, Netherlands J. Pl. Pathol. 94: 143, 1988.

Colonies reaching 14–16 mm diam. in 10 days, white, with brownish cream reverse, moderately slow-growing. Phialides arising mostly in whorls of 2–5 on prostrate hyphae, measuring 13–30 × 1–2 µm, tapering distally to 0.5 µm. Conidia held together in heads, 1.8–2.5 µm diam., with irregularly polyhedral to tuberculate outline. Dictyochlamydospores irregular, mostly submerged, formed as lateral branches with swollen cells. Crystals absent. Temperature optimum: 18°C (CBS 611.89 with 4–5 mm diam.) or

21°C (CBS 891.72 with 21 mm diam.). No growth at 27°C (CBS 611.89) or 30°C (CBS 891.72).

STRAINS EXAMINED:
CBS 891.72, ex *Pulcherricium caeruleum*, Germany, 1972, J.A. Stalpers, **ex-neotype** (GAMS 1988).
CBS 611.89, ex litter of *Pinus sylvestris*, Germany, 1989, G. Kraepelin.

DRECHSLER (1942) found this fungus attacking cysts and eggs of *Heterodera* species and as an endoparasite of *Rhabditis terricola*. No living strain isolated from nematodes is available at the moment.

The species can be distinguished from all other taxa of the genus by its isodiametric-polyhedral and smaller conidia. The two isolates vary in the degree of lobulation of the conidia and differ strongly in growth rate, but have identical RFLP patterns of the ITS region and the β-tubulin gene.

7. ***Pochonia bulbillosa*** (W. Gams & Malla) Zare & W. Gams, Nova Hedwigia 73: 73, 2001. Fig. 5-12

≡ *Verticillium bulbillosum* W. Gams & Malla, *Cephalosporium*-artige Schimmelpilze, p. 189, 1971 (basionym).
= *Verticillium cephalosporum* W. Gams, ibid., p. 180, 1971.

Colonies reaching 20–35 mm diam. in 10 days, conidiophores and phialides similar to those of *P. chlamydosporia* var. *chlamydosporia*. Conidia produced in globose heads, of two types: falcate with blunt ends, 4–6.3 × 1.5–2.0 µm, and subglobose to ovoid, 2.2–3.0 × 1.3–2.0 µm. Dictyochlamydospores more or less scanty, produced on the agar surface, irregular or sometimes forming chains or reduced to irregularly swollen hyphae. Crystals absent. Temperature optimum: 21–24°C (21–30 mm diam.). No growth at 30°C.

STRAINS EXAMINED:
CBS 247.68, ex agricultural soil, Germany, 1964, W. Gams, **ex-type** of *V. cephalosporum*.
CBS 145.70, ex root of *Picea abies*, Denmark, 1970, D.S. Malla, **ex-type**.
CBS 578.78, ex plant material, New Zealand, 1978, G.F. Laundon.
CBS 426.81, ex paramo soil, Colombia, 1981, sample taken by T. van der Hammen & R. Jaramillo, isol. W. Gams.

This taxon can be readily distinguished from all other taxa of *Pochonia* by its falcate conidia. They are generally shorter and blunter than those of *Lecanicillium psalliotae* and related species, in which the conidia typically sit transversely on the phialide tip. The synonymy of *V. cephalosporum* (at least for the ex-type strain) was demonstrated by GAMS (1988).

Pochonia bulbillosa is commonly isolated from forest soils. Synthetically produced mycorrhizae of *Picea abies* with *Laccaria laccata* were damaged by toxic metabolites and mycoparasitism, but mycorrhizae formed with *Hebeloma crustuliniforme* were not (MARCHETTI & VARESE 1997).

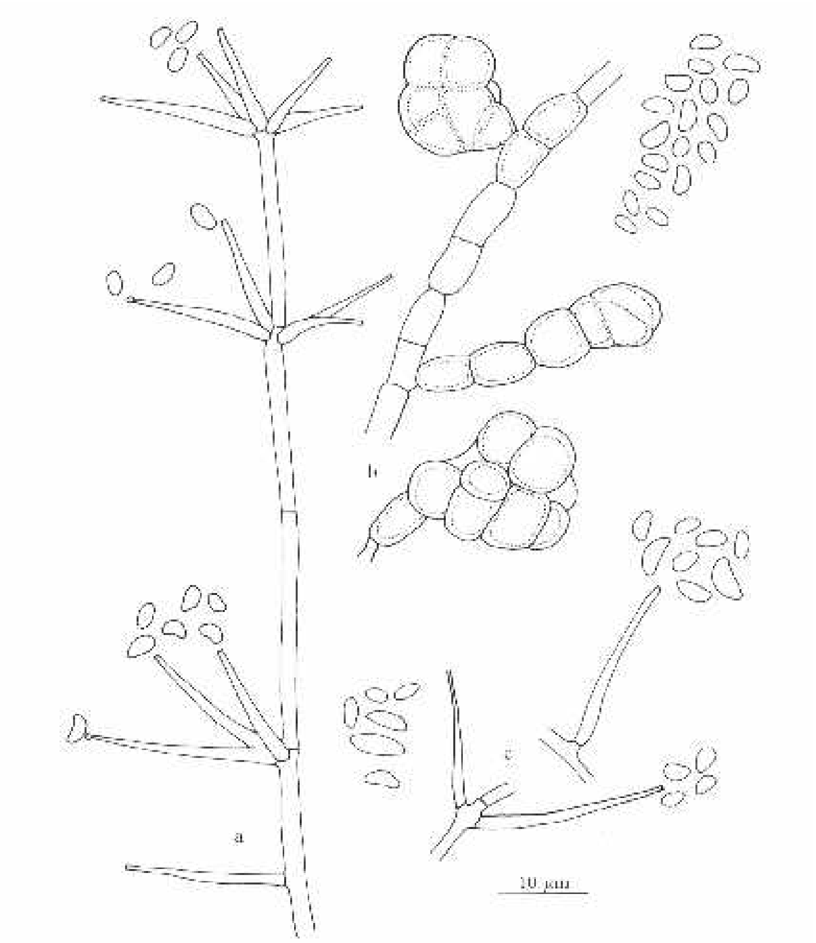


Fig. 5-12. *Pochonia bulbillosa*. a. Conidiophore and conidia of PD 82/32, b. irregular dictyo-chlamydospores and conidia of CBS 145.70 on OA, c. phialides and conidia of CBS 145.70.

8. ***Pochonia microbactrospora*** W. Gams & Zare, Nova Hedwigia 73: 73, 2001.

Figs 5-13, 5-14

Colonies reaching up to 15 mm diam. in 10 days, thin, pale yellow to white, reverse yellow, rather slow-growing. Phialides solitary or verticillate up to 2–3 per node, swollen at the base and narrowed in the middle. Conidia produced in small slimy heads, ellipsoidal to rod-shaped, 2–2.5 × 0.7–1.0 µm. Dictyochlamydospores of irregular shape, in chains or loosely aggregated cells. Octahedral crystals present. Temperature optimum: 21°C (7 mm diam.). No growth at 33°C.

STRAIN EXAMINED:
CBS 101433, isolated by S.L. Glockling, ex rotifers in pine litter, Japan, 1996, **ex-type** (Holotype: herb. CBS).

GLOCKLING (1998a) identified this strain as *Verticillium bactrosporum* (Drechsler) Subram., but, because of its smaller and non-adhesive conidia, and also based on molecular evidence (see Fig. 5-1), we considered this identification inappropriate and described the very distinct fungus as a new species of *Pochonia*. This species resembles *Verticillium campanulatum* GLOCKLING & DICK (1997), but it has conidia that are only of one type and are smaller than those of *V. campanulatum*. DRECHSLER (1941) described and illustrated *Acrostalagmus bactrosporus* with an intermediate size (2–3 × 1.3–1.6 µm) of adhesive conidia. That species is currently unavailable but obviously belongs to *Haptocillium*.

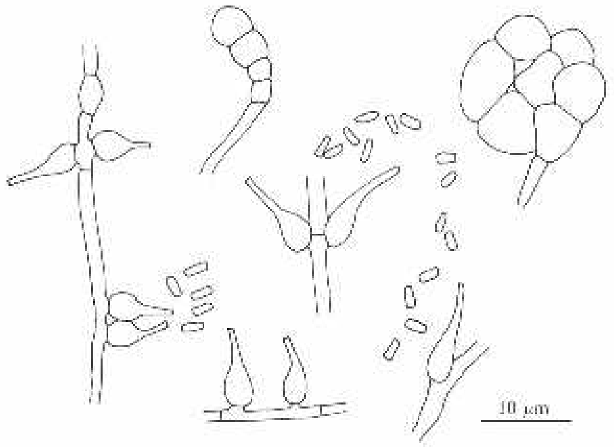


Fig. 5-13. *Pochonia microbactrospora*. Incipient dictyochlamydospores, phialides and conidia of CBS 101433 on PCA and SEA.

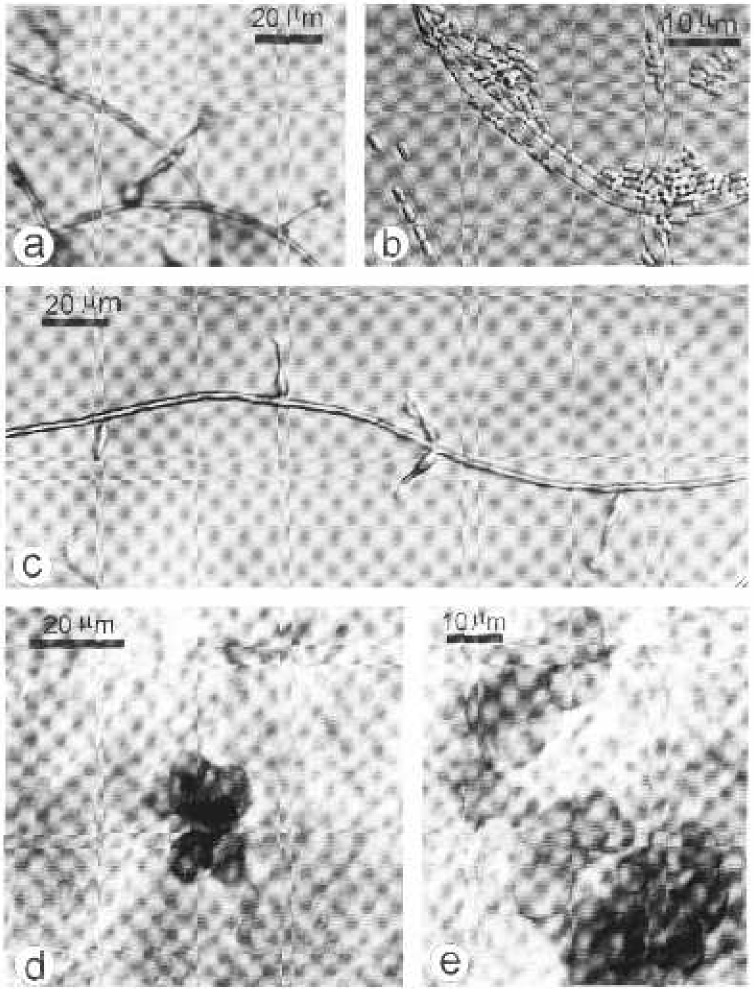


Fig. 5-14. *Pochonia microbactrospora*. a. Conidial heads, b. conidia, c. phialides, d, e. dictyochlamydospores stained in cotton blue. a–e. CBS 101433, DIC.

Table 5-II. Differences between *Pochonia* and *Rotiferophthora*.

Feature	*Pochonia*	*Rotiferophthora*
Hosts	nematode cysts and eggs	only rotifers
Dictyochlamydospores	more or less globose	usually flat
Verticillate phialides	long, gradually tapering from base to apex	more or less short, usually flask-shaped with a swollen base and a narrow cylindrical neck
Intercalary phialides	absent	present
Conidia	without mucoid sheath or oil droplet	held in a mucoid sheath, with a large oil droplet

ROTIFEROPHTHORA G.L. Barron, Canad. J. Bot. 69: 495, 1991.

Colonies very slow-growing. Conidiophores simple or sparingly branched; phialides either discrete, single, paired or in whorls, and flask-shaped, or intercalary, with short narrow fertile necks, and overtopped by a terminal phialide. Conidia globose, short-ellipsoidal to fusiform, clavate or lunate, containing one or more conspicuous oil droplets which are visible in water and lactic acid mounts. Dictyochlamydospores usually abundant, more or less applanate.

Type species: *Rotiferophthora globispora* G.L. Barron.

The genus *Rotiferophthora* was introduced by BARRON (1991) to include parasites of bdelloid rotifers. A few species had previously been classified in *Acrostalagmus* or *Diheterospora*. Over 25 species have been described, mostly based on minute differences in conidial shape (DRECHSLER 1942, BARRON 1973, 1980, 1985, 1991, GLOCKLING & DICK 1997, GLOCKLING 1998b). Very few of them have been grown in pure culture and at the moment only a small number of species are available in culture collections. Representatives of *Rotiferophthora* are exclusively known as specialized endoparasites of bdelloid rotifers, mainly *Adineta* species (BARRON 1985). Conidia and chlamydospores are produced outside the host body (GLOCKLING 1998b). All species produce dictyochlamydospores. Conidia adhering in heads are produced from flask-shaped or elongate phialides and often also from short cylindrical conidiogenous necks of intercalary phialides (BARRON 1991, GLOCKLING 1998a). The animals become infected by ingesting the conidia (BARRON 1980, 1985).

Rotiferophthora and *Pochonia* are considered closely related. The differences between the two genera are summarized in Table 5-II (extracted from BARRON 1985, GLOCKLING & DICK 1997, GLOCKLING 1998b).

Strains of two species received from Dr S.L. GLOCKLING were included in this study. For a key to all species described we refer to GAMS & ZARE (2003).

Rotiferophthora angustispora (G.L. Barron) G.L. Barron, Canad. J. Bot. 69: 495, 1991.

≡ *Diheterospora angustispora* G.L. Barron, Canad. J. Bot. 63: 214, 1985.

Colonies reaching 15 mm diam. in 10 days, cream-coloured, with pale-brown reverse. Conidiophores more or less erect, up to 200 µm tall, 2.5–4 µm wide, bearing lateral branches in whorls, especially near the base. Phialides solitary or up to 5 on each node, 13–22 × 2.0–2.5 µm, swollen near the base and tapering gradually to a narrow apex which is often irregularly reflexed upwards. Intercalary phialides with a short neck, infrequent, appr. 6 × 0.8–1.3 µm. Conidia narrowly cylindrical or sausage-shaped, 6.5–8 × 1.0–1.3 µm. Because of the narrow conidia, the oil droplet at the distal end is difficult to see. Dictyochlamydospores flat, 15–25 × 10–12 µm. Temperature optimum 18–21°C (10–12 mm diam.). No growth at 27°C.

STRAIN EXAMINED:
CBS 101437, isolated by S.L. Glockling from a rotifer in decaying straw, England, 1997.

This species was originally recovered from bdelloid rotifers in farmyard soil in Guelph, Canada (BARRON 1985).

Rotiferophthora minutispora S.L. Glockling, Mycol. Res. 102: 1145, 1998.

Colonies very slow-growing, reaching 6 mm diam. in 10 days, pinkish red, with dark cream-coloured reverse. Conidiophores up to 300 µm tall, with up to 6 whorls of phialides, which are elongate and tapering, measuring 16 × 2 µm. Conidia produced in heads, spherical, 2.0–2.2 µm diam. Dictyochlamydospores 14–16 µm diam., bulbous. Temperature optimum: 21–24°C (about 6 mm diam.). No growth at 33°C.

STRAIN EXAMINED:
CBS 101436 = IMI 372229, isolated by S.L. Glockling from a rotifer in Japan, **ex-type**.

RESIDUAL GROUP

Verticillium incurvum W. Helfer, Libri Bot. 1: 77, 1991. Fig. 5-15

Colonies very slow-growing, reaching 5 mm diam. in 10 days, white, with brownish-cream reverse. Phialides in whorls, measuring 16–26 × 1.0–1.7 µm, producing single conidia at the apex. Conidia falcate, gently curved and blunt-ended, 0(–1)-septate, 9–15 × 1.5–2.0 µm. Chlamydospores produced abundantly in the mycelium, of irregular shape, consisting of small globose cells, each cell 8–11 µm diam.

STRAIN EXAMINED:
CBS 460.88, isolated by I. Nuss from *Ganoderma lipsiense*, Bayerischer Wald, Germany, 1987, **ex-type**.

These observations are very close to HELFER's (1991) original description, who would have classified the species in sect. *Albo-erecta* of *Verticillium* if he had not seen 'dictyochlamydospores'. Therefore we compared this species with *Pochonia* species. Its monomorphic conidia are much longer than those of *P. bulbillosa*. Its abundant catenate chlamydospores differ from the dictyochlamydospores of *Pochonia*. The ITS sequences of this species were so different from *Pochonia* species that they could not be aligned and analysed together. According to LSU + SSU sequences of rDNA, the species is classified in the Nectriaceae (SUNG *et al*. 2001), thus *Verticillium* sect. *Albo-erecta*.

Verticillium epiphytum Hansford, Proc. Linn. Soc. Lond. 155: 41, 1943.
Figs 5-16, 5-17

?= *Cephalosporium curtipes* var. *uredinicola* Sukap. & Thirum., Bull. Torrey Bot. Club 93: 307, 1966.

Colonies rather fast-growing, reaching 15–30 mm diam. in 10 days, thin, white, reverse yellow to yellowish cream, with brownish tinges. Most strains producing a yellow pigment diffusing into the agar. Hyphae hyaline, septate, 1.0–1.5 µm wide. Phialides relatively long, 30–60 × 1.0–2.5 µm, usually solitary, arising from prostrate hyphae, forming globose to ellipsoidal conidial heads. Conidia falcate with blunt ends, always 1-celled, measuring 3.5–10.5 × 1–2 µm. Microconidia absent. Chlamydospores mostly present (though scanty),

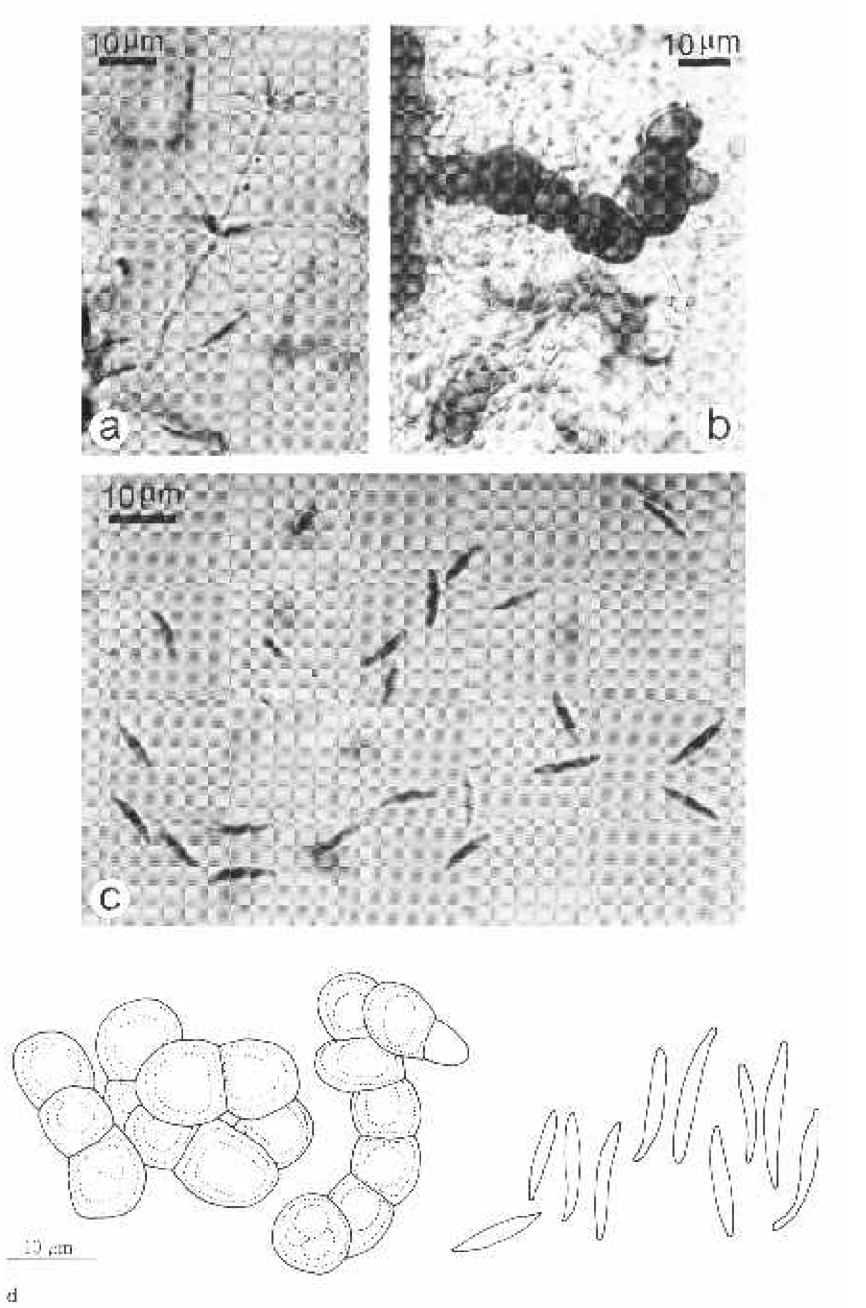


Fig. 5-15. *Verticillium incurvum*. a. Conidiophore, b. chlamydospores, c. conidia, DIC, b, c. stained in cotton blue. d. chlamydospores and conidia. a–d. CBS 460.88.

terminal, generally 1–celled, globose to subglobose to reniform or slightly constricted in the middle, sometimes slightly curved and appearing 1-septate with a small second cell, hyaline, thick-walled with a rough chromophilic surface, measuring 4–12 × 3.5–11.5 µm. Crystals present or absent. Temperature optimum: 24(–27)°C (16 mm diam., CBS 384.81, or 26 mm diam., CBS 154.61). No growth at 33°C.

STRAINS EXAMINED:
CBS 154.61 = IMI 090246 = ATCC 14494 = HACC 105, isolated by M.J. Thirumalachar from India from uredinia of a rust in 1961, ex-type of *Cephalosporium curtipes* var. *uredinicola*.
IMI 071315 = CBS 384.81 = ATCC 22593, isolated by E.F. Vestal from the coffee rust, *Hemileia vastatrix*, in Thailand in 1957, the only authentic strain of *V. epiphytum*.
IMI 286186 = CBS 101285, ex *Phakopsora pachyrhizi*, Thailand, 1984.
CBS 650.85 = ATCC 16541 = HACC 188, India, 1985, deposited as *Cephalosporium pimprina* Thirum. ined., producer of antiamoebin.
IMI 338015, ex *Hemileia vastatrix*, Uganda, 1990, C. Prior.
IMI 346243 = CBS 101296, ex rust pustules, India, 1991, D.H. Smith.

HERBARIUM MATERIAL:
Type material of *V. epiphytum*, numbered 771, collected by Hansford in Kampala, Uganda, on *Helminthosporium triumfettae* on *Triumfetta* sp., 1926 (K). A specimen numbered 1187, on *Cladosporium herbarum*, collected by Hansford on *Lantana trifolia* in 1930, Kampala, Uganda (K).

Conidia of the type specimen were slightly shrunken and smaller than in CBS 384.81. The specimen is not in good condition and we could not find any chlamydospores. On HANSFORD's specimen 1187 from Uganda it was almost impossible to find the fungus.

Cephalosporium curtipes var. *uredinicola* could not clearly be separated from *V. epiphytum* using morphological and molecular (sequences of ribosomal RNA genes and spacers) features. Although *Lecanicillium psalliotae* has also been isolated from rust fungi, it seems that most foliicolous verticillium-like mycoparasites from tropical countries belong to this taxon.

SUKAPURE & THIRUMALACHAR (1966) did not mention the thick-walled chlamydospores in *Cephalosporium curtipes* var. *uredinicola*; however, the ex-type of this taxon does produce them. The strains showed some variation: IMI 346243 has larger conidia than the average. Strains IMI 346243 and IMI 338015 did not produce any chlamydospores, and no crystals were observed in CBS 650.85 and IMI 338015.

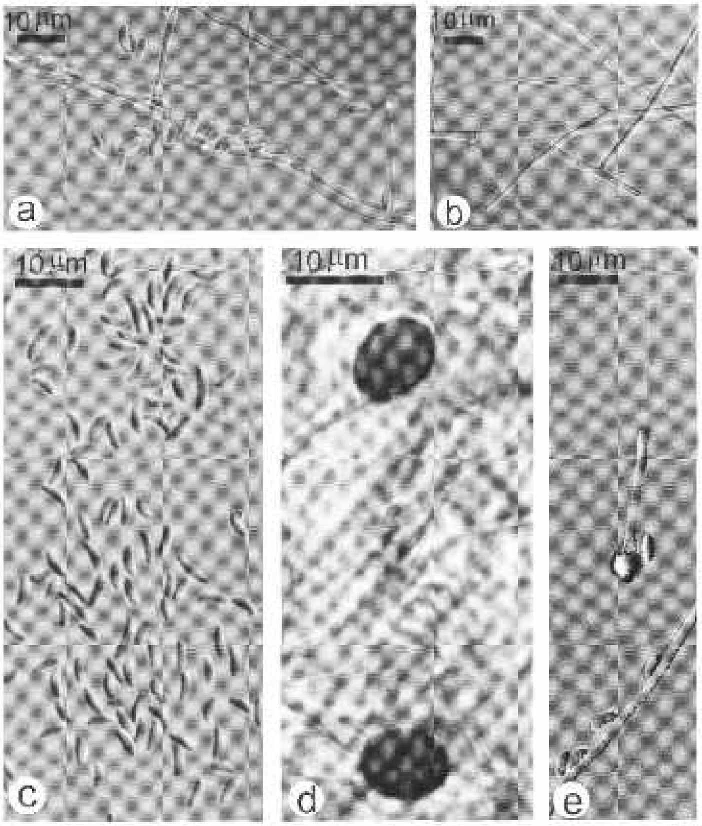


Fig. 5-16. *Verticillium epiphytum*. a, c. Conidia, b. phialides, d, e. chlamydospores. a, b, e. CBS 384.81; c, d. CBS 650.85, a–e. DIC.

GAMS (1971) placed this species under *Verticillium psalliotae*. *Verticillium epiphytum* is, however, distinct in its blunt-ended conidia held in globose heads, wider hyphae and phialides, longer phialides, presence of terminal chlamydospores and the lack of red pigment diffusing into the agar. The same features differentiate this fungus from all other previously described species.

Verticillium epiphytum appeared close to *Pochonia* in a phylogeny inferred from ITS sequences (see part 1). According to sequences of LSU + SSU (see part 2), it is sufficiently distinct from *Pochonia* and not monophyletic with it. Because we have not seen much material and the phylogenetic position is so far poorly resolved, we refrain from a formal classification. This may be one of the many verticillium-like taxa that are very difficult to distinguish generically and can be left, for the time being, in an informal aggregate of *Verticillium*.

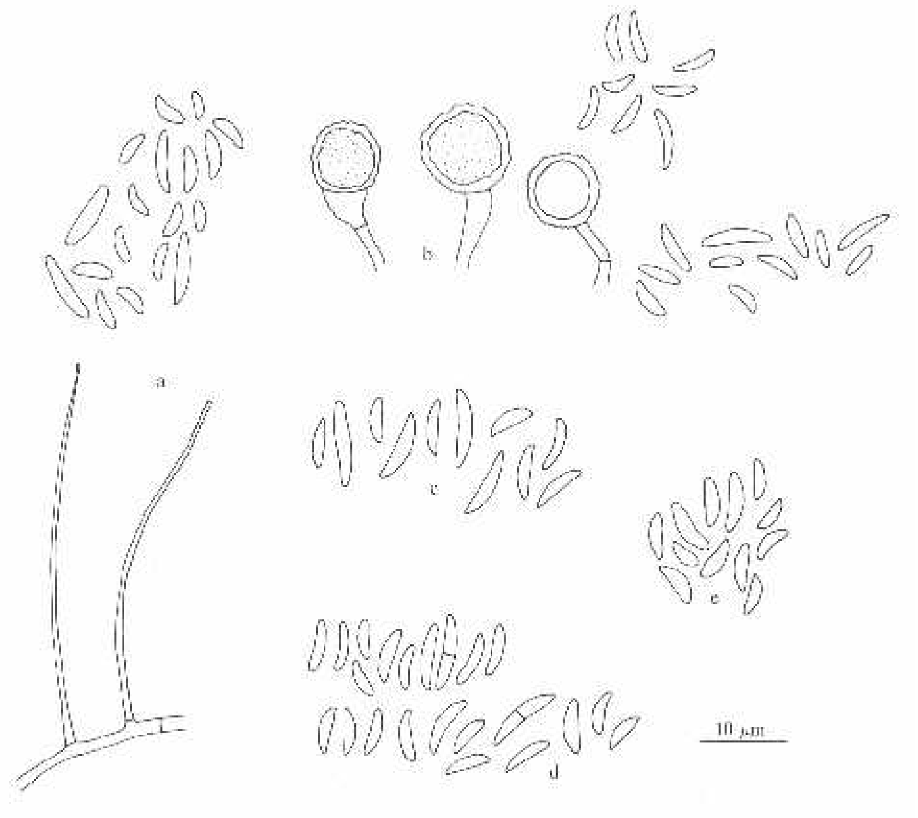


Fig. 5-17. *Verticillium epiphytum*. a. Phialides and conidia of CBS 154.61 on PCA, b. chlamydospores and conidia of CBS 650.85 on PCA, c–e. conidia of CBS 101296, CBS 384.81 and IMI 338015 on various media.

Verticillium pseudohemipterigenum H.C. Evans & Y. Jun, Mycol. Res. 101: 1245, 1997.

Figs 5-18, 5-19

Colonies reaching 25 mm diam. in 10 days, compact, white, with cream to brownish cream reverse, without discoloration of the agar. Phialides almost exclusively in whorls of 4–8(–10) on erect conidiophores, hyaline, smooth-walled, (8–)10–16(–20) × 2–5 µm, often inflated at the base, tapering to a needle-like, less than 0.3 µm wide, 4–10 µm long neck. Solitary phialides awl-shaped scanty, up to 25 µm in length, tapering gradually from 1–1.5 to 0.3–0.5 µm. Conidia produced singly, hyaline, aseptate, smooth, narrowly fusiform to ellipsoidal, often in the shape of an orange segment (3.5–)4.5–6.5 × 1.5–2 µm. Teleomorph unknown. Temperature optimum: 24–27°C (24–26 mm diam.). No growth at 33°C (but 2 days at this temperature are survived).

STRAINS EXAMINED:
IMI 331563 = I 96–1013 = CBS 102069, isolated by C. Prior from *Coccus viridis* (coffee green scale) in Trinidad, 1985, **ex-type.**
I 96-1014 = CBS 102070, ex insect, Surinam, 1996. H.C. Evans.

This taxon is not closely related to any other taxa in this group. Its position could not be resolved using morphology and sequences of ITS regions and 5.8S gene. Therefore, the species is retained in its original genus for the time being. It resembles *Verticillium hemipterigenum* Petch (PETCH 1932), especially in the shape and arrangement of phialides and solitary production of conidia, but it is distinguished by its asymmetrically falcate conidia. The two species were studied in detail by HYWEL-JONES *et al*. (1997). *Verticillium hemipterigenum* has not been studied with molecular methods.

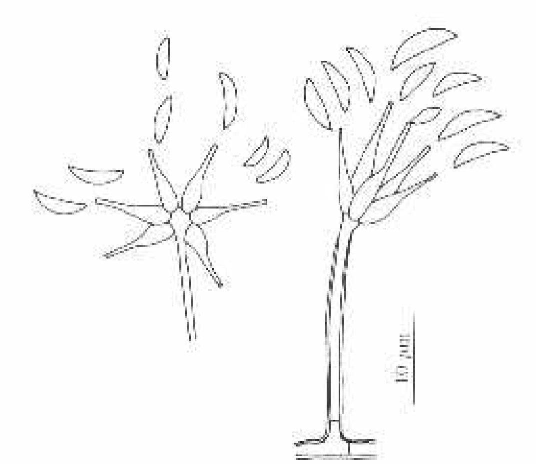


Fig. 5-18. *Verticillium pseudohemipterigenum*. Conidiophores and conidia, IMI 331563, 8 days on PCA.

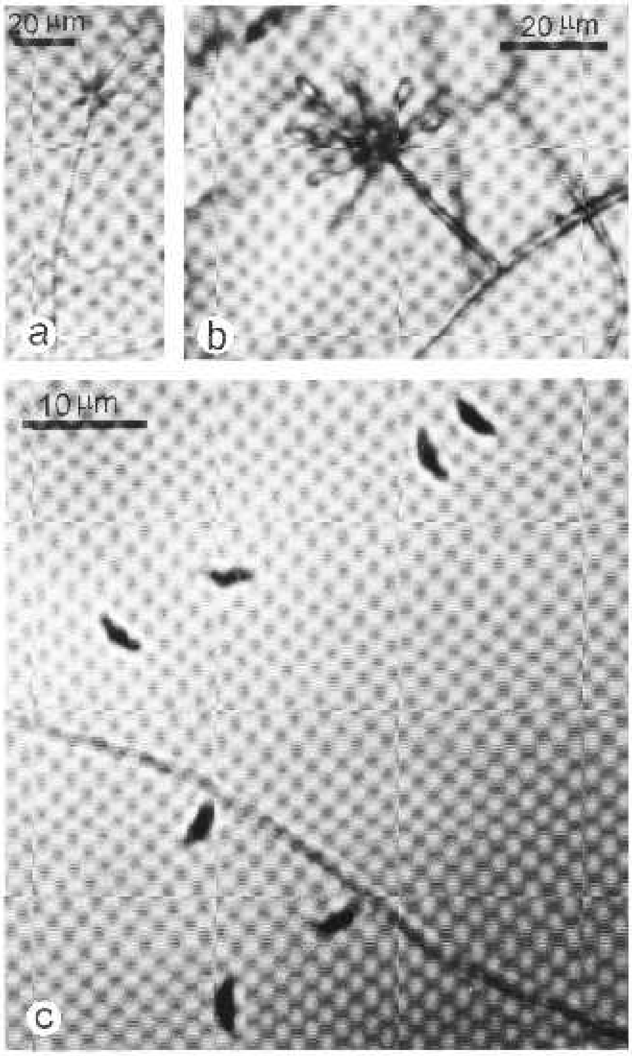


Fig. 5-19. *Verticillium pseudohemipterigenum*. a, b. Conidiophores, c. conidia in cotton blue. IMI 331563, DIC.

PART SIX:
THE GENUS *HAPTOCILLIUM*

Cluster C in Fig. 1-1 is particularly distinct and monophyletic. It was named *Haptocillium* W. Gams & Zare and typified by *H. balanoides* (Drechsler) Zare & W. Gams (GAMS & ZARE 2001 – part 3). The generic distinction of *Haptocillium* is supported by 97% Jackknife value as a monophyletic group based on parsimony analysis of ribosomal RNA sequences (see parts 1 & 2). It is regarded as belonging to the *Cordyceps ophioglossoides* clade (SUNG *et al*. 2001 – part 2). A cladogram (Fig. 6-1), based on sequences of the ITS regions and the 5.8S gene of the nuclear ribosomal DNA, shows the relationships of the seven recognized species.

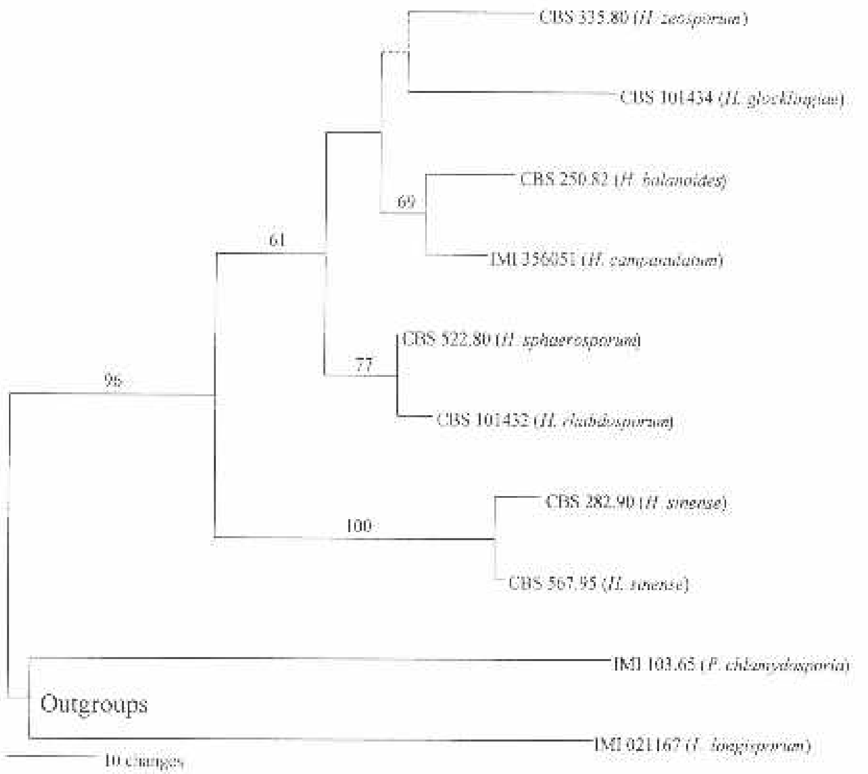


Fig. 6-1. One of three equally parsimonious trees recovered using sequences of ITS regions and the 5.8S gene. Dashed lines indicate the branches that collapsed in the strict consensus tree. Figures above branches indicate the Jackknife support values. CI = 0.82, HI = 0.17, RI = 0.62, RC = 0.51, tree length = 251. *H.* = *Haptocillium*, *L.* = *Lecanicillium*, *P.* = *Pochonia*. The position of *H. rhabdosporum* could be ascertained only from its ITS-2 sequence.

The adhesive nematophagous conidia are similar to the more differentiated adhesive conidia of *Drechmeria* W. Gams & H.-B. Jansson (GAMS & JANSSON 1985, GAMS 2003), which are produced on mostly intercalary phialides. That this fungus is a close relative of *Haptocillium* was shown by GERNANDT *et al.* (1997).

To resolve controversies around the species concept, molecular methods were invoked. The results clearly indicate the distinctness of several taxa that are now included in *Haptocillium*. The exact number of species to be distinguished is not yet settled. Our treatment takes a rather conservative approach in recognizing seven species so far, and more may turn out to be distinct in the future.

A systematic representation of RFLP patterns is shown in Table 6-I. A reconstruction of the phylogeny of the species distinguished here, based on ITS sequences, is given in Fig. 6-1.

Table 6-I. RFLP fragment sizes and patterns of the ITS region and mtDNA of *Haptocillium* species. Symbols for different patterns in the second column: ITS patterns = Capital letters, mtDNA = Arabic figures.

Taxon	Pattern	RFLPs of the ITS region			RFLPs of mtDNA
		Msp I	*Hinf* I	*Hae* III	*Hae* III
H. balanoides CBS 250.82	A,1	260,220,140	250,190,100	360,180,100	>12, 9, 8, 4.4, 2.4, 1.8, 1.4
H. sphaerosporum CBS 589.92	A',2	250,140,110,70	280,220,100	260,170,160	11.5, 4.1, 4, 3.6, 3, 2.8, 1.9, 1.6
H. sphaerosporum CBS 781.84, CBS 257.83	A',3	250,140,110,70	280,220,100	260,170,160	>12, 8.5, 5.6, 2.9
H. sphaerosporum CBS 552.80* CBS 889.85	A',4	250,140,110,70	280,220,100	260,170,160	5.4, 5.2, 4.5, 3.1, 1.8, 1.6
H. sphaerosporum CBS 787.96	A'',4'	290,150,150	260,240,100	260,170,160	12, 5.1, 4.9, 3.2, 1.8
H. sphaerosporum CBS 614.82	A''',5	250,140,110,70	280,220,100	280,150,150	>12, 8.5, 5.6, 2.8
H. zeosporum CBS 335.80*	B,6	240,180,110,70	380,330,100	420,170	6, 5.5, 5.2, 4.1, 2.6, 2, 1.8
H. campanulatum IMI 356051/ CBS 101230	C,7	270,140,120,80	280,220,100	260,150,150	8.2, 7.2, 5.5, 2.3, 1
H. sinense CBS 101431, CBS 567.95, CBS 131.95	D,8	240,140,120, 90	330,220, 50	260,170,160	5.5, 5, 4.2, 4, 1.9, 1.5
H. sinense CBS 282.95	D,9	240,140,120, 90	330,220, 50	260,170,170	7.6, 5.4, 4.9, 3.2, 1.6
H. glockingiae CBS 101434	E,10	220,140,120,70	290,170, 90,70	290,190,140	>>12, 5.5, 4.4, 4.3, 3.6, 2.4, 2, 1.9
H. rhabdosporum CBS 101432	F,11	300,150,100, 50	340,180, 80	280,170,170	8, 7.5, 5, 4.8, 3.2

* These strains were labelled *H. balanoides* in the cladograms of our papers I-III

Phialides of *Haptocillium* species are verticillate or solitary, slenderly aculeate or flask-shaped with an inflated basal part. The morphology of the phialides can vary with the medium. GAMS (1988) noticed in strain CBS 522.80, which is now classified as *H. sphaerosporum*, a predominance of solitary, swollen phialides on water agar with added nematodes, while verticillate aculeate phialides dominated in the same isolate on oatmeal or cornmeal agars. Similar observations were made for *H. balanoides* by DOWSETT *et al.* (1982). Sympodially proliferating polyphialides have already been described and illustrated by DRECHSLER (1946) for *Acrostalagmus zeosporus*, but we observed them in several other species as well. In certain species like *H. zeosporum* they seem to be more frequent than in others. Conidia are typically acorn-shaped (balanoid) to subglobose, but they can also be more elongate and conoid. Conidia have typically a flattened top that becomes conspicuously thick-walled; this is the adhesive surface with which the conidia are fastened to their prey. Conidia are either monomorphic or dimorphic. In the latter case, the second conidial type is an elongate or otherwise larger form that does not show the apical wall thickening. The distally thickened portion of the wall shows a characteristic vertical striation in transmission electron microscopy (DIJKSTERHUIS 1993, SJOLLEMA *et al*. 1993). This structure is characteristic of the adhesive layer seen in several other fungi that attach themselves to nematodes, e.g. *Drechmeria* (SAIKAWA 1982). The shape and size of conidia and their arrangement on conidiophores are used for specific distinction.

Dictyochlamydospores similar to those seen in *Pochonia* but morphologically simpler were observed by GAMS (1988) in isolates now classified in *H. sphaerosporum* and *H. zeosporum* grown on water agar with nematodes. DOWSETT *et al.* (1982) saw intercalary swollen cells suggestive of chlamydospores in *Verticillium balanoides* on unspecified agar media. None of the strains examined in this study was seen to produce structures similar to dictyochlamydospores (as are mostly produced by the species of *Pochonia*), under the conditions used here, although some of these strains had been shown to produce them in the past on water agar with nematodes (GAMS 1988). None of the species described under this genus is known to produce crystals.

HAPTOCILLIUM W. Gams & Zare, Nova Hedwigia 72: 334, 2001.

Colonies slow-growing, reaching 5-15 mm diam. on PDA after 10 days at 20°C. Conidiophores erect or prostrate, bearing verticillate or solitary phialides, which are usually swollen near the base and taper into a narrow neck in which collarette and periclinal wall thickening are hardly visible. Conidia balanoid, campanulate to cylindrical, subglobose to irregularly angular, mostly with a distal flattened adhesive surface (visible as a wall thickening), produced in heads or short chains or both. Crystals absent. Species known as endoparasites of free-living nematodes, which they penetrate starting from externally adhering conidia.

Type species *H. balanoides* (Drechsler) Zare & W. Gams

Teleomorph unknown.

Etymology: Greek *haptein* = to attach, and *-cillium*, suffix taken from *Verticillium*.

The seven species described in this genus, apart from *H. sinense* and *H. glocklingiae*, are interrelated and not sharply differentiated from each other. RFLP patterns of the ITS region and mtDNA (Table 6-I) do not offer much more information than the ITS sequences (Fig. 6-1). The species are rather easily distinguished from each other morphologically. The commonest and not quite sharply delimited species, *H. sphaerosporum*, has small balanoid conidia, while those of *H. glocklingiae* are similar but larger. Conidia of *H. balanoides* are more triangular and elongate conidia are particularly frequent in this species. *Haptocillium zeosporum* has larger balanoid conidia, which are terminally broader than in the other species. *Haptocillium campanulatum* produces two types of conidia: small conidia are campanulate to cylindrical, produced in heads, and large conidia are subglobose, sometimes produced in chains. *Haptocillium rhabdosporum* produces monomorphic, cylindrical conidia, larger than those of *H. campanulatum*, and has unusually broad vegetative hyphae. *Haptocillium sinense* produces small conidia that are irregularly angular and are arranged in chains and heads.

KEY TO THE SPECIES OF *HAPTOCILLIUM*

1\. Conidia at least partly in chains, sometimes in heads **2**
1´. Conidia always in heads, never in chains **3**

2\. Conidia slightly irregularly angular, small, 1.3–2.0 × 1.0–1.7 µm, in heads and short chains........ 6. *H. sinense*
2´. Conidia of two types; campanulate to cylindrical, 2.7–3.0 × 1.3–1.5 µm, in heads, and globose to subglobose, 4.2–4.5 × 3–3.5 µm, often catenate .... 5. *H. campanulatum*
[2´´. Conidia subglobose to nut-shaped, 1.6-1.9 x 1.4-1.5 µm, mostly catenate........ '*V.*' *seriatum* Glockling 1997]

3\. Conidia cylindrical **4**
3´. Conidia balanoid to elongate balanoid **5**
[3´´. Conidia globose with mostly 5 apical adhesive buds, 3.5-5.0 x 2.2-2.5 µm........ '*V.*' *coronatum* G.L. Barron 1989]

4\. Conidia cylindrical to campanulate, 2.7–3 × 1.3–1.5 µm.....see 5. *H. campanulatum*
4´. Conidia cylindrical, 3.8–4.0 × 1.3–1.5 µm........ 7. *H. rhabdosporum*

5\. Conidia triangular to elongate-balanoid, measuring 3.0–4.0 × 1.7–2.0 µm; elongate conidia absent, balanoid conidia with inconspicuous terminal wall thickening........ 4. *H. zeosporum*
5´. Balanoid conidia (2.2–3.5 × 1.3–3.0 µm) mostly with pronounced terminal wall-thickening, elongate conidia (5.5–6.5 × 2.0–2.2 µm) scarce or frequent........ **6**

6\. Balanoid to triangular conidia mostly 2.5–3.0 × 1.5–2.0 µm; elongate conidia frequent; phialides slender, aculeate on OA; polyphialides absent... 1. *H. balanoides*
6´. Balanoid to subglobose conidia 2.2–3.2 × 1.3–2.3 µm; elongate conidia scarce (if present); phialides slender or swollen on OA; polyphialides usually absent .... 2. *H. sphaerosporum*
6´´. Balanoid conidia 3.2–3.6 × 2.5–3.0 µm, elongate conidia rather frequent, 5.7–6.5 × 2.0–2.2 µm; polyphialides present........ 3. *H. glocklingiae*

1. ***Haptocillium balanoides*** (Drechsler) Zare & W. Gams, Nova Hedwigia 72: 335, 2001.

Fig. 6-2

≡ *Cephalosporium balanoides* Drechsler, Phytopathology 31: 786, 1941 (basionym).
≡ *Acremonium balanoides* (Drechsler) Subramanian, Kavaka 5: 98, 1978 ('1977').
≡ *Verticillium balanoides* (Drechsler) Dowsett, J. Reid & Hopkin, Mycologia 74: 690, 1982.
≡ *Tolypocladium balanoides* (Drechsler) Bissett, Canad. J. Bot. 61: 1313, 1983.
= ? *Verticillium sphaerosporum* var. *bisporum* T. Watanabe, Ann. Phytopathol. Soc. Japan 46: 600, 1980.

Colonies thin, cream to whitish-cream, reverse uncoloured, slow-growing, reaching 6–15 mm diam. in 10 days on PDA. Vegetative hyphae normally 1.3–2.5 µm, sometimes up to 4–5.6 µm wide, staining purple in lactic acid-cotton blue. Phialides usually slender, aculeate and diverging, on PCA sometimes more inflated, 14–18 × 1.5–2.0 µm in the lower part, gradually tapering to about 1 µm; inflated phialides up to 3 µm wide in the lower part. Conidia almost triangular with thin or slightly thickened apical wall, 2.0–3.0 × 1.5–1.8 µm, elongate conidia rather frequent, 5.0–7.5 × 1.5–2.0 µm, lacking an apical wall thickening. Dictyochlamydospores not observed, but chlamydospore-like thickened cells reported by DOWSETT *et al*. (1982). Temperature optimum: 21°C (6 mm diam.). No growth at 27°C (see also ZARE & GAMS 2003 a).

STRAIN EXAMINED:
CBS 250.82, ex nematode in soil, Winnipeg, 1982, J. Reid, **ex-neotype**, designated by GAMS (1988).

Haptocillium balanoides differs from *H. sphaerosporum* by somewhat more triangular short and rather frequent elongate conidia. Phialides are mostly rather slender and diverge broadly. In the ITS sequence the species is placed together with *H. campanulatum* on a branch different from *H. sphaerosporum*. The RFLP patterns of *H. balanoides* differ considerably from those of *H. sphaerosporum*.

The feature of dimorphic conidia that gave rise to the variety *bisporum* T. Watanabe occurs in several taxa distinguished here and does not seem specific for any of them, but it is particularly frequent in the ex-neotype of *H. balanoides*. Unfortunately, no type material could be obtained from the author and the identity of the variety remains uncertain.

Most of the ecological data to be compiled under this name (see also GAMS 1988, ZARE & GAMS 2003 a) may equally apply to *H. sphaerosporum*, because the identity of the material studied by various authors cannot be assessed with certainty. The species has also been reported as a parasite of *Ditylenchus dipsaci* in white clover in New Zealand (HAY 1995). Contrasting with *Hirsutella rhossiliensis* Minter & Brady, whose conidia are infectious only while attached to a conidiophore (McINNIS & JAFFEE 1989), the conidia of *Haptocillium* species are equally infectious after liberation and bind to a rather wide range of nematode species. Consequently, *H. balanoides*, when applied as a suspension of conidia and hyphal fragments, had a much greater effect than *H. rhossiliensis* in controlling *D. dipsaci* and promoting growth of clover, both under gnotobiotic conditions (HAY & REGNAULT 1995) and in pot cultures (HAY & BATESON 1997). According to these authors, the fungus has a low saprophytic ability and does not survive in the soil for prolonged periods without added nematodes. SJOLLEMA *et al.* (1993) studied the penetration of hyphae from germinating conidia into the nematode body using transmission electron-microscopy. ATKINSON & DÜRSCHNER-PELZ (1995) inoculated the fungus onto several species of nematodes: *Ditylenchus dipsaci*, *Globodera rostochiensis*, and *Panagrellus redivivus*. Conidia adhered to all species; some of them were removed while the animals moved through a layer of wet sand. Colonized individuals produced different quantities of conidia, viz. appr. 16,000, 11,700, and 840 for the above species, in the order mentioned. WATANABE (2000) found the species (or possibly *H. zeosporum*, but no polyphialides were mentioned) on dead needles of *Pinus densiflora* in Tsukuba, Japan.

2. ***Haptocillium sphaerosporum*** (J.B. Goodey) Zare & W. Gams, Nova Hedwigia 73: 278, 2001. Figs 6-3, 6-4

≡ *Verticillium sphaerosporum* J.B. Goodey, Trans. Br. Mycol. Soc. 34: 272, 1951 (basionym).
= ? *Acrostalagmus obovatus* Drechsler, Phytopathology 31: p. 784, 1941.
≡ *Verticillium obovatum* (Drechsler) Subramanian, Kavaka 5: 98, 1978 ('1977').

Colonies thin, cream to whitish-cream, reverse brown to pale brown, slow-growing, reaching 6–15 mm diam. in 10 days on PDA. Vegetative hyphae 1.3–2.5 µm wide, often swollen up to 5 µm, thick-walled and somewhat roughened. Conidiophores more or less erect, bearing phialides singly or 2–4 in whorls. Phialides on water agar with nematodes mostly solitary with a strongly swollen base, on richer agar media more regularly subulate and more densely verticillate, sometimes sympodially proliferating (polyphialides), measuring 7–19 × 1.7–3.2 µm. Conidia acorn-shaped (balanoid), at least partly truncate and thick-walled at the tip, measuring 2.2–3.2 × 1.3–2.3 µm. Elongate conidia rarely present, 6.0–7.5 × 2.2–2.5 µm. Dictyochlamydospores recently not observed, but described for several isolates by GAMS (1988) in cultures on water agar with *Panagrellus redivivus*. Temperature optimum: 18°C in CBS 787.96, otherwise 21–24°C (5–9 mm diam.). No growth at 30°C, except that CBS 381.84 grew moderately and CBS 257.83 grew minimally. No isolate grew at 33°C.

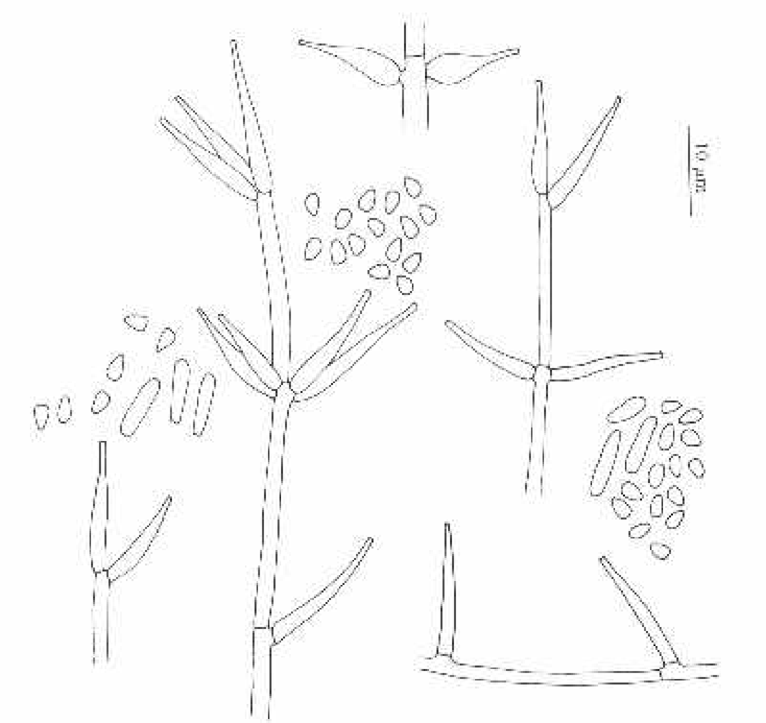


Fig. 6-2. *Haptocillium balanoides*. CBS 250.82, Conidiophores, balanoid and elongate conidia. Slender phialides drawn from colonies grown on OA and PCA, two swollen phialides from colony on PCA.

STRAINS EXAMINED:
CBS 522.80, ex *Ditylenchus triformis*, Germany, 1980, U. Dürschner, **epitype**, designated in Nova Hedwigia 73: 279, 2001 (herb. CBS).
CBS 614.82, ex nematodes, Sweden, 1982, H.B. Jansson.
CBS 257.83, ex nematodes, Germany, 1983, A.-R. Fritsch.
CBS 381.84, ex nematodes, California, 1984, R. Mankau.
CBS 889.85, ex *Panagrellus redivivus*, Germany, 1985, M. Hashem.
CBS 589.92, ex nematodes, Netherlands, 1992, H. Velvis.
CBS 787.96, ex soil, Russia, 1996, R. Bergero & A. M. Luppi-Musca.

The seven strains fall into three subgroups according to their ITS RFLP patterns, among which five mtDNA RFLP patterns. Each of the remaining species had unique ITS and mtDNA RFLP patterns (Table 6-I).

GAMS (1988) synonymized *V. sphaerosporum* with *V. balanoides*. When comparing DRECHSLER's (1941) description with that given by GOODAY (1951) for *V. sphaerosporum* and after examination of GOODAY's type material, this synonymy seemed quite obvious. The neotypification of *H. balanoides* with the isolate preserved as CBS 250.82 by GAMS (1988), however, necessitates a redisposition, because that isolate clearly differs from all others available.

The synonyms listed above might equally refer to *H. sphaerosporum* or *H. balanoides*. *Acrostalagmus obovatus* seems to be a fungus with predominantly more elongate conidia and inconspicuous apical wall thickening, as exemplified by CBS 381.84. Strains CBS 614.82 and CBS 257.83 formed some broad hyphae (4–5.6 µm wide), staining purple in lactic acid-cotton blue. To evaluate the importance of these differences, more numerous isolates will have to be compared with sequences of additional DNA portions.

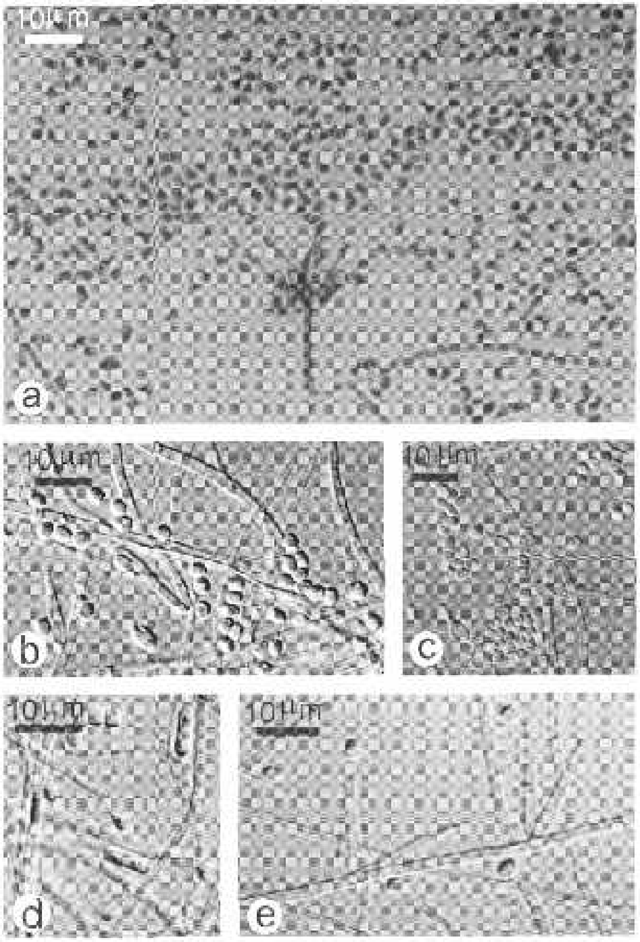


Fig. 6-3. *Haptocillium sphaerosporum.* a–e. Balanoid conidia, phialides and conidiophores, d. elongate conidia. a. CBS 522.80; b, c. CBS 614.82; d, e. CBS 381.84; a. in cotton blue, a–e. DIC.

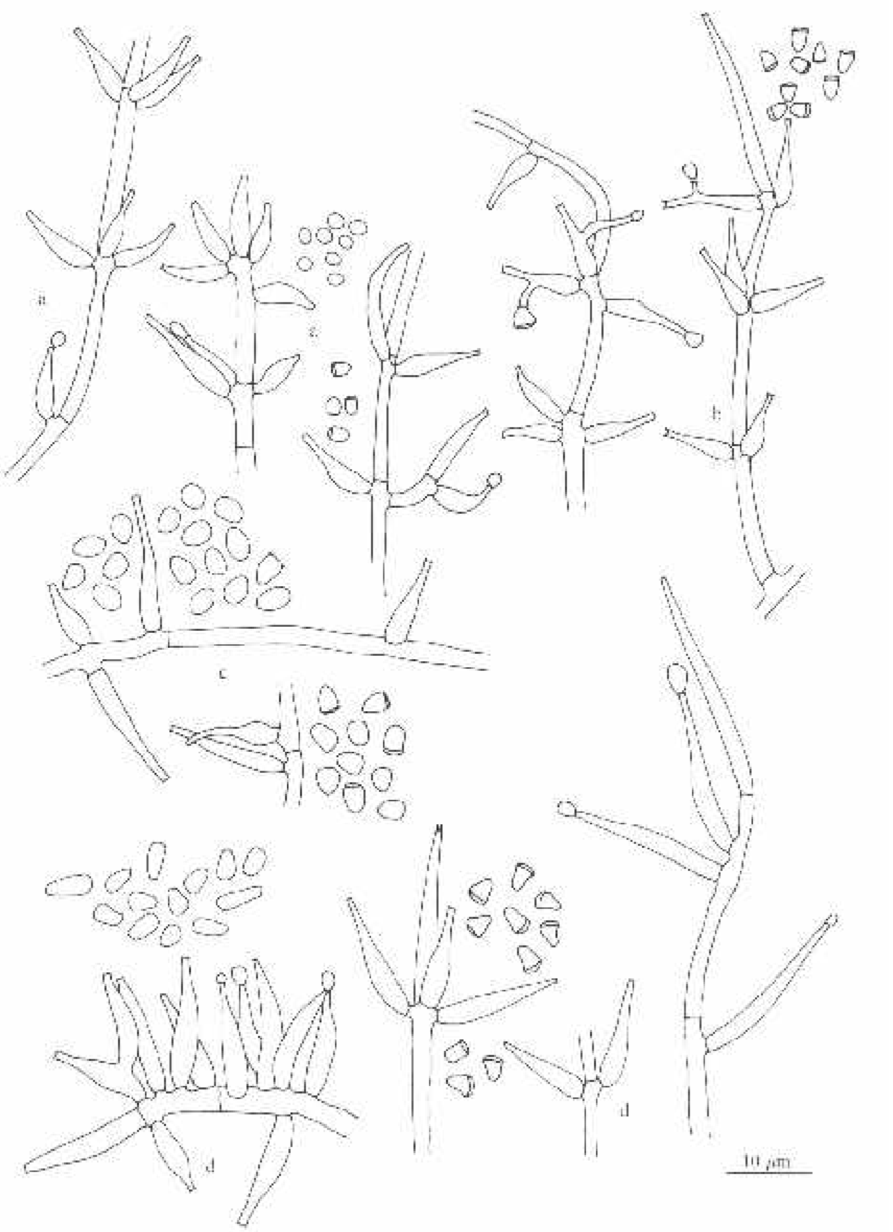


Fig. 6-4. *Haptocillium sphaerosporum.* a. From type material, b. CBS 889.85, drawn from nematode material on water agar, c. CBS 381.84, from cultures on MEA and OA, d. CBS 589.92, dense conidiophores on OA, less dense on PCA.

3. ***Haptocillium glocklingiae*** Zare & W. Gams, Nova Hedwigia 73: 281, 2001.

Figs 6-5, 6-6

Colonies thin, cream-coloured, reverse cream-coloured, without discoloration of the agar, reaching 8 mm diam. in 10 days on PDA. Vegetative hyphae 1.5–2.5(–3.0) µm wide. Phialides rather long, 17–21 × 2.0–2.5 µm, slightly swollen at the base and tapering gradually to the apex, frequently producing dichotomous ends and sometimes proliferating sympodially and producing another conidiogenous opening at the end (polyphialides). Conidia of two types, balanoid and elongate. Balanoid conidia measuring 3.2–3.5 × 2.5–3.0 µm, with upper surface visibly thick-walled, often adhering in round, rosette-like aggregates; elongate conidia 5.7–6.5 × 2.0–2.2 µm. Dictyochlamydospores not observed. Temperature optimum: 21–24°C (4 mm diam.). No growth at 30°C.

STRAIN EXAMINED:
CBS 101434, ex nematode in rabbit dung, Japan, 1996, S. Glockling, **ex-type** (Type: in herb. CBS).

The strain studied here of *Haptocillium glocklingiae* was mentioned and illustrated by GLOCKLING & YAMADA (1997) as *V. balanoides*. The species is close to *H. balanoides* in the shape of balanoid conidia, but has larger balanoid conidia which characteristically adhere to each other in a kind of rosette. The presence of elongate conidia as well as frequent polyphialides also distinguishes this species.

This species is distinct from other *Haptocillium* species in its ITS sequences and RFLPs (Fig. 6-1, Table 6-I).

4. ***Haptocillium zeosporum*** (Drechsler) Zare & W. Gams, Nova Hedwigia 73: 282, 2001.

Figs 6-7, 6-8

≡ *Acrostalagmus zeosporus* Drechsler, Phytopathology 36: 216, 1945 (basionym).
≡ *Verticillium zeosporum* (Drechsler) Glockling, Nordic J. Bot. 17: 658, 1997.

Colonies thin, white, with brown reverse, without discoloration of the agar, very slow-growing, reaching 5 mm diam. in 10 days on PDA. Vegetative hyphae 1.5–2.5(–3.0) µm wide. Phialides solitary or rarely up to 2–3 at each node. Phialides measuring 6.5–7.0 × 2.3–2.8 µm, constricted at the base and swollen immediately above, becoming very narrow towards the apex, producing small numbers of conidia in small heads. Conidia acorn-shaped to triangular, measuring 3.0–4.0 × 1.7–2.0 µm at the broadest part. Dictyochlamydospores not observed, but seen by GAMS (1988). Temperature optimum: 21°C (4–5 mm diam.). No growth at 30°C.

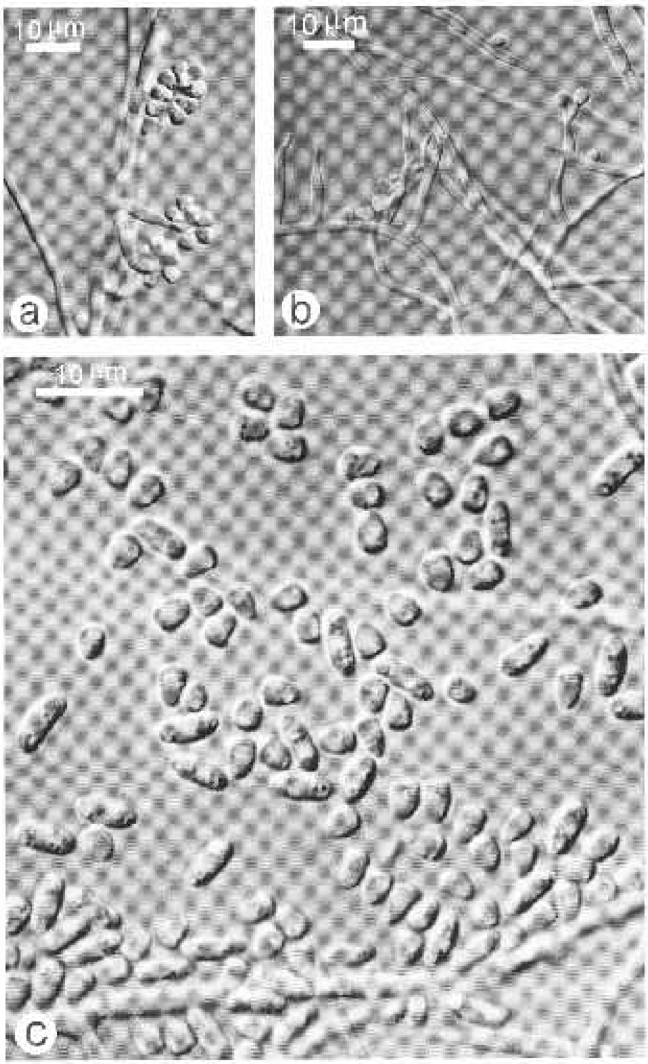


Fig. 6-5. *Haptocillium glocklingiae*. a, b. Conidiophores and phialides, c. balanoid and elongate conidia. CBS 101434, DIC.

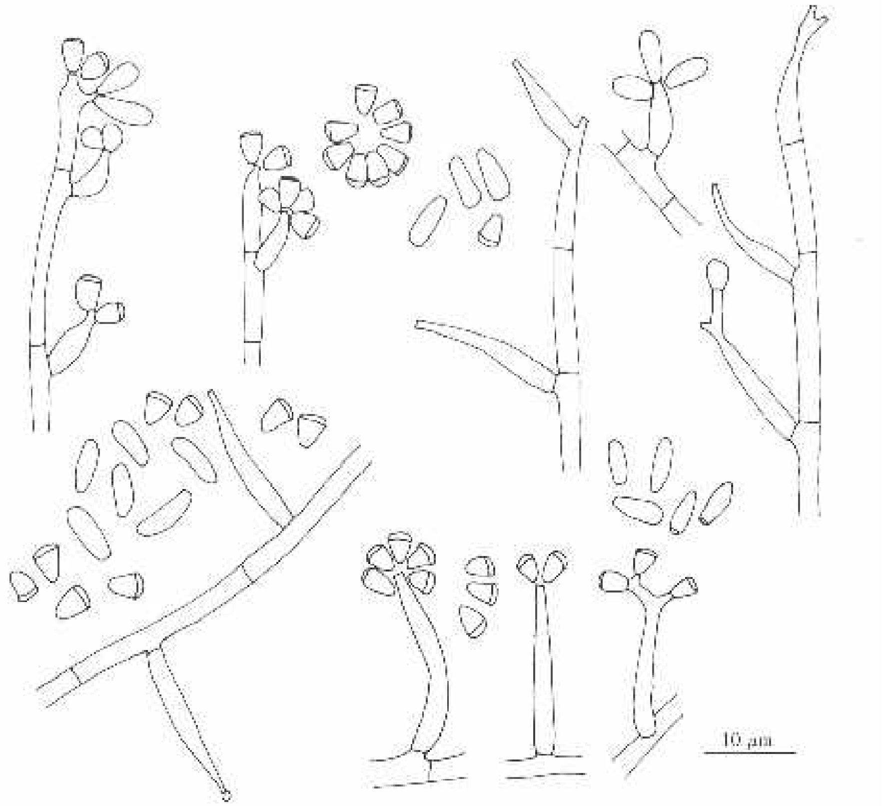


Fig. 6-6. *Haptocillium glocklingiae*. Conidiophores, often with polyphialides, adhesive conidia, often in rosettes, and elongate conidia. CBS 101434, grown on PCA, phialides and conidia bottom left on OA.

STRAIN EXAMINED:
CBS 335.80 = IMI 239515, isolated from nematode in soil by I.S. Damirdagh in Iraq (dried culture as neotype in Herb. CBS, designated in Nova Hedwigia 73: 283, 2001).

Longer and terminally broader conidia distinguish *H. zeosporum* from *H. balanoides*.

5. ***Haptocillium campanulatum*** (Glockling) Zare & W. Gams, Nova Hedwigia 73: 285, 2001. Figs 6-9, 6-10

≡ *Verticillium campanulatum* Glockling, Nordic J. Bot. 17: 655, 1997 (basionym).

Colonies compact, cream-coloured above and in reverse, without discoloration of the agar, reaching 15 mm diam. in 10 days on PDA. Vegetative hyphae 1.3–2.0(–2.5) µm wide. Phialides flask-shaped, produced singly, in pairs or whorls, tapering towards the apex,

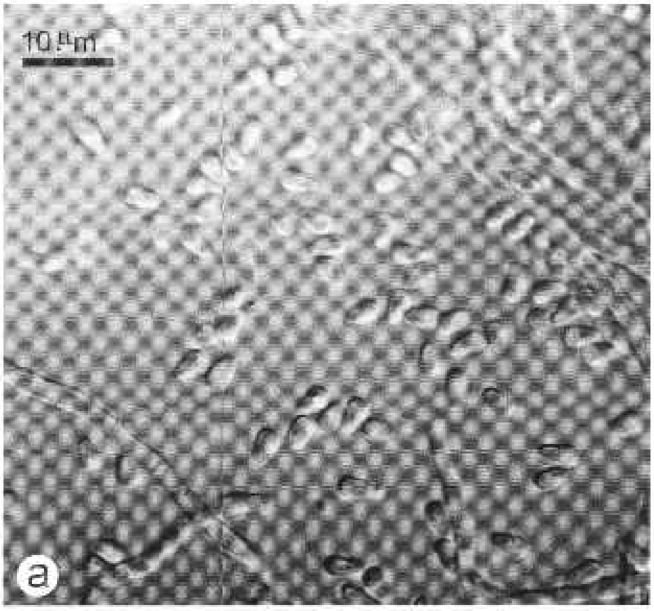


Fig. 6-7. *Haptocillium zeosporum*. Conidia. CBS 335.80, DIC.

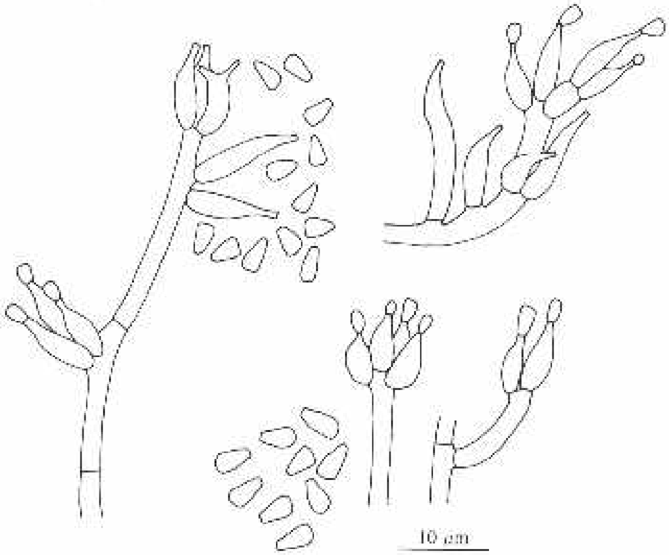


Fig. 6-8. *Haptocillium zeosporum*. Conidiophores and conidia. CBS 335.80, grown on MEA and PCA.

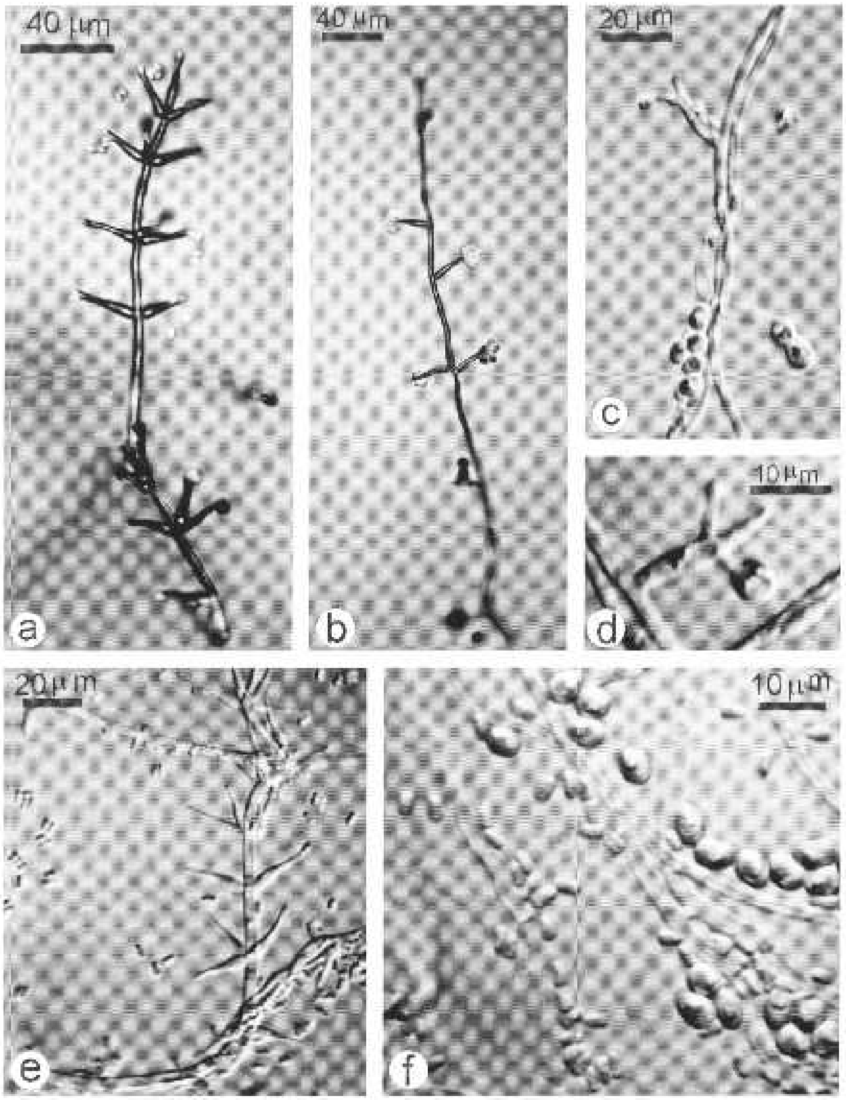


Fig. 6-9. *Haptocillium campanulatum*. a, b. Conidiophores, in b. campanulate and subglobose conidia produced on different phialides, c–e. mono- and polyphialides, f. campanulate and subglobose conidia. a–f. IMI 356051, DIC.

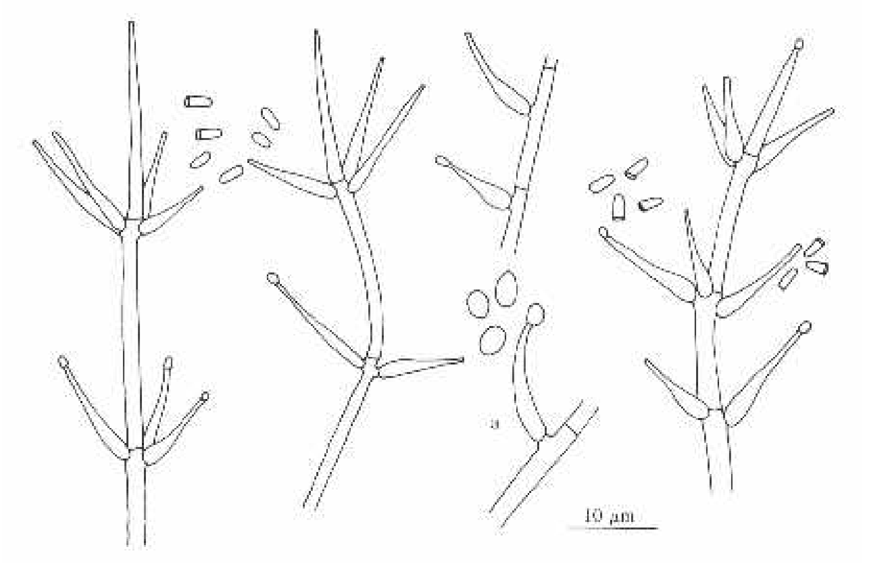


Fig. 6-10. *Haptocillium campanulatum*. Conidiophores and adhesive conidia, in a, some larger ovoid conidia. CBS 101240 (IMI 356051), drawn from PDA culture.

4.0–4.5 × 1.5–2.0 µm. Conidia of two types: smaller conidia, campanulate, cylindrical to obovate, 2.7–3.0 × 1.3–1.5 µm, with adhesive wall thickening at the slightly broader end, produced in heads, and larger conidia, broadly obovate, nearly broadly ellipsoid to subglobose, measuring 4.2–4.5 × 3.0–3.5 µm, produced in heads and chains. Temperature optimum: 21–24°C (7–8 mm diam.). No growth at 33°C.

STRAIN EXAMINED:
IMI 356051 = CBS 101240, ex nematode excretions, England, 1992, S.L. Glockling, **ex-type**.

GLOCKLING & DICK (1997) reported two types of conidia produced on different conidiogenous cells. Production of small campanulate to obovate conidia in combination with large subglobose conidia in chains and in heads is diagnostic for this taxon. The smaller, campanulate to almost cylindrical conidia are close to those described by DRECHSLER (1941) for *Acrostalagmus bactrosporus*, and the delimitation of the present species against DRECHSLER's will remain uncertain until additional strains from nematodes become available.

This species is very close to *H. balanoides* on the basis of ITS sequences, but has unique RFLP patterns (Fig. 6-1, Table 6-I).

The isolate CBS 101433 that GLOCKLING & DICK (1997) identified as *Acrostalagmus bactrosporus*, was identified by ZARE *et al.* (2001) as *Pochonia microbactrospora*. Among similar species with cylindrical conidia, this species has the smallest conidia, 2.0–2.5 × 0.7–1.0 µm; *Haptocillium rhabdosporum* CBS 101432 has the largest conidia among species with cylindrical conidia, measuring 3.8–4.0 × 1.3–1.5 µm. The conidial size of *H. campanulatum* matches that described for *A. bactrosporus* (2–3 × 1.3–1.6 µm) most closely.

6. ***Haptocillium sinense*** (K.G. Zhang, L. Cao & Z.Q. Liang) Zare & W. Gams, Nova Hedwigia 73: 287, 2001. Figs 6-11, 6-12

≡ *Verticillium sinense* K.G. Zhang, L. Cao & Z.Q. Liang, Mycol. Res. 100: 1481, 1996 (basionym).

Colonies whitish, thin, with brown reverse, without discoloration of the agar, slow-growing, reaching 9–12 mm diam. in 10 days on PDA. Vegetative hyphae 1.5–2.0(–2.5) µm wide, often with some swollen parts. Conidiophores erect, hyaline, bearing phialides singly or in whorls of 2–6. Phialides swollen in the lower part, measuring 5–12 × 1.5–2.7 µm, tapering towards the apex to 0.5–0.7 µm. Conidia formed in heads or short chains, 1-celled, hyaline, acorn-shaped to irregularly angular, measuring 1.3–2.0 × 1.0–1.7 µm. Dictyochlamydospores unknown. Temperature optimum (18–) 21–24°C (3–6 mm diam.). No growth at 30°C.

STRAINS EXAMINED:
CBS 567.95 = GAUZ 412, ex nematode near root of *Actinidia deliciosa*, China, 1991, K.Q. Zhang, **ex-type**.
CBS 131.95, ex bark near root of *Actinidia deliciosa*, China, 1991, K.Q. Zhang.
CBS 282.90, ex bacteriovorous nematode from soil, Netherlands, CBS 1990, P.H.F. van den Boogert.
CBS 101431, ex nematode on rotten tubers, Japan, 1995, S. Glockling, originally identified as *V. coccosporum.*

This species is known to destroy nematodes of the species *Meloidogyne javanica* (ZHANG *et al*. 1996). *Haptocillium sinense* is distinct from *H. balanoides* by producing small, irregularly angular conidia both in heads and short chains. Strain CBS 101431 was identified by GLOCKLING & SHIMAZU (1997) as *Verticillium coccosporum* (Drechsler) W. Gams.

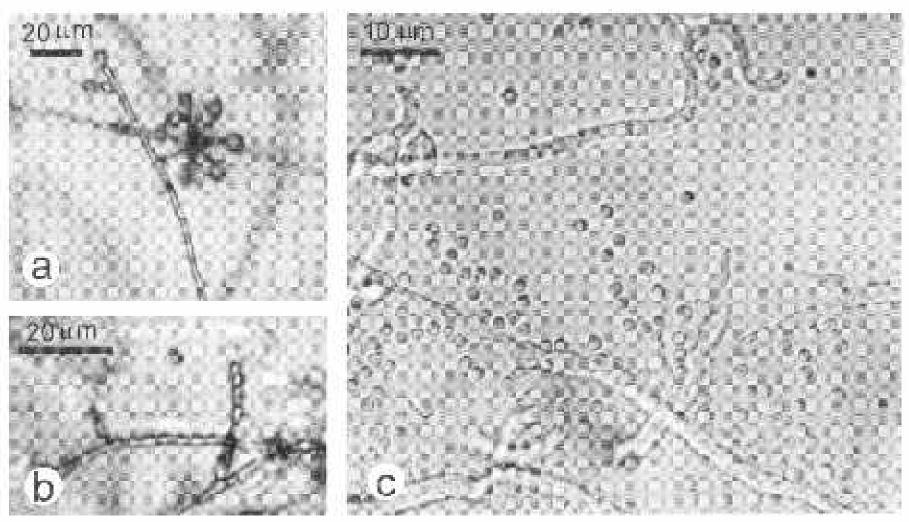


Fig. 6-11. *Haptocillium sinense*. a. Conidial heads, b. conidial chain, c. conidia and phialides. a. CBS 101431; b, c. CBS 282.90; a–c. DIC.

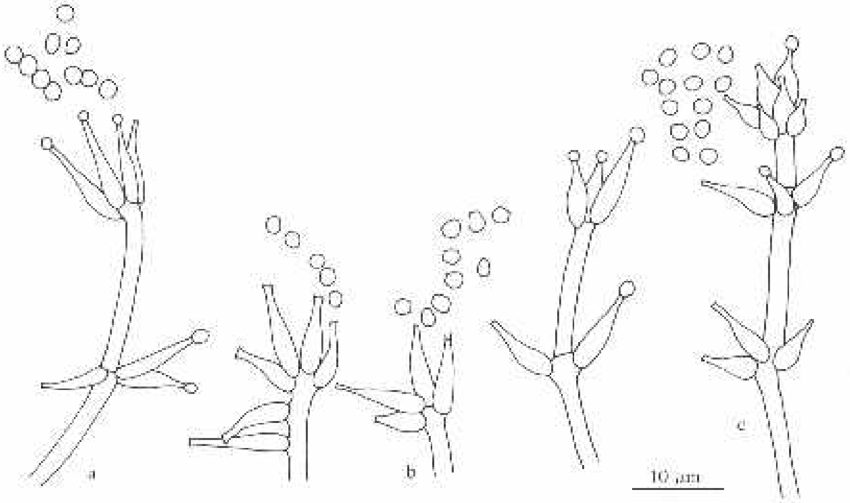


Fig. 6-12. *Haptocillium sinense*. Conidiophores and conidia. a. CBS 282.90, b. CBS 131.95, c. CBS 567.95, from colonies on PCA and OA.

The present taxon seems indeed the most likely candidate for *V. coccosporum*, while the isolates identified as this species by GAMS (1988) had larger conidia and are now identified as *Pochonia suchlasporia* var. *catenata*. We retain here the name *H. sinense* which is properly typified and seems to deviate from the diagnosis of *V. coccosporum* by the somewhat irregular shape of the conidia. *Verticillium coccosporum* was described as having perfectly regular rounded conidia, 1.3–1.7 µm diam., adhering in chains of up to 20 conidia. *Verticillium seriatum* Glockling (in GLOCKLING & DICK 1997) also has long conidial chains, with conidia given as measuring 1.6–1.9 × 1.4–1.5 µm. Unfortunately, information about whether these conidia are adhesive is missing, and the species cannot be identified to genus.

The strains of *H. sinense* studied here had identical ITS- and mtDNA-RFLP patterns, but the Dutch isolate CBS 282.90 had a different mtDNA RFLP (Table 6-I).

7. ***Haptocillium rhabdosporum*** Zare & W. Gams, Nova Hedwigia 73: 288, 2001.

Figs 6-13, 6-14

Colonies whitish, thin, with brown reverse, reaching 8 mm diam. in 10 days on PDA. Vegetative hyphae generally 2.0–3.0 µm wide, in some parts widened to 4.0–4.5 µm, staining purple when mounted in lactic acid-cotton blue, particularly at the septa. Phialides solitary or verticillate up to 3–4 at each node, rather wide, swollen at the base and tapering towards the apex, measuring 11.0–14.5 × 2.0–2.7 µm. Conidia produced in small heads, rod-like with truncate ends, usually one end slightly broader, measuring 3.8–4.0 × 1.3–1.5 µm. Dictyochlamydospores not observed. Temperature optimum: 21–24°C (5–7 mm diam.). No growth at 30°C.

STRAIN EXAMINED:
CBS 101432, isolated by S.L. Glockling from nematodes in cow dung, in England in 1997 (**ex-type**), originally identified as *V.* cf. *bactrosporum*. (Type in herb. CBS).

Morphological features (presence of broad hyphae, cylindrical, truncate conidia with one end broader than the other) characterize this fungus as a distinct species of *Haptocillium*. RFLP patterns of ITS region and mtDNA were unique (Table 6-I). Attempts to sequence the whole ITS region of strain CBS 101432 failed and only the ITS2 region could be sequenced (AF375050). According to this sequence, the species is closest to *H. balanoides* (Fig. 6-1).

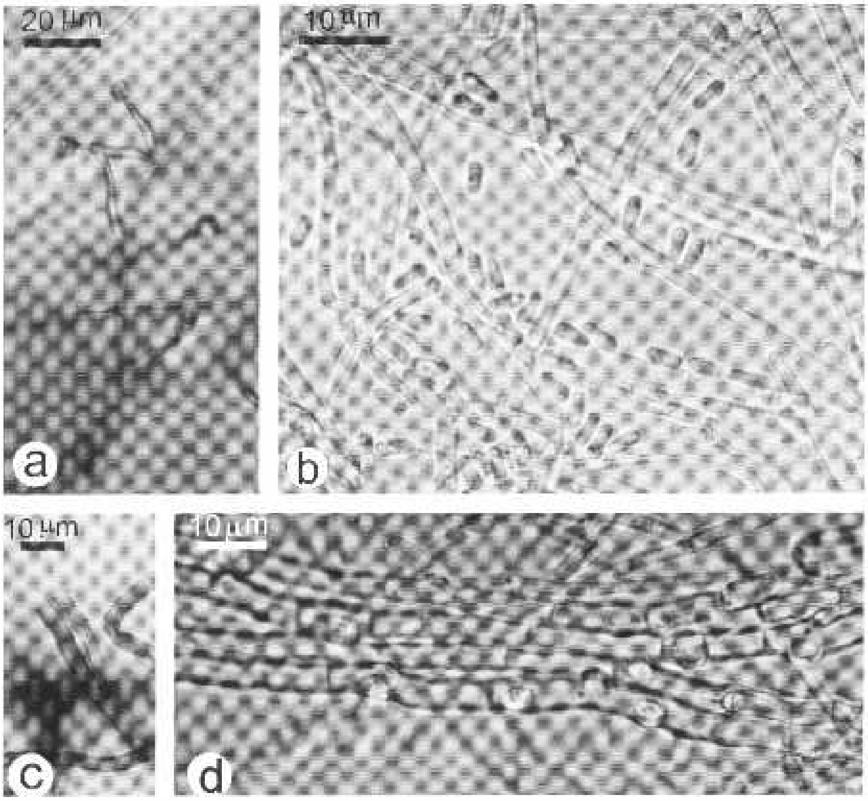


Fig. 6-13. *Haptocillium rhabdosporum*. a. Conidial heads, b. conidia, c, d. mycelium. a–d. CBS 101432, DIC.

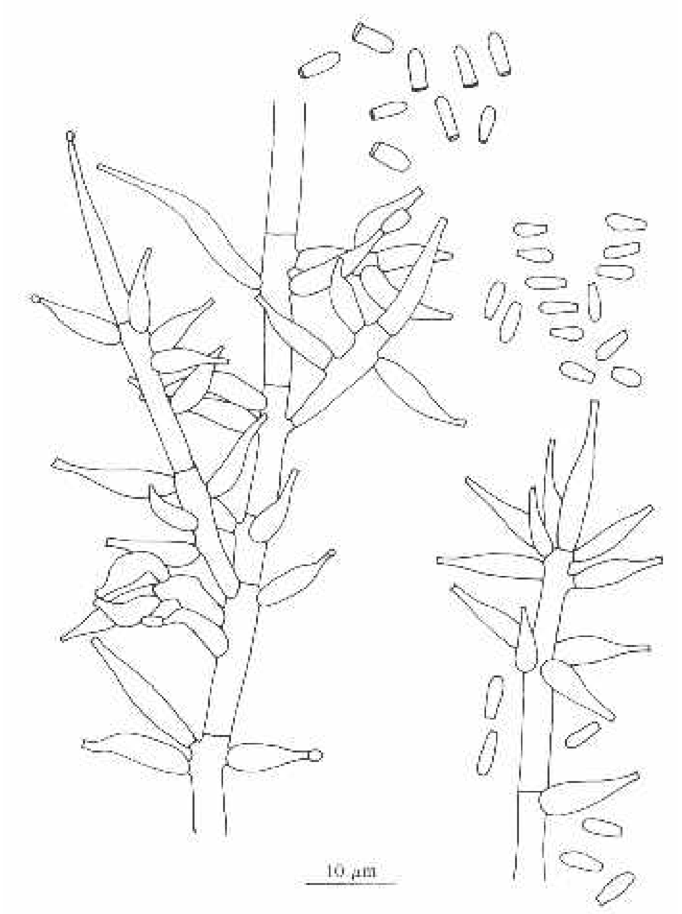


Fig. 6-14. *Haptocillium rhabdosporum*. Conidiophores and conidia, CBS 101432. Drawn from cultures on SEA and PCA.

Acknowledgements

Besides all the mycologists who contributed material by depositing strains in the cultures collections, we are indebted to Dr HARRY C. EVANS (CABI Bioscience) for his valuable contributions about teleomorphs and ecology included here. We have received a number of strains from Dr SALLY GLOCKLING (Newcastle). Dr M.E. NOORDELOOS searched in the Leiden herbarium for OUDEMANS's specimen of *Acrostalagmus aphidum*. Dr DAVID CHANDLER (Horticulture Research International) provided strains of 'Vertalec' (1.72) and 'Mycotal' (19.79). Drs K.A. SEIFERT, J.W. SPATAFORA, D. van der MEI, R.C. SUMMERBELL and Dr A. CULHAM kindly contributed to improving parts of the text. TOM GRÄFENHAN made special efforts to provide an ITS sequence of CBS 101432. Mrs C. van den TWEEL-VERMEULEN inked the drawings and Mrs A. van IPEREN carried out the temperature experiments.

References

ARORA, D.K., HIRSCH, P.R. and KERRY, B.R. 1996. PCR-based molecular discrimination of *Verticillium chlamydosporium* isolates. Mycol. Res. 100: 801–809.

ATKINSON, H.J. and DÜRSCHNER-PELZ, U. 1995. Spore transmission and epidemiology of *Verticillium balanoides*, an endozoic fungal parasite of nematodes in soil. J. Invert. Pathol. 65: 237–242.

BAŁAZY, S. 1973. A review of entomopathogenic species of the genus *Cephalosporium* Corda (Mycota, Hyphomycetes). Bull. Soc. Amis Sci. Lett. Poznan 14: 101–137.

BARBARA, D.J. and CLEWES, E. 2003. Plant pathogenic *Verticillium* species: how many of them are there? Molec. Pl. Path. 4(4): 297–305.

BARRON, G.L. 1973. A new species of *Verticillium* parasitic on rotifers. Mycopathol. Mycologia Appl. 50: 271–274.

BARRON, G.L. 1977. Predators and parasites of microscopic animals. pp. 167–200 *In* Biology of Conidial Fungi. Vol. 2 (eds COLE, G.T. and KENDRICK, B.). Academic Press, New York.

BARRON, G.L. 1980. Fungal parasites of rotifers: two new verticillate endoparasites with aerial conidiophores. Canad. J. Bot. 58: 432–438.

BARRON, G.L. 1985. Fungal parasites of bdelloid rotifers: *Diheterospora*. Canad. J. Bot. 63: 211–222.

BARRON, G.L. 1989. New species and new records of fungi that attack microscopic animals. Canad. J. Bot. 67: 267–271.

BARRON, G.L. 1991. A new genus, *Rotiferophthora*, to accommodate the *Diheterospora*-like endoparasites of rotifers. Canad. J. Bot. 69: 494–502.

BARRON, G.L. and ONIONS, A.H.S. 1966. *Verticillium chlamydosporium* and its relationships to *Diheterospora*, *Stemphyliopsis*, and *Paecilomyces*. Canad. J. Bot. 44: 861–869.

BATISTA, A.C. and FONSECA, O.M. 1965. *Pochonia humicola* n. gen. e n. sp., uma curiosa entidade fungica dos solos do Nordeste do Brasil. Publ. Inst. Micol. Recife 462: 1–11.

BIDOCHKA, M.J., ST.LEGER, R.J., STUART, A. and GOWANLOCK, K. 1999. Nuclear rDNA phylogeny in the fungal genus *Verticillium* and its relationship to insect and plant virulence, extracellular proteases and carbohydrases. Molec. Biol. 145: 955–963.

BLACKWELL, M. and SPATAFORA, J.W. 1994. Molecular data sets and broad taxon sampling in detecting morphological convergence. pp. 243–248. *In* Ascomycete Systematics: Problems and Perspectives in the Nineties (eds

REYNOLDS, D. and HAWKSWORTH, D.L.). Plenum Press, London.

BOOTH, C. 1959. Studies of Pyrenomycetes. Mycol. Pap. 73: 107-109.

BRADY, B.L.K. 1979. *Verticillium lecanii.* CMI Descriptions of Pathogenic Fungi and Bacteria No. 610, Kew, Surrey.

BROUGHTON, R.E., STANLEY, S.E. and DURRETT, R.T. 2000. Quantification of homoplasy for nucleotide transitions and transversions and a reexamination of assumptions in weighted phylogenetic analysis. Syst. Biol. 49: 617–627.

BRUNS, T.D., PALMER, J.D., SHUMARD, D.S., GROSSMAN, L.I. and HUDSPETH, M.E.S. 1988. Mitochondrial DNAs of *Suillus*: three-fold size change in molecules that share a common gene order. Curr. Genet. 13: 49–56.

BURSNALL, L.A. and TRIBE, H.T. 1974. Fungal parasitism in cysts of *Heterodera*. II. Egg parasites of *H. schachtii*. Trans. Br. Mycol. Soc. 62: 595–601.

CAMPBELL, W.P. and GRIFFITHS, D.A. 1975. The development and structure of thick-walled, multicellular, aerial spores in *Diheterospora chlamydosporia* (= *Verticillium chlamydosporium*). Canad. J. Microbiol. 21: 963–971.

CARDER, J.H., SEGERS, R., BUTT, T.M., BARBARA, D.J., VON MENDE, N. and COOSEMANS, J. 1993. Taxonomy of the nematophagous fungi *Verticillium chlamydosporium* and *V. suchlasporium* based on secreted enzyme activities and RFLP analysis. J. Invert. Pathol. 62: 178–184.

CHANDLER, D., HEALE, J.B. and GILLESPIE, A.T. 1993. Competitive interaction between strains of *Verticillium lecanii* on two insect hosts. Ann. Appl. Biol. 122: 435–440.

CHEN, J.D., LIU, J.G., CHEN, S.S., CAI, F.J. and ZHANG, Z.C. 1984. Studies on *Aphanocladium aranearum* (Petch) Gams var. *sinensis* var. nov., a fungus parasitic on *Agaricus bisporus* (Lange) Sing. Acta Mycol. Sin. 3: 96–101.

CHEN, J.D., LIU, J.G., CHEN, S.S., CAI, F.J. and ZHANG, Z.C. 1985. A new species of *Aphanocladium* on *Agaricus bisporus*. Acta Mycol. Sin. 4: 227–233.

CLAY, K. 1988. Clavicipitaceous endophytes of grasses. Coevolution and the change from parasitism to mutualism. pp. 79–105. *In* Coevolution of Fungi with Plants and Animals (eds HAWKSWORTH, D.L. and PIROZYNSKI, K.). Academic Press, London.

COWIE, R.H. 2001. Can snails ever be effective and safe biocontrol agents? Int. J. Pest Manag. 47: 2340.

DACKMAN, C. and NORDBRING-HERTZ, B. 1985. Fungal parasites of the cereal cyst nematode *Heterodera avenae* in Southern Sweden. J. Nematol. 17: 50–55.

DAYAL, R. and BARRON, G.L. 1970. *Verticillium psalliotae* as a parasite of *Rhopalomyces*. Mycologia 62: 626–830.

DE HOOG, G.S. and VAN OORSCHOT, C.A.N. 1985. Taxonomy of the *Dactylaria* complex, VI. Key to the genera and check-list of epithets. Stud. Mycol. 26: 97–122.

DE LEIJ, F.A.A.M. 1992. The significance of ecology in the development of *Verticillium chlamydosporium* as a biological control agent against root-knot nematodes (*Meloidogyne* spp.). Dissertation, University of Wageningen.

DE LEIJ, F.A.A.M. and KERRY, B.R. 1991. The nematophagous fungus *Verticillium chlamydosporium* as a potential biological control agent for *Meloidogyne arenaria*. Rev. Nématol. 14: 157–164.

DIEHL, W.W. 1950. *Balansia* and the *Balansiae* in America. Agriculture Monograph No. 4. U.S.D.A., Washington. 82 pp.

DIJKSTERHUIS, J. 1993. Nematode-fungal interactions; structure-function relationships. Diss., Univ. Groningen. 139 pp.

DOWSETT, J.A., REID, J. and HOPKIN, A. 1982. On *Cephalosporium balanoides* Drechsler. Mycologia 74: 687–690.

DRECHSLER, C. 1941. Some hyphomycetes parasitic on free-living terricolous nematodes. Phytopathology 31: 773–802.

DRECHSLER, C. 1942. Two zoophagous species of *Acrostalagmus* with multicellular *Desmidiospora*-like chlamydospores. J. Washington Acad. Sci. 32: 343–350.

DRECHSLER, C. 1946. A new hyphomycete parasitic on a species of nematode. Phytopathology 36: 213–217.

EKBOM, B. and AHMAN, I. 1980. The fungus *Verticillium fusisporum* as an insect pathogen. J. Invert. Pathol. 36: 136–138.

EVANS, H.C. and HYWEL-JONES, N.L. 1997. Entomopathogenic fungi. pp. 3–27. *In* Soft Scale Insects: Their Biology, Natural Enemies and Control (eds BEN-DOV, Y. and HODGSON, C.J.). Elsevier Science, Amsterdam.

EVANS, H.C. and SAMSON, R.A. 1982. Entomogenous fungi from Galápagos Islands. Canad. J. Bot. 60: 2325–2333.

EVANS, H.C. and SAMSON, R.A. 1984. The genus *Verticillium*: taxonomic problems in species with invertebrate hosts. pp. 186-189. *In* Fundamental and Applied Aspects of Invertebrate Pathology (eds SAMSON, R.A., VALK, J.M. and PETERS, D.). Foundation of the Fourth International Colloquium of Invertebrate Pathology, Wageningen, Netherlands.

FASSATIOVÁ, O. and LYSEK, H. 1982. Ovicidal fungi in soil ecological system. Acta Univ. Carol., Biologica 9: 297–334.

FELSENSTEIN, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Syst. Zool. 27: 401–410.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

GAMS, W. 1971. *Cephalosporium*-artige Schimmelpilze (Hyphomycetes). Gustav Fischer Verlag, Stuttgart.

GAMS, W. 1973. Phialides with solitary conidia? Remarks on conidium ontogeny in some hyphomycetes. Persoonia 7: 161–169.

GAMS, W. 1988. A contribution to the knowledge of nematophagous species of *Verticillium*. Netherl. J. Pl. Pathol. 94: 123–148.

GAMS, W. 2003. *Drechmeria coniospora*. IMI Descriptions of Fungi and Bacteria 1561: 3 pp.

GAMS, W., DIEDERICH, P. and PÕLDMAA, K. 2004. Fungicolous fungi. Chapter 17 *In* Measuring and Monitoring Biological Diversity: Standard Methods for Fungi (eds MÜLLER, G., BILLS, G.F. and FOSTER, M.S.). Academic Press, New York (in press).

GAMS, W., HOEKSTRA, E.S. and APTROOT, A. (eds) 1998. CBS Course of Mycology. Centraalbureau voor Schimmelcultures, Baarn, Delft.

GAMS, W., HOOG, G.S. DE, SAMSON, R.A. and EVANS, H.C. 1984. The hyphomycete genus *Engyodontium*. A link between *Verticillium* and *Aphanocladium*. Persoonia 12: 135–147.

GAMS, W. and JANSSON, H.-B. 1985. The nematode parasite *Meria coniospora* Drechsler in pure culture and its classification. Mycotaxon 22: 33–38.

GAMS, W., O'DONNELL, K., SCHROERS, H.J. and CHRISTENSEN, M. 1998. Generic classification of some more hyphomycetes with solitary conidia borne on phialides. Canad. J. Bot. 76: 1570–1583.

GAMS, W. and ZAAYEN, A. VAN 1982. Contribution to the taxonomy and pathogenicity of fungicolous *Verticillium* species. I. Taxonomy. Netherlands J. Pl. Pathol. 88: 57–78.

GAMS, W. and ZARE, R. 2001. A revision of *Verticillium* sect. *Prostrata.* III. Generic classification. Nova Hedwigia 72: 329–337.

GAMS, W. and ZARE, R. 2003. A taxonomic review of the clavicipitaceous anamorphs parasitizing nematodes and other microinvertebrates. pp. 17–73. *In* Clavicipitalean Fungi. Evolutionary Biology, Chemistry, Biocontrol, and Cultural Impacts (eds WHITE, J.F., jr, BACON, C.W., HYWEL-JONES, N.L. and SPATAFORA, J.W.). Marcel-Dekker, New York, Basel.

GARDES, M. and BRUNS, T.D. 1993. ITS primers with enhanced specificity for Basidiomycetes; application to the identification of mycorrhizae and rusts.

Molec. Ecol. 2: 113–118.

GERNANDT, D.S., CAMACHO, F.J. and STONE, J.K. 1997. *Meria laricis*, an anamorph of *Rhabdocline*. Mycologia 89: 735–744.

GERNANDT, D.S. and STONE, J.K. 1999. Phylogenetic analysis of nuclear ribosomal DNA places the nematode parasite, *Drechmeria coniospora*, in Clavicipitaceae. Mycologia 91: 993–1000.

GINTIS, B.O., MORGAN-JONES, G. and RODRÍGUEZ-KÁBANA, R. 1983. Fungi associated with several developmental stages of *Heterodera glycines* from an Alabama soybean field soil. Nematropica 13: 181–200.

GLASS, N.L. and DONALDSON, G.C. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. Appl. Envir. Microbiol. 61: 1323–1330.

GLENN, A.E., BACON, C.W., PRICE, R. and HANLIN, R.T. 1996. Molecular phylogeny of *Acremonium* and its taxonomic implications. Mycologia 88: 369–383.

GLOCKLING, S.L. 1998a. Two new species of rotifer-attacking fungi, *Rotiferophthora*, from Japan and records of *Verticillium bactrosporum* in rotifer hosts. Mycol. Res. 102: 145–150.

GLOCKLING, S.L. 1998b. Three new species of *Rotiferophthora* attacking bdelloid rotifers in Japan. Mycol. Res. 102: 1142–1148.

GLOCKLING, S.L. and DICK, M.W. 1997. Two new species of nematophagous *Verticillium* (Hyphomycetes) forming catenulate conidia, with a key to the nematophagous *Verticillium* species. Nord. J. Bot. 17: 653–662.

GLOCKLING, S.L. and SHIMAZU, M. 1997. Culturing of three species of endoparasitic fungi infecting nematodes. Mycol. Res. 101: 55–60.

GLOCKLING, S.L. and YAMADA, F. 1997. A survey of fungi which kill microscopic animals in the dung of the Amami rabbit. Mycologist 11: 113–120.

GODDARD, N.H. 1913. Can fungi living in agricultural soil assimilate free nitrogen. Bot. Gaz. 56: 249–305.

GODOY, G., RODRÍGUEZ-KÁBANA, R. and MORGAN-JONES, G. 1982. Parasitism of eggs of *Heterodera glycines* and *Meloidogyne arenaria* by fungi isolated from cysts of *H. glycines*. Nematropica 12: 111–119.

GOODAY, J.B. 1951. A new species of hyphomycete attacking the stem eelworm *Ditylenchus dipsaci*. Trans. Br. Mycol. Soc. 34: 270–272.

GRAYBEAL, A. 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? Syst. Biol. 47: 9–17.

HALL, R.A. 1981. The fungus *Verticillium lecanii* as a microbial insecticide against aphids and scales. pp. 483–498. *In* Microbial Control of Pests and Plant Diseases (ed. BURGES, H.D.). Academic Press, London.

HALL, R.A. 1982. Control of whitefly *Trialeurodes vaporariorum* and cotton aphid *Aphis gossypii* in glasshouses by two isolates of the fungus *Verticillium lecanii*. Ann. Appl. Biol. 101: 1–11.

HALL, R.A. and BURGES, H.D. 1979. Control of aphids in glasshouses with the fungus *Verticillium lecanii*. Ann. Appl. Biol. 93: 235–246.

HANSFORD, C.G. 1943. Contribution towards the fungus flora of Uganda V. Fungi Imperfecti. Proc. Linn. Soc. London 155: 34–67.

HARRINGTON, T.C., STEIMEL, J.P., WORKNEH, F. and YANG, X.B. 2000. Molecular identification of fungi associated with vascular discoloration of soybean in the North Central United States. Pl. Dis. 84: 83–89.

HAY, F.S. 1995. Endoparasites infecting nematodes in New Zealand. New Zealand J. Bot. 33: 401–407.

HAY, F.S. and BATESON, L. 1997. Effect of the nematophagous fungi *Hirsutella rhossiliensis* and *Verticillium balanoides* on stem nematode (*Ditylenchus dipsaci*) in white clover. Australas. Pl. Pathol. 26: 142–147.

HAY, F.S. and REGNAULT, L. 1995. Introduction of the endoparasitic nematophagous fungus *Verticillium balanoides* into white clover (*Trifolium repens*) by its host, stem nematode (*Ditylenchus dipsaci*). Australas. Pl. Pathol. 24: 249–251.

HELFER, W. 1991. Pilze auf Pilzfruchtkörpern. Untersuchungen zur Ökologie, Systematik und Chemie. IHW-Verlag, Eching. Libri Bot. 1: 1–157.

HELYER, N., GILL, G. and BYWATER, A. 1992. Control of chrysanthemum pests with *Verticillium lecanii*. pp. 9-12. *In* Non-chemical Approaches to Crop Protection in Horticulture (eds SUNDERLAND, K.D., CRUTE, I.R., BURCHILL, R.T. and BEN-ARIE, R.). Horticulture Research International, Wellesbourne, UK.

HODGE, K.T. 2003. Clavicipitaceous anamorphs. pp. 75–123. *In* Clavicipitalean Fungi. Evolutionary Biology, Chemistry, Biocontrol, and Cultural Impacts (eds WHITE, J.F., jr, BACON, C.W., HYWEL-JONES, N.L. and SPATAFORA, J.W.). Marcel-Dekker, New York, Basel, USA.

HODGE, K.T., GAMS, W., SAMSON, R.A., KORF, R.P. and SEIFERT, K.A. 2004. Lectotypification and status of *Isaria* Pers. : Fr. Taxon: In press.

HUGHES, S.J. 1951. Studies on microfungi. XI: Some hyphomycetes which produce phialides. Mycol. Pap. 45: 136.

HYWEL-JONES, N.L., EVANS, H.C. and JUN, Y. 1997. A re-evaluation of the leafhopper pathogen *Torrubiella hemipterigena*, its anamorph *Verticillium hemipterigenum* and *V. pseudohemipterigenum* sp. nov. Mycol. Res. 101: 1242–1246.

IRVING, F. and KERRY, B.R. 1986. Variation between strains of the nematophagous fungus, *Verticillium chlamydosporium*. Nematologica 32: 474–485.

JACKSON, C.W. and HEALE, J.B. 1985. Relationship between DNA content and spore volume in sixteen isolates of *Verticillium lecanii* and two new diploids of *V. dahliae* (= *V. dahliae* var. *longisporum* Stark). J. Gen. Microbiol. 131: 3229–3236.

JOHNSTON, J.R. 1918. Algunos hongos entomógenos de Cuba. Memorias Soc. Cubana Hist. Nat. 3: 61–82.

JONES, R.T. and FREDERICK, L. 1971. *Cephalosporium salvinae* sp. nov., a pathogen of *Salvinia rotundifolia* Willd. Mycopathol. Mycol. Appl. 43: 195–200.

JUHL, M. 1982. Cereal cyst nematode, soil heating and fungal parasitism. EPPO Bull. 12: 505–511.

JUN, Y., BRIDGE, P.D. and EVANS, H.C. 1991. An integrated approach to the taxonomy of the genus *Verticillium.* J. Gen. Microbiol. 137: 1437–1444.

KAMYSCHKO, O.P. 1962. De Monilialibus terrestribus novis notula. Bot. Mater. Otd. Sporov. Rast. Bot. Inst. Komarova Akad. Nauk S.S.S.R. (Not. Syst. Sect. Cryptog. Inst. Bot. Komarovii Acad. Sci. URSS). 15: 137–141.

KERRY, B.R. 1995. Ecological considerations for the use of the nematophagous fungus, *Verticillium chlamydosporium,* to control plant parasitic nematodes. Canad. J. Bot. 73: 65–70.

KERRY, B.R. and CRUMP, D.H. 1977. Observations on fungal parasites of females and eggs of the cereal cyst-nematode, *Heterodera avenae*, and other cyst nematodes. Nematologica 23: 193–201.

KERRY, B.R., IRVING, I. and HORSEY, J.C. 1986. Variation between strains of the nematophagous fungus, *Verticillium chlamydosporium* Goddard. I: Factors affecting growth *in vitro*. Nematologica 32: 461–473.

KOBAYASI, Y. 1941. The genus *Cordyceps* and its allies. Science Rep. Tokyo Bunrika Daigaku, Sect. B 5 (84): 53–260.

KOBAYASI, Y. 1982. Keys to the taxa of the genera *Cordyceps* and *Torrubiella.* Trans. Mycol. Soc. Japan 23: 329–364.

KOUVELIS, V.N., ZARE, R., BRIDGE, P.D. and TYPAS, M.A. 1999. Differentiation of mitochondrial subgroups in the *Verticillium lecanii* species complex. Lett. Appl. Microbiol. 28: 263–268.

KUSHWAHA, R.K.S. 1980. A new species of *Verticillium.* Curr. Sci. 49: 948–949.

LA TOUCHE, C.J. 1947. Passive movements of newly formed acrogenous spores in *Verticillium* and some other genera of the hyphomycete fungi. Nature, London 160: 679.

LIECKFELDT, E. and SEIFERT, K.A. 2000. An evaluation of the use of ITS sequences in the taxonomy of the Hypocreales. Stud. Mycol. 45: 35–44.

LIM, T.K. and WAN, Z.W.N. 1983. Mycoparasitism of the coffee rust pathogen, *Hemileia vastatrix*, by *Verticillium psalliotae* in Malaysia. Pertanika 6: 23–25.

LÓPEZ-LLORCA, L.V. 1990. Purification and properties of extracellular proteases produced by the nematophagous fungus *Verticillium suchlasporium*. Canad. J. Microbiol. 36: 530–537.

LÓPEZ-LLORCA, L.V. and DUNCAN, G.H. 1988. A study of fungal endoparasitism of the cereal cyst nematode (*Heterodera avenae*) by scanning electron microscopy. Canad. J. Microbiol. 34: 613–619.

LÓPEZ-LLORCA, L.V., MOYA, M. and LLINARES, A. 1994. Effect of pH on growth and pigment production of nematophagous and entomogenous fungi. Micol. Veg. Mediterr. 8: 107–112.

LÓPEZ-LLORCA, L.V. and OLIVARES-BERNABEU, C. 1998. Metabolites influencing pathogenicity of nematophagous fungi. pp. 171–186. *In* Molecular Variability of Fungal Pathogens (eds BRIDGE, P., COUTEAUDIER, Y. and CLARKSON, J.). CAB International, Wallingford, UK.

MADDISON, W.P. and MADDISON, D.R. 1992. MacClade: analysis of phylogeny and character evolution. Version 3.0. Sinauer Associates, Sunderland, Massachusetts.

MAINS, E.B. 1949. New species of *Torrubiella*, *Hirsutella* and *Gibellula*. Mycologia 41: 303–310.

MAINS, E.B. 1958. North American entomogenous species of *Cordyceps*. Mycologia 50: 169–222.

MANKAU, R. 1980. Biocontrol: fungi as nematode control agents. J. Nematol. 12: 213–232.

MARCHETTI, M. and VARESE, G.C. 1997. Azione di *Verticillium bulbillosum* sulla formazione in vitro di micorrize di *Laccaria laccata* ed *Hebeloma crustuliniforme* con *Picea abies*. Allionia 34: 45–54.

McINNIS, T.M. and JAFFEE, B.A. 1989. An assay for *Hirsutella rhossiliensis* spores and the importance of phialides for nematode inoculation. J. Nematol. 21: 229-234.

MESSNER, R., SCHWEIGKOFLER, W., IBL, M., BERG, G. and PRILLINGER,

H. 1996. Molecular characterization of the plant pathogen *Verticillium dahliae* Kleb. using RAPD-PCR and sequencing of the 18SrRNA-gene. J. Phytopathol. 144: 347–354.

MILLER, T.C. and GUBLER, W.D. 1988. Field application of *Verticillium lecanii* controls *Sphaerotheca macularis* f. sp. *fragariae* in California strawberry fruit pathogen. Phytopathology 88: S63 (Abstr.).

MINTER, D.W., BRADY, B.L. and HALL, R.A. 1983. Five hyphomycetes isolated from eriophyid mites. Trans. Br. Mycol. Soc. 81: 455–471.

MONCALVO. J.M., LUTZONI, F.M., REHNER, S.A., JOHNSON, J. and VILGALYS, R. 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Syst. Biol. 49: 278–306.

MORGAN-JONES, G. and GAMS, W. 1982. Notes on Hyphomycetes. XLI. An endophyte of *Festuca arundinacea* and the anamorph of *Epichloë typhina*, new taxa in one of two new sections of *Acremonium*. Mycotaxon 15: 311–318.

MORGAN-JONES, G., GODOY, G. and RODRÍGUEZ-KÁBANA, R. 1981. *Verticillium chlamydosporium*, fungal parasite of *Meloidogyne arenaria* females. Nematropica 11: 115–119.

MORGAN-JONES, G., WHITE, J.F. JR. and RODRÍGUEZ-KÁBANA, R. 1983. Phytonematode pathology: ultrastructural studies: 1. Parasitism of *Meloidogyne arenaria* eggs by *Verticillium chlamydosporium*. Nematropica 13: 245–260.

MUGNAI, L., BRIDGE, P.D. and EVANS, H.C. 1989. A chemotaxonomic evaluation of the genus *Beauveria*. Mycol. Res. 92: 199–209.

MUGNIER, J. 1994. PCR Catalogue: Fungi. Rhone-Poulenc Agro: France.

MULLIS, K.B. and FALLONA F.A. 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. Methods Enzymol. 155: 335–350.

NIKOH, N. and FUKATSU, T. 2000. Interkingdom host jumping underground: phylogenetic analysis of entomoparasitic fungi of the genus *Cordyceps*. Mol. Biol. Evol. 17: 629–638.

O'DONNELL, K. and GRAY, L.E. 1995. Phylogenetic relationships of the soybean sudden death syndrome pathogen *Fusarium solani* f. sp. *phaseoli* inferred from rDNA sequence data and PCR primers for its identification. Molec. Pl. Microbe Interact. 8: 709–716.

PALM, M.E., GAMS, W. and NIRENBERG, H.I. 1995. *Plectosporium*, a new genus for *Fusarium tabacinum*, the anamorph of *Plectosphaerella cucumerina*. Mycologia 87: 397–406.

PARKIN, J. 1906. Fungi parasitic upon scale insects (Coccidae and Aleurodidae): a general account with special reference to Ceylon forms. Ann. Roy. Bot. Gard. Peradeniya, Ceylon 3: 11–81.

PATERSON, R.R.M. and BRIDGE, P.D. 1994. Biochemical techniques for filamentous fungi (IMI Technical Handbooks No. 1). CAB International, Wallingford, Oxon, UK.

PETCH, T. 1925. Studies in entomogenous fungi. VI: *Cephalosporium* and associated fungi. Trans. Br. Mycol. Soc. 10: 152–182.

PETCH, T. 1931. Notes on entomogenous fungi. Trans. Br. Mycol. Soc. 16: 55–75.

PETCH, T. 1932. Notes on entomogenous fungi. Trans. Br. Mycol. Soc. 16: 209–245.

PETCH, T. 1939. Notes on entomogenous fungi. Trans. Br. Mycol. Soc. 23: 127–148.

PETHYBRIDGE, G.H. 1919. Notes on some saprophytic species associated with diseased potato plants and tubers. Trans. Br. Mycol. Soc. 6: 104–120.

QUINLAN, R.J. 1988. Use of fungi to control insects in glasshouses. pp. 19–36. *In* Fungi in Biological Control Systems (ed. BURGE, M.N.). Manchester Univ. Press, England.

RAEDER, U. and BRODA, P. 1985. Rapid preparation of DNA from filamentous fungi. Lett. Appl. Microbiol. 1: 17–20.

ROGERSON, C. T. 1970. The Hypocrealian fungi (Ascomycetes, Hypocreales). Mycologia 62: 865–910.

SAIKAWA, M. 1982. An electron microscope study of *Meria coniospora*, an endozoic nematophagous hyphomycete. Canad. J. Bot. 60: 2019–2023.

SAKSIRIRAT, W. and HOPPE, H.-J. 1991. Secretion of extracellular enzymes by *Verticillium psalliotae* Treschow and *Verticillium lecanii* (Zimml.) Viégas during growth on uredospores of the soybean rust fungus (*Phakopsora pachyrhizi* Syd.) in liquid cultures. J. Phytopathol. 131: 161–173.

SAMSON, R.A., EVANS, H.C. and HOEKSTRA, E.S. 1982. Notes on entomogenous fungi from Ghana VI. The genus *Cordyceps*. Proc. Kon. Ned. Akad. Wetensch. C 85: 589–605.

SAMUELS, G.J. 1988. Fungicolous, lichenicolous, and myxomyceticolous species of *Hypocreopsis*, *Nectriopsis*, *Nectria*, *Peristomialis*, and *Trichonectria*. Mem. New York Bot. Gard. 48: 1–78.

SANGER, F., NICKLEN, S. and COULSEN, A.R. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 85: 3608–3612.

SCHARDL, C.L., LEUCHTMANN, A., CHUNG, K.-R., PENNY, D. and SIEGEL, M.R. 1997. Coevolution by common descent of fungal symbionts (Epichloe spp.) and grass hosts. Mol. Biol. Evol. 14: 133–143.

SCHARDL, C.L., LIU, J., WHITE, J.F. JR., FINKEL, R.A., AN, Z. and SIEGEL, M.R. 1991. Molecular phylogenetic relationships of nonpathogenic grass mycosymbionts and clavicipitaceous plant pathogens. Pl. Syst. Evol. 178: 27–41.

SCHULER, T., HOMMES, M., PLATE, H.-P. and ZIMMERMANN, G. 1991. *Verticillium lecanii* (Zimmermann) Viégas (Hyphomycetales: Moniliaceae): Geschichte, Systematik, Verbreitung, Biologie und Anwendung im Pflanzenschutz. Mitt. Biol. Bundesanst. Land- & Forstw. 269: 1–153.

SEIFERT, K.A., LOUIS-SEIZE, G. and SAVARD, M.E. 1997. The phylogenetic relationships of two trichothecene-producing hyphomycetes, *Spicellum roseum* and *Trichothecium roseum*. Mycologia 89: 250–257.

SJOLLEMA, K., DIJKSTERHUIS, J., VEENHUIS, M. and HARDER, W. 1993. An electron microscopical study of the infection of the nematode *Panagrellus redivivus* by the endoparasitic fungus *Verticillium balanoides*. Mycol. Res. 97: 479–484.

SMITH, F.E.V. 1924. Three diseases of cultivated mushrooms. Trans. Br. Mycol. Soc. 10: 81–97.

SPATAFORA, J.W. and BLACKWELL, M. 1993. Molecular systematics of unitunicate perithecial ascomycetes: The Clavicipitales-Hypocreales connection. Mycologia 85: 912–922.

SPATAFORA, J.W., VOLKMANN-KOHLMEYER, B. and KOHLMEYER, J. 1998. Independent terrestrial origins of the Halosphaeriales (marine Ascomycota). Amer. J. Bot. 85: 1569–1580.

STEENBERG, T. and HUMBER, R.A. 1999. Entomopathogenic potential of *Verticillium* and *Acremonium* species (Deuteromycotina: Hyphomycetes). J. Invert. Pathol. 73: 309–314.

SUBRAMANIAN, C.V. 1977. Revisions of Hyphomycetes I. Kavaka 5: 93-98.

SUH, S.-O., SPATAFORA, J.W., OCHIEL, G.R.S., EVANS, H.C. and BLACKWELL, M. 1998. Molecular phylogenetic study of a termite pathogen *Cordycepioideus bisporus*. Mycologia 90: 611–617.

SUKAPURE, R.S. and THIRUMALACHAR, M.J. 1966a. Studies of *Cephalosporium* species from India II. Bull. Torrey Bot. Club 93: 305-311.

SUKAPURE, R.S. and THIRUMALACHAR, M.J. 1966b. Conspectus of species of *Cephalosporium* with particular reference to Indian species. Mycologia 58: 351-360.

SUNG, G.-H., SPATAFORA, J.W., ZARE, R., HODGE, K.T. and GAMS, W. 2001. A revision of *Verticillium* sect. *Prostrata*. II. Phylogenetic analyses of SSU and

LSU nuclear rDNA sequences from anamorphs and teleomorphs of the Clavicipitaceae. Nova Hedwigia 72: 311-328.

SWOFFORD, D.L. 1998. Phylogenetic analysis using parsimony 4.0b2 beta version. Sinauer, Sunderland, Massachusetts.

TAYLOR, J.W. 1993. A contemporary view of the holomorph: nucleic acid sequence and computer databases are changing fungal classification. pp. 3–13. *In* The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics (eds REYNOLDS, D.R. and TAYLOR, J.W.). CAB International, Wallingford, UK.

TAYLOR, J.W. 1995. Making the Deuteromycota redundant: a practical integration of mitosporic and meiosporic fungi. Canad. J. Bot. 73 (suppl. 1): S754–S759.

TEHLER, A., FARRIS, J.S., LIPSCOMB, D.L. and KALLERSJO, M. 2000. Phylogenetic analyses of the fungi based on large rDNA data sets. Mycologia 92: 459–474.

TRESCHOW, C. 1941. The *Verticillium* diseases of cultivated mushrooms. Dansk Bot. Arkiv 11(1): 1–31.

TRIBE, H. T. 1977. Pathology of cyst nematodes. Biol. Rev. 52: 477–507.

TSUNEDA, A. SKOROPAD, W.P. and TEWARI, J.P. 1976. Mode of parasitism of *Alternaria brassicae* by *Nectria inventa*. Phytopathology 66: 1056–1064.

TYPAS, M.A., GRIFFEN, A.M., BAINBRIDGE, B.W. and HEALE, J.B. 1992. Restriction fragment length polymorphisms in mitochondrial DNA and ribosomal RNA gene complexes as an aid to the characterization of species and sub-species populations in the genus *Verticillium*. FEMS Microbiol. Lett. 95: 157–162.

VERHAAR, M.A., HIJWEGEN, T. and ZADOKS, J.C. 1996. Glasshouse experiments on biocontrol of cucumber powdery mildew (*Sphaerotheca fuliginea*) by the mycoparasites *Verticillium lecanii* and *Sporothrix rugulosa*. Biol. Control 6: 353–360.

VESTERGAARD, S., GILLESPIE, A.T., BUTT, T.M., SCHREITER, G. and EILENBERG, J. 1995. Pathogenicity of the hyphomycete fungi *Verticillium lecanii* and *Metarhizium anisopliae* to the western flower thrips, *Frankliniella occidentalis*. Biocontrol Sci. Technol. 5: 185–192.

VIÉGAS, A.P. 1939. Um amigo do fazendeiro. *Verticillium lecanii* (Zimm.) n. comb., o causador do halo branco do *Coccus viridis* (Green). Rev. Inst. Café Est. São Paulo 14: 754.

VILGALYS, R. and SUN, B.L. 1994. Ancient and recent patterns of geographic speciation in the oyster mushroom *Pleurotus* revealed by phylogenetic analysis of ribosomal DNA sequences. Proc. Natl. Acad. Sci., USA 91: 4599–4603.

WAINWRIGHT, M., BETTS, R.P. and TEALE, D.M. 1986. Antibiotic activity of oosporein from *Verticillium psalliotae*. Trans. Br. Mycol. Soc. 86: 168–170.

WATANABE, T. 2000. *Verticillium balanoides*, a nematode endoparasite associated with pine needles of collapsing Japanese red pine trees in Tsukuba. Mycoscience 41: 283–285.

WHITE, J.F., jr., BACON, C.W., HYWEL-JONES, N.L. and SPATAFORA, J.W. (eds) 2003. Clavicipitalean Fungi. Evolutionary Biology, Chemistry, Biocontrol, and Cultural Impacts. Marcel-Dekker, New York, Basel, USA.

WHITE, T.J., BRUNS, T., LEE, S. and TAYLOR, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes from phylogenetics. pp. 315–322. *In* PCR Protocols (eds INNES, M.A., GELFAND, D.H., SNINSKY, J.S. and WHITE, T.J.). Academic Press: London, UK.

WILLCOX, J. and TRIBE, H.T. 1974. Fungal parasitism in cysts of *Heterodera*. I: Preliminary investigations. Trans. Br. Mycol. Soc. 62: 585–594.

YIP, H.-Y. and RATH, A.C. 1989. *Microhilum oncoperae* gen. et sp. nov. (Deuteromycotina: Hyphomycetes): The cause of mycosis on the larvae of *Oncopera intricata* (Lepidoptera: Hepialidae). J. Invert. Pathol. 53: 361–364.

YUN, Y., BRIDGE, P.D. and EVANS, H.C. 1991. An integrated approach to the taxonomy of the genus *Verticillium*. J. Gen. Microbiol. 137: 1437–1444.

ZAAYEN, A. VAN and GAMS, W. 1982. Contribution to the taxonomy and pathogenicity of fungicolous *Verticillium* species. II. Pathogenicity. Netherlands J. Pl. Pathol. 88: 143–154.

ZARE, R. 2000. Contributions to the taxonomy of *Verticillium*. PhD thesis, Univ. Reading.

ZARE, R. 2003. A revision of plant-associated *Verticillium* species. Rostaniha 4: 29–54.

ZARE, R. and GAMS, W. 2001a. A revision of *Verticillium* section *Prostrata*. IV. The genera *Lecanicillium* and *Simplicillium* gen. nov. Nova Hedwigia 73: 1–50.

ZARE, R. and GAMS, W. 2001b. A revision of *Verticillium* section *Prostrata*. VI. The genus *Haptocillium*. *Nova Hedwigia* 73: 271–292.

ZARE, R. and GAMS, W. 2003a. *Haptocillium balanoides*. IMI Descriptions of Fungi and Bacteria 1562: 3 pp.

ZARE, R. and GAMS, W. 2003b. *Lecanicillium aphanocladii*. IMI Descriptions of Fungi and Bacteria 1563: 2 pp.

ZARE, R. and GAMS, W. 2003c. *Lecanicillium dimorphum*. IMI Descriptions of Fungi and Bacteria 1564: 2 pp.

ZARE, R. and GAMS, W. 2003d. *Lecanicillium lecanii*. IMI Descriptions of Fungi and Bacteria 1565: 3 pp.

ZARE, R. and GAMS, W. 2003e. *Lecanicillium longisporum*. IMI Descriptions of Fungi and Bacteria 1566: 2 pp.

ZARE, R. and GAMS, W. 2003f. *Lecanicillium muscarium*. IMI Descriptions of Fungi and Bacteria 1567: 6 pp.

ZARE, R. and GAMS, W. 2003g. *Lecanicillium psalliotae*. IMI Descriptions of Fungi and Bacteria 1568: 2 pp.

ZARE, R. and GAMS, W. 2003h. *Pochonia chlamydosporia*. IMI Descriptions of Fungi and Bacteria 1569: 4 pp.

ZARE, R. and GAMS, W. 2003i. *Pochonia suchlasporia*. IMI Descriptions of Fungi and Bacteria 1570: 2 pp.

ZARE, R., GAMS, W. and CULHAM, A. 2000. A revision of *Verticillium* sect. *Prostrata*. I. Phylogenetic studies using ITS sequences. Nova Hedwigia 71: 465–480.

ZARE, R., GAMS, W. and EVANS, H.C. 2001. A revision of *Verticillium* section *Prostrata*. V. The genus *Pochonia* with notes on *Rotiferophthora*. Nova Hedwigia 73: 51–86.

ZARE, R., GAMS, W. and SCHROERS, H.-J. 2004. The type species of *Verticillium* is not congeneric with the plant-pathogenic species placed in *Verticillium* and it is not the anamorph of *Nectria inventa*. Mycol. Res. 108: 576-582.

ZARE, R., KOUVELIS, V.N., TYPAS, M.A. and BRIDGE, P.D. 1999. Presence of a 20 bp insertion/deletion in the ITS1 region of *Verticillium lecanii*. Lett. Appl. Microbiol. 28: 258–262.

ZHANG, K.Q., CAO, L. and LIANG, Z.Q. 1996. A new nematophagous species of *Verticillium* from China. Mycol. Res. 100: 1481–1482.

ZIMMERMANN, A. 1899. Over een Schimmelepidemie der groene Luizen. Korte Berichten uit 's Lands Plantentuin. Teysmania 9: 240–243.

Index

Note: Bolded page numbers indicate main entries, those with an asterisc refer to illustrations. Italicized numbers indicate synonyms.