

## *Phaeoacremonium* gen. nov. associated with wilt and decline diseases of woody hosts and human infections

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**Abstract:** A new hyphomycete genus, *Phaeoacremonium*, is proposed for five new species, *P. aleophilum*, *P. angustius*, *P. chlamydosporum*, *P. inflatipes*, *P. rubrigenum*, and the type species, *P. parasiticum*, which was formerly accommodated in *Phialophora*. Morphologically, the genus is intermediate between *Acremonium* and *Phialophora*. It is distinguished from *Phialophora* by its aculeate conidiogenous cells and inconspicuous collarettes, and from *Acremonium* by its pigmented vegetative hyphae and conidiophores. Because species of *Phaeoacremonium* have been associated with human infections and disease symptoms of several woody hosts, this new genus is considered to represent an ecologically important group of fungi.

**Key Words:** hyphomycetes, medical mycology, *Phialophora*, systematics

### INTRODUCTION

Grape diseases named Esca, apoplexy or Black Measles, with an extensive range of symptoms, have been described to occur in Italy (e.g. Baldacci et al., 1962), France (Viala, 1926; Dubos and Larignon, 1988), and California (Chiarappa, 1959). From grapevines (*Vitis vinifera* L.) showing these symptoms, similar fungi have been isolated including *Stereum hirsutum* (Willd.) Pers., *Phellinus igniarius* (L.: Fr.) Quél. and

a number of *Cephalosporium*-like fungi (Dubos and Larignon, 1988).

Since 1940 a slow die-back disease has been encountered sporadically in grapevines growing in the Cape Province of South Africa. Symptoms include unequal to stunted growth, eventually leading to die-back. The organism most commonly associated with these rootstocks has been identified as *Phialophora parasitica* Ajello et al. (Ferreira et al., 1994). In inoculation experiments on vine rootstocks, *P. parasitica* causes a plugging of the xylem vessels, similar to that observed under field conditions (Ferreira et al., 1994).

*Phialophora parasitica* was originally isolated from a subcutaneous infection of a human patient who had undergone a kidney transplant (Ajello et al., 1974). However, Hawksworth et al. (1976) noted that this fungus had earlier been associated with disease conditions on various woody hosts. They also reported that the decline of oaks in Texas, originally attributed to a *Cephalosporium* species (Halliwell, 1966), was in fact caused by *P. parasitica*. *Phialophora parasitica* has also been associated with various disease conditions of a *Nectandra* sp. in Costa Rica, *Phoenix dactylifera* L. in Iraq, *Prunus armeniaca* L. in Tunisia (Hawksworth et al., 1976), a *Cupressus* sp., *Aquilaria agalocha* Roxb. (Hawksworth and Gibson, 1976) and olive trees (*Olea europaea* L.) (Thanassouloupoulos and Thanassouloupoulos, 1984). A similar fungus (*Cephalosporium* sp.) has also been obtained from grapevines (Chiarappa, 1959). Hawksworth et al. (1976) re-examined the strain CBS 239.74, received from Chiarappa, and concluded that this fungus could represent a new species. They based this conclusion on the fact that the conidiophore stipes were darker and more sharply differentiated from the paler phialides than those of *P. parasitica*. The conidia were also smaller,  $2.0\text{--}5.0 \times 0.7\text{--}2.5 \mu\text{m}$ , and mostly straight, whereas those of *P. parasitica* were larger,  $2.5\text{--}6.0\text{--}8.0 \times 1.0\text{--}2.0\text{--}4.0 \mu\text{m}$ , and more allantoid. These authors refrained from describing it as a new taxon owing to the lack of sufficient strains for detailed study.

The genera *Acremonium* Link:Fr. and *Phialophora* Medlar are generally considered to be heterogeneous (Schol-Schwarz, 1970; Gams, 1971; Cole and Kendrick, 1973). The structures used to discriminate spe-

cies in these complexes are poorly differentiated. For this reason, and due to a general lack of cultures of many of the species, there has been a tendency to assign intermediate strains to either one or the other of these generic aggregates. Schol-Schwarz (1970) included several species with curved conidia in *Phialophora*, most of which apparently will have to be excluded. Species with strongly curved, sickle-shaped conidia and thin, fast-growing colonies are distinctive and characteristic of anamorphs of *Gaeumannomyces* Arx & D.L. Olivier. For this group, which also includes *Cephalosporium maydis* Samra et al., no generic name is yet available. Less curved, allantoid conidia are described for the "*Phialophora lagerbergii* group".

The strains presently available for the *P. lagerbergii* group in the CBS collection have been reexamined. Only the ex type strains, CBS 266.33 of *P. lagerbergii* (Melin & Nannf.) Conant, and CBS 294.39 of *P. repens* (R.W. Davidson) Conant, can be recognized as representing these species. Both are similar to each other, having rather broadly spreading colonies with a brown center and broad hyaline margin; partly brown, incrustated vegetative hyphae, up to 5–6  $\mu\text{m}$  wide; and rather fine, sub-hyaline, ampulliform phialides that are often aggregated in dense clusters. Conidia are tiny, normally not exceeding  $5 \times 1 \mu\text{m}$  (at least when grown on oatmeal agar for not more than 2 wk). These species seem to take a rather isolated position in *Phialophora*; they can be easily distinguished from the "*P. parasitica* group" by their rather short and ampulliform phialides. The only remaining species with conspicuous ampulliform phialides and allantoid conidia that really fits in *Phialophora* is *P. botulisporea* G.T. Cole & W.B. Kendr., which has clustered, lageniform phialides with very pronounced collarettes and conidia,  $2.5\text{--}7.0 \times 1.0\text{--}2.0 \mu\text{m}$  (Cole and Kendrick, 1973).

*Phialophora bubakii* (Laxa) Schol-Schwarz was also described as having partly allantoid conidia. This species deviates from others in *Phialophora* not only by lacking a prominent collarette but, particularly, by acropleurogenous branching of the pigmented conidiophores. Slightly curved, allantoid conidia are also described for the anamorphs of *Ascocoryne* J.W. Groves & D.E. Wilson, for which the most adequate genus is *Coryne* Gray, with several other synonyms (Groves and Wilson, 1967; Roll-Hansen and Roll-Hansen, 1979). These fungi are characterized by slimy colonies, often with some purple pigmentation and a tendency to form gelatinous conidiomata in old colonies; the hyaline phialides lack any trace of a light-microscopically visible collarette. Another group of species with slimy, generally nonpigmented or only partially pigmented colonies (sometimes with brown chlamydospores), comprise the anamorphs of

*Coniochaeta* (Sacc.) Cooke, for which the genus *Lecythophora* Nannf. has been reintroduced by Gams and McGinnis (1983). These fungi are characterized by the predominant occurrence of intercalary phialides (adelophialides of Gams, 1971).

In a step towards establishing clearer generic boundaries, Gams and McGinnis (1983) erected the genus *Phialemonium* W. Gams & McGinnis to accommodate another group of fungi intermediate between *Acremonium* and *Phialophora*. *Phialemonium* is characterized by having hyaline conidiogenous structures, and prominent, slender, lateral conidiiferous pegs (if discrete phialides are present they are slender near the base and slightly inflated near the middle). Furthermore, its very narrow vegetative hyphae (0.5  $\mu\text{m}$  in width) also distinguishes *Phialemonium* from the otherwise similar *Lecythophora*.

There is an increasing amount of evidence, based on molecular data, that the type species of *Phialophora*, *P. verrucosa* Medlar, has affinities to the Herpotrichiaceae (Masclaux et al., 1995), a family of loculoascomycetes (Untereiner et al., 1995). This group of species differs widely from the *P. parasitica* group by having very darkly pigmented hyphae and conidiophores, and slower growing colonies. Moreover, there is no indication whatsoever of any relationship of the *P. parasitica* group with the Herpotrichiaceae.

As more isolates of *P. parasitica* and similar fungi accumulated, it became obvious that they form a cluster of related and morphologically well-defined species that cannot be adequately accommodated in *Phialophora*. Although they are dematiaceous, such as is true of *Phialophora*, their phialides are aculeate and the collarettes tend to be inconspicuous and not flaring, not really resembling *Phialophora* (FIGS. 1–6). Conidiophore stipes and part of the vegetative hyphae are obviously pigmented, unlike those of *Acremonium*. Furthermore, in an analysis of restriction fragment length polymorphism and sequence data of rDNA from various strains identified as *P. parasitica*, Yan et al. (1995) showed that this species represents a heterogeneous assortment of taxa, which is not related to other species classified in *Phialophora*. The aim of this study was, therefore, to clarify the taxonomic position of strains determined as *P. parasitica* that are associated with human infections and disease symptoms on a diversity of woody substrates.

After more than 30 years of collecting strains of these fungi at CBS, we are now in the position to describe a new genus for these fungi which, besides the type species, comprises five new species. It is worth noting that strains of four of these have been isolated from grapevine. Their causal effects in connection with Esca disease, singly and in association

