DNA phylogeny reveals polyphyly of *Phoma* section *Peyronellaea* and multiple taxonomic novelties

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Abstract: Species of the anamorph genus *Phoma* are commonly isolated from a wide range of ecological niches. They are notoriously difficult to identify due to the paucity of morphological features and the plasticity of these when cultivated on agar media. Species linked to *Phoma* section *Peyronellaea* are typified by the production of dictyochlamydospores and thus have additional characters to use in taxon delineation. However, the taxonomy of this section is still not fully understood. Furthermore the production of such chlamydospores also is known in some other sections of *Phoma*. DNA sequences were generated from three loci, namely ITS, actin, and β-tubulin, to clarify the phylogeny of *Phoma* taxa that produce dictyochlamydospores. Results were unable to support section *Peyronellaea* as a taxonomic entity. Dictyochlamydospore formation appears to be a feature that developed, or was lost, many times during the evolution of *Phoma*. Furthermore, based on the multigene analyses, five new *Phoma* species could be delineated while a further five required taxonomic revision to be consistent with the genetic variation observed.

Key words: actin, β-tubulin, coelomycetes, dictyochlamydospores, ITS, multigene phylogeny, taxonomy

INTRODUCTION

Although the genus *Phoma* Sacc. emend Boerema & Bollen is widely distributed and omnipresent, it is still poorly understood and generally considered to be a taxonomically difficult group of mitosporic ascomycetes. *Phoma* is characterized by the production of single-celled, hyaline conidia in monophialidic, doliform to flask-shaped conidiogenous cells in thinned pycnidia (Boerema and Bollen 1975). The present concept however also includes species that produce thick-walled pycnidia, or form septate conidia in addition to continuous conidia in pure culture (Boerema et al 2004). Specimens have been isolated mainly from soil and from a wide range of plant hosts where they reside as primary pathogens, opportunists, saprobes or endophytes (Aveskamp et al 2008). The existing subgeneric classification was defined by Boerema (1997) after a 40 y study of the morphological characters. The genus was divided into nine sections that are based on their morphological appearance: *Phoma*, *Heterospora*, *Paraphoma*, *Peyronellaea*, *Phyllostictoides*, *Sclerophomella*, *Plenodomus*, *Macrospora* and *Pilosa*. Although this subdivision is extremely helpful in identifying strains up to species level, it remains artificial because several taxa exhibit features that are representative of different sections.

One of the most confusing sections in this regard is *Peyronellaea*, even though it has been studied intensively (Boerema 1993, Boerema et al 1965, 1968, 1971, 1973, 1977, Morgan-Jones and Burch 1987, Morgan-Jones and White 1983, White and Morgan-Jones 1983, 1986, 1987). *Peyronellaea* was incorporated into genus *Phoma* in 1990 (van der Aa et al 1990). It accommodates fungi producing pycnidial conidiomata with phialidic conidiogenous cells as well as dictyochlamydospores, having both transverse and longitudinal septa. Three types of dictyochlamydospores are distinguished: (i) alternarioid to irregular botryoid, that is those that resemble the conidia of genus *Alternaria* and that often also develope in chains (Luedemann 1961); (ii) epicoccoid, resembling the

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conidia of the genus *Epicoccum* and (iii) pseudosclerotoid, resembling pseudosclerotia, often developing in aggregates of many unicellular chlamydospores (Boerema et al. 2004).

The section currently comprises 12 species and five infraspecific taxa (Boerema et al. 2004). However, several species accommodated in other sections of *Phoma* are also capable of producing comparable chlamydospores, including *P. gardeniae* (sect. *Paraphoma*), *P. eumartitana* and *P. narcissi* (sect. Heterospora), *P. multistriata* and *P. eufyrena* (sect. *Phoma*) and *P. zeae-maydis* and *P. boeremae* (sect. *Macrospora*). Furthermore *P. exigua* had been included erroneously in sect. *Peyronellaea*, but in contrast to the taxa mentioned above it does not produce multicellular chlamydospores (Boerema et al. 1977).

Several taxa that currently are incorporated in *Phoma* sect. *Peyronellaea* have features in common with other sections, or even with other genera. For example two species from North America, *P. americana* and *P. subglomerata*, are characterized by the incidental production of uniseptate conidia, a key character for species placed in *Phoma* sect. *Phyllostictoides* (van der Aa et al 1990). Furthermore one species, *P. episcocina*, produces thick-walled poroid pycnidia resembling those of *Phoma* sect. *Sclerophomella* (Boerema and de Gruyter 1998). Some strains of the type species of this section, *P. glomerata*, produce conidia that become pigmented after maturation. This feature is uncommon in *Phoma* and actually is regarded as a character of *Microsphaeropsis*, *Coniothyrium* or *Paraconiothyrium* (Verkley et al. 2004, Damm et al. 2008).

In the present paper we studied the genetic and morphological diversity among the taxa currently accommodated in *Peyronellaea*. A further aim was to clarify the phylogenetic relation with several chlamydospore-producing species currently accommodated in other sections or that still remain to be described.

MATERIALS AND METHODS

Cultural and morphological studies.—A total of 122 strains (Table 1) were obtained from the culture collections of CBS (Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands), PD (Plant Protection Service, Wageningen, the Netherlands), IMI (International Mycological Institute, Kew, UK) and LEV (Plant Health and Diagnostic Station, Auckland, New Zealand). Freeze-dried strains were revived overnight in 2 mL malt/peptone (50/50%) liquid medium. Cultures were transferred and maintained on oatmeal agar (OA, Gams et al. 2007) at 10 C and in complete darkness. Morphological studies of the strains were performed on OA, malt extract agar (MEA) and cherry decoction agar (CHA, Gams et al. 2007). Cultures were incubated as described in Boerema et al. (2004). Eight days after inoculation colony growth was measured. Colony colors were rated 15 d after incubation with Rayner’s color chart (1970). Morphological features were studied after sporulation. Fungal structures were mounted in tap water with a scalpel blade and examined under a Nikon 80i light microscope. Sizes of the various structures were determined by averaging the measurements of 30 examples of each structure, except for conidiogenous cells, of which the size range was estimated based on ca. five structures. Fifth and 95th percentiles were determined for all measurements and are provided in parentheses. The production of metabolite E+ was determined by application of a droplet of IN NaOH (Dorenbosch 1970, Noordeloos et al. 1993). The structure of the pycnidial wall and shape of conidiogenous cells were studied with microtome sections 6 µm thick, prepared with a Leica CM3050 freezing microtome and mounted in lactic acid. Taxonomic novelties and descriptions were deposited in MycoBank (www.mycobank.org, Crous et al. 2004).

Molecular studies. DNA extraction, PCR and sequencing.—Actively growing mycelium was scraped from culture plates and transferred to 2 mL collection tubes from the Ultra-Clean™ Microbial DNA Kit (MoBio Laboratories Inc., Carlsbad, California). DNA isolation was carried out according to the manufacturer’s recommendations. DNA samples were checked for purity and integrity by gel electrophoresis, after which the samples were diluted 10× and stored at 4 C before further handling. The ITS1-5.8S-ITS2 region (ITS) of the nuclear ribosomal DNA operon was amplified with the V9G (de Hoog and Gerrits van den Ende 1998) and ITS4 (White et al. 1990) primer pair. The actin gene (ACT) was partly amplified with primer pair ACT-512F and ACT-783R (Carbone and Kohn 1999). Two newly designed primers, TUB2Fd (5’- GTB CAC CTY CAR ACC GGY CAR TG – 3’) and TUB4Rd (5’- CCR GAY TGR CCR AAR ACR AAG TTG TG – 3’), were used to amplify a part of the β-tubulin (TUB) gene. For the two housekeeping genes ACT and TUB, each PCR reaction had a total volume of 12.5 µL and contained 0.5 µL 10× diluted gDNA, 1 × PCR Buffer, 2 mM MgCl2, 100 µM of each of the dNTP, 0.2 µM of each of the primers and 0.5 units Taq DNA polymerase (Bioline, Luckenwalde, Germany). The reaction mixture prepared for ITS amplification was similar, except for a double concentration of dNTP. The polymerase chain reactions (PCR) were conducted in a 2720 Thermal Cycler (Applied Biosystems, Foster City, California) with an initial denaturation at 95 C for 5 min, followed by 40 cycles of denaturation (95 C for 30 s), annealing (48 to 55 C for 30 s depending on the locus) and extension (72 C for 80 s). The final extension phase was conducted at 72 C for 7 min. Annealing temperatures varied per reaction and were set at 48 C, 52 C, and 55 C for ITS, TUB and ACT respectively.

Both strands of the amplified DNA fragments were sequenced with the same PCR primers and the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) according to the manufacturer’s recommendations. Sequence products were purified using a 96-well multiscreen HV plate (Millipore, Billerica, Massachusetts) and Sephadex G-50 superfine columns (Amersham Biosciences, Roosendaal, the Netherlands). The products were analyzed on an
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<td>FJ426936 FJ427046 FJ427156</td>
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<td><em>P. paspali</em></td>
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<td><em>Paspalum dilatatum</em></td>
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<td><em>P. pinodella</em></td>
<td>CBS 518.90</td>
<td><em>Pisum sativum</em></td>
<td>Netherlands</td>
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<td><em>Heracleum dissectum</em></td>
<td>Russia</td>
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<td><em>Triticum sp.</em></td>
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<td><em>P. schachtii</em></td>
<td>CBS 502.84</td>
<td><em>Heterodera schachtii</em></td>
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<td>CBS 179.80</td>
<td><em>Sorghum vulgare</em></td>
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<td>Guinea-Bissau</td>
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ABI Prism 3700 DNA Sequencer (Applied Biosystems). A consensus sequence was assembled from the forward and reverse sequences with the BioNumerics v4.5 software package (Applied Maths, St-Martens-Latem, Belgium). Sequences were deposited in GenBank (Table I).

Phylogenetic analysis.—The consensus sequences were aligned with BioNumerics and adjusted by hand where necessary. The best nucleotide substitution models were determined with MrModeltest v2.2 (Nylander 2004). A Bayesian tree inference (BI) analysis was performed with MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001). One tree was saved per 100 generations, and the run was automatically ended when the standard deviation of split frequencies was below 0.01. To avoid suboptimal trees being taken into account for the consensus tree, a burn-in of 25% of the saved trees was used. The resulting “50% majority rule consensus” trees were printed with TreeView v1.6.6 (Page 1996) and are lodged with TreeBASE (www.treebase.org).

To obtain further evidence for branch supports, a series of neighbor joining (NJ) analyses was conducted in PAUP (phylogenetic analysis using parsimony) v4.0b10 (Swofford 2003) with the uncorrected (“p”), the Kimura-2-parameter and the HKY85 substitution models. Alignment gaps were treated as missing data, and all characters were unordered and of equal weight. Ties were broken randomly.

A third measure of branch support was obtained by conducting a maximum likelihood (ML) analysis using RAxML (randomized accelerated maximum likelihood) software (Stamakis et al 2008) through the CIPRES Website (www.phylo.org). The same three partitions were used as in the BI and NJ tests, but because RAxML implements only the GTR substitution model the symmetrical model for the ITS partition was waived. The robustness of trees in the NJ and ML analyses was evaluated by 1000 bootstrap replications.

To test whether the three different loci could be used in a combined analysis phylogenies were estimated with maximum likelihood analyses for each data partition (ML bootstrap values > 70%) and compared by eye for congruency. Congruence of these trees was further determined with the Shimodaira-Hasegawa test (SH test, Shimodaira and Hasegawa 1999), which is implemented in PAUP. The topology of the concatenated ML tree was compared to the topology of the ML trees obtained for each partition in a one-tailed bootstrap test using 1000 replications with full likelihood maximization to determine whether the trees were significantly different.

The SH test also was used to determine whether the species that currently are linked with Phoma section Peyronellaea represent a monophyletic group. Therefore a constraint tree in which such a phylogeny was simulated was compared to the consensus tree obtained from the RAxML analysis of the ITS dataset. These trees subsequently were compared as described above.
RESULTS

ITS phylogeny.—Due to alignment difficulties of the housekeeping genes, two alignments of DNA sequences were subjected to phylogenetic analyses. The first alignment consisted of 122 ITS sequences generated in this study and six obtained from GenBank. This ITS alignment consisted of 566 characters including alignment gaps, of which 237 were variable and 329 were constant. A GenBank sequence of Pyrenochaeta romeroi (DQ836802) was used as outgroup. The BI analysis was run using the best model and parameters as determined, which were the symmetrical (SYM) substitution model with inverse gamma rates and equal dirichlet base frequencies. The temperature value set at 0.4. The analysis run of the ITS sequence matrix in MrBayes resulted in 11 039 trees, from which the burn-in was discarded and the consensus tree and posterior probabilities were calculated. The topology and support values of the BI tree were in congruence with those of the trees obtained by NJ and the optimal tree obtained in the ML analysis. The reconstructed phylogeny with the ITS dataset revealed 16 heterogeneous strains to be a paraphyletic basal assemblage to a major clade consisting of 106 strains (Fig. 1). The majority of the taxa belonging to Phoma sect. Peyronellaea were found in this major clade (support values 1, 100% and 95% for BI posterior probability, NJ and ML bootstrap supports respectively), although several type species of other Phoma sections also were accommodated here, such as P. herbarum (section Phoma), P. exigua var. exigua (section Phyllistictoides) and P. Zeae-maydis (sect. Macrospora). Further the Peyronellaea species P. chrysanthemicola and P. violicola were located among the basal lineages, indicating that section Peyronellaea does not represent a monophyletic clade. This is supported by the SH test conducted on the ITS dataset, in which the hypothesis that the tree (Fig. 1) is in congruence with monophyly of Peyronellaea is rejected (P < 0.01).

Concatenated phylogeny.—The second alignment included 104 taxa, including one outgroup taxon (CBS 560.81 P. paspali), which was found to be basal to the major clade (Fig. 1). No strongly conflicting nodes were detected in the phylogenies of the separate loci (ML bootstrap values > 70%). Topologies were congruent for each partition, although ITS showed a lower degree of resolution of the terminal taxa. Also the results of the SH tests (Table II) suggest that the ITS tree differs most from the concatenated tree, although this is not significant (P = 0.073). Based on the similarity in topologies and the nonsignificant SH tests, the partitions used in the second dataset (ITS, ACT, TUB) could be concatenated.

The concatenated alignment had a total length of 1148 characters (ITS: 500, ACT: 300, TUB: 348) including alignment gaps. Of these characters 383 (ITS: 96, ACT: 156, TUB: 131) were variable and 767 (ITS: 406, ACT: 144, TUB: 217) were constant. The SYM+I+G model was found to be optimal for the ITS partition, whereas the best substitution model for the ACT and TUB sequence matrix was determined to be GTR+I+G. The temperature value was set at 0.5 for the BI analysis. The MrBayes run of the second dataset resulted in 3340 trees, from which the burn-in was discarded and the consensus tree and posterior probabilities were calculated (Fig. 2). Trees supporting the same clades were obtained irrespective of the analysis method used. Further phylogenetic results are discussed below where applicable.

TAXONOMY

Most Peyronellaea taxa and other chlamydospore-forming species studied here appeared to be properly described in the past. However, five novel dictyochlamydospore-forming species of Phoma could be identified in the present study. These species are described below. One species, P. infossa, was already known to science, but its description is amended as it appeared to produce dictyochlamydospores. Furthermore five new combinations are proposed.

Phoma calidophila Aveskamp, Gruyter & Verkley, nom. nov. pro Sphaeronaema sahariense Faurel & Schotter. Mycobank MB512566.
Fig. 1. Fifty percent majority rule consensus tree from a BI analysis of ITS sequences of *Phoma* sect. *Peyronella* (n = 122). At the nodes the BI Posterior Probabilities are presented above the branch, and bootstrap percentages of the NJ analysis using the HKY85 substitution model and ML analysis are given below the branch. Branches that were less than 50% supported in the NJ and ML analyses are indicated with a hyphen, whereas asterisks indicate full support. The bar indicates the number of substitutions per site. The tree is rooted with *Pyrenochaeta romeroi*.
**Phoma calorpreferens** (Boerema, Gruyter & Noordel.) Aveskamp, Gruyter & Verkley, comb. nov. MycoBank MB512567.

= **Phoma pomorum** var. *calorpreferens* Boerema, Gruyter & Verkley, comb. nov. MycoBank MB512567.

**Notes.** This taxon was considered to be a warm-preferring variety of *P. pomorum* because it can grow at temperatures above 30°C. The phylogenetic studies however reveal that both taxa are only distantly related. Therefore *P. pomorum* var. *calorpreferens* is elevated to species level here. It shares many characters with *P. pomorum*, but pycnidia are generally smoother indicated with a hyphen, whereas asterisks indicate full support. The bar indicates the number of substitutions per site. The tree is rooted to *Phoma paspali* CBS 560.81.
and the conidial matrix is pinkish instead of cream-white (Boerema 1993). Furthermore conidia (4–)5–8.5(-12) × 2–3(-3.5) μm and chlamydopores (< 25 μm) are generally larger than those of *P. pomorum* (Boerema 1993).

**Phoma coffeae-arabicae** Aveskamp, Verkley & Gruyter, sp. nov. MycoBank MB512568  Figs. 3a–c, 4  Conidia ellipsoidea usque ovoidea, hyalina, continua, (4–)4.5–6(-7) × (2.5–)3–4(-4.5) μm, eguttulata, vel guttulis polaribus minutis 1–4. Chlamydosporae multicellulares immersae, pseudosclerotioideae, dictyosporae, intercalares, solitariae, (23–)40–100(-190) × (11–)15–30 μm.  

Pycnidia mostly solitary or in chains, on the agar surface or submerged, variable in shape and size, mostly ovoid but also (sub)globose or elongated, glabrous, (100–)150–310 × (100–)110–200(–240) μm, papillate or with an elongated neck, mostly uni- or bi-ostiolate. Ostioles variable in size, but sometimes

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**Fig. 3.** Two-week old colonies on OA (top), MEA (middle) and CHA (bottom). a–c. *Phoma coffeae-arabicae* CBS 123380. d–f. *P. infossa* CBS 123395. g–i. *P. microchlamydospora* CBS 105.95. j–l. *P. omnivirens* CBS 341.86. m–o. *P. sancta* CBS 281.83. p–q. *P. schachtii* CBS 502.84.
relatively wide (< 30 μm diam). *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 3–5 layers, 10–17 μm thick. *Conidiogenous cells* phialic, hyaline, simple, smooth, flask-shaped to globose, ca. 6–7.5 × 5.5–7 μm. *Conidia* ellipsoid to ovoid, thin-walled, smooth, hyaline, always aseptate, variable in length, (4–)4.5–6(–7) × (2.5–)3–4(–4.5) μm, eguttulate or with 1–4 minute apolar guttules. *Conidial matrix* salmon to flesh. *Multicellular chlamydospores* immersed, brown, pseudosclerotioid, dictyosporous, intercalary, solitary, but often with 2–3 elements on a single hypha, (23–)40–100(–190) × (11–)15–30 μm.

Colonies on OA 61–66 mm diam, with entire, smooth margins. Aerial mycelium sparse or absent, tufted, white. Immersed mycelium hyaline or greenish olivaceous, fuscous-black near center. Reverse concolorous. Colonies on MEA 57–70 mm diam, with entire, smooth, sharp margin. Aerial mycelium condensed, white with rosylvineaceous tinges. Agar surface iron-gray. Reverse fulvous to amber, but leaden black in zones with abundant pycnidia. Colonies on CHA similar growth rate to MEA. Aerial mycelium compact or tufted, primrose to citrine-green, pale greenish glaucous near center, and leaden-gray near margin. Reverse leaden-black.

**Etymology**: Named after the host from which it was isolated, *Coffea arabica*.


**Notes.** Multiple *Phoma* species have been found in association with *Coffea arabica*, such as *P. coffeicola*, *P. coffeiphila*, *P. costarricensis*, *P. excelsa*, *P. pereupyrena* and *P. tarda*. However none of those species produces multicellular chlamydospores, although unicellular, perennial structures have been described in *P. pereupyrena* (de Gruyter et al 1993). Furthermore the conidia of these species are more elongated than those of *P. coffae-arabicae* (Saccas 1981, Boerema et al 2004).

Although *Phoma coffae-arabicae* forms pseudosclerotioid chlamydospores, it is phylogenetically related to a group that mainly comprises *Peyronellaea* species forming alternarioid-botryoid chlamydospores (Fig. 2). It is easily recognized by its conspicuously...
wide ostiole, comparable to that of *P. macrostoma* (White and Morgan-Jones 1984).

**Phoma infossa** Ellis & Everh., J. Myc. 4:102. 1888.

*Pycnidia* mostly solitary on the agar surface, subglobose to elongated, but sometimes somewhat tapering toward the ostiolum, glabrous, (170–)190–250 (–305) × (105–)140–180(–200) μm. *Ostioles* mostly single, (22–)40–75(–105) μm diam, papillate, or with an erumpent and obtusely-conic neck. *Pycnidal wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 5–9 layers, 28.5–55 μm thick. *Micropycnidia* sometimes emerge from pycnidia but also solitary, globose to subglobose, 45–80 μm diam. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped, ca. 5.5–8(–11) × 5.5–7(–7) μm. *Conidia* from both pycnidial types indistinguishable, ovoid, thin-walled, smooth, hyaline but incidentally brown, aseptate, (4–)4.5–6 × 2.5–3.5 μm, eguttulate, or with (1–)3–6 minute polar guttules. Conidial matrix rosy-buff to salmon. *Multicellular chlamydospores* honey to cinnamon, commonly alternariaid-botryoid, dictyosporous, but sometimes also phragmosporous, solitary or coalescing into long chains of up to five elements, terminal on hyphae, but occasionally intercalary, abundantly in the aerial mycelium, 18–32(–55) × 11.5–17(–22) μm.

Colonies on OA 45–55 mm diam, with entire, smooth margins; aerial mycelium occurring in sections, tufted, floccose, lavender-gray or white, ca. 2–3 mm high; immersed mycelium gray to gray-olivaceous; near colony margin becoming hyaline or citrine, with zones of olivaceous-black mycelium. Reverse slate-blue with dark mouse-gray tinges. Colonies on MEA 42–49 mm diam, with entire, smooth, sharp margins. Aerial mycelium compact, tufted, smoke-gray, but olivaceous or primrose near the center and rosy-vinaceous near the margin; sometimes with zones in which the aerial mycelium is absent and where the surface is covered by abundant black pycnidia. Occasionally sectors occur with more developed white to pale mouse-gray aerial mycelium. Reverse black, but primrose near the center and sienna at the margins. Colonies on CHA similar to MEA, but with moderate aerial mycelium occurring; reverse violaceous-black.

Notes. The obtusely-conic, erumpent ostioles that are produced abundantly together with the simple, papillate ones are characteristic for *P. infossa*. This species is only rarely observed and has been found before on dead limbs of *Fraxinus* in New York state (Ellis and Everhart 1888). To our knowledge however this is the first time this species has been cultivated and preserved.

Phoma microchlamydospora Aveskamp & Verkley, sp. nov. MycoBank MB512569

Conidia subglobose usque ellipsoidae, hyalina, continua, (4–)4.5–6.5(–7) × 3.5–4.5(–5.5) μm, a *P. pimprina* guttulis majoribus differentia. Chlamydosporae unicellulares (sub)globoseae, 4.5–6.5 μm diam, intercalares, plerumque cate


**Pycnidia** solitary or confluent, globose, glabrous, dark mouse-gray to black, immersed or superficial on the agar surface, as well as in the aerial mycelium, (110–) 150–260(–380) × (110–) 150–260(–340) μm. **Ostioles** 1–3(–5), papillate, but often on an elongated neck. **Pycnidial wall** pseudoparenchymatous, composed of oblong to isodiametric cells, 2–5 layers, 10–18 μm thick. **Microsclerotia** abundant, pale brown, solitary, globose to elongated, (27.5–)35.5–71 × (27–)31–62(–70) μm. **Conidiogenous cells** phialidic, hyaline, simple, smooth, flask-shaped or broadly cymbiform, ca. 11 × 6 μm. **Conidia** from both pycnidial types indistinguishable, subglobose to ellipsoidal, hyaline, smooth, aseptate, (4–)4.5–6.5(–7) × 3.5–4.5(–5.5) μm, eguttulate or with up to 4(–6) small guttules. Conidial matrix rosy-buff to rosy-vinaceous. **Unicellular chlamydospores** (sub)globose, tan-brown, intercalary, often in chains, relatively small, 4.5–6.5 μm diam, with many small to medium-sized guttules. **Multicellular chlamydospores** sparse, botryoid-dictyosporous, brown, consisting of up to seven cells, globose, intercalary but sometimes laterally branched from hyphal strands, always solitary, 4–13 μm diam, eguttulate or with many medium-sized guttules.

**Conidia** from both pycnidial types indistinguishable, subglobose to ellipsoidal, hyaline, smooth, aseptate, (4–)4.5–6.5(–7) × 3.5–4.5(–5.5) μm, eguttulate or with up to 4(–6) small guttules. Conidial matrix rosy-buff to rosy-vinaceous. **Unicellular chlamydospores** (sub)globose, tan-brown, intercalary, often in chains, relatively small, 4.5–6.5 μm diam, with many small to medium-sized guttules. **Multicellular chlamydospores** sparse, botryoid-dictyosporous, brown, consisting of up to seven cells, globose, intercalary but sometimes laterally branched from hyphal strands, always solitary, 4–13 μm diam, eguttulate or with many medium-sized guttules.

Colonies on OA 36–40 mm diam, with entire, smooth,
sharp margins. Aerial mycelium normally absent, or dark aerial hyphae may appear near center. Immersed mycelium hyaline; reverse olivaceous. Sometimes with a saffron discoloration of the agar due to a diffusible pigment, which persists after application of NaOH. Colonies on MEA 28–34 mm diam, with entire, smooth, sharp margin. Immersed mycelium fuscous-black. Sometimes sectors with white compact aerial mycelium are present; reverse concolorous. Colony on CHA as on MEA, although sometimes a thin, pale olivaceous-gray to iron-gray mycelial mat is covering the surface.

*Etymology:* Named after its relatively small chlamydospores.

*Specimens examined:* UNITED KINGDOM. From leaves of *Eucalyptus* sp., 1994, A.M. Ainsworth (HOLOTYPE CBS H-20147) (culture CBS 105.95); From an unknown vegetable plant, 1990, D. Hyall (CBS H-20148) (culture CBS 491.90).

*Notes.* The chlamydospores of *Phoma microchlamydospora* are extremely small compared to most other botryoid dictyochlamydospora producing species, which produce on average structures 8–20 μm diam (Boerema *et al* 2004). Conidia are similar in shape and size to those of *P. pimprina*, but the guttules are larger in the present species. Phylogenetically, this species clusters with *P. calidophila*, although distinctive differences exist in pycnidial and chlamydospore morphology.


≡ *Sphaeronaema multirostratum* P.N. Mathur, S.K. Menon & Thirum., Sydowia 13:146. 1959 [as *Sphaeronaema multirostrata*].


For an extended synonymy see Boerema *et al* (2004).

*Pycnidia* solitary or confluent, globose to subglobose or irregular, glabrous, brown to black, superficial or immersed, variable in size, 150–350(–720) μm diam. *Ostioles* multiple, conspicuous (10–25 μm diam), nonpapillate or on elongated necks, up to 260 μm long. *Pycnidal wall* pseudoparenchymatous, composed of oblong to cylindrical or elongated cells, 4–5 layers, ca. 9–14.5 μm thick. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped. *Conidia* oblong to ellipsoidal, thin-walled, smooth, hyaline, aseptate, highly variable in size, (3.5–)4.5–6.5(–8.5) × (1.5–)2–2.5(–3) μm, with 0–3(–4) polar guttules. Conidial matrix white to buff or rosy-buff.

*Chlamydoospores* mostly unicellular, 5–15 μm diam, ellipsoidal to oblong to somewhat pyriform, olivaceous or pale brown with greenish guttules, solitary or in chains, intercalary but incidentally also terminal. A bunch of clustered unicellular chlamydoospores can be observed regularly, especially in older cultures. These structures are easily mistaken for pseudosclerotoid chlamydoospores as in *P. violicola*.

Colonies on OA (60–)65–70(–80) mm diam, with entire, smooth, sharp margins. Aerial mycelium sparse, floccose or tufted, white to gray or absent. Agar surface olivaceous to chestnut with colorless sectors. Reverse concolorous. Colony on MEA 60–75 mm diam, with entire, smooth, sharp margin. Aerial mycelium fely, floccose or wooly, olivaceous to olivaceous-buff. Agar surface glaucous-gray. Reverse leaden-gray to olivaceous-black. Colony on CHA 65–75 mm diam, with entire, smooth, sharp margin. Aerial mycelium floccose, white to gray, absent near the margin of the colony. Agar surface dark mouse-gray to greenish black. Reverse concolorous.


*Notes.* The three varieties of *P. multirostrata* recognized by Boerema (1986), var. *multirostrata*, *macrospora* and *microspora* can no longer be retained as separate taxonomic entities. Taxonomic characters distinguish these varieties insufficiently, forcing Boerema *et al* (2004) already to state that “intermediate variants commonly occur.” Furthermore no genetic differences consistent with those distinguishing the varieties were found in the DNA analysis in the present study. Therefore all varieties are synonymized with the original species, *P. multirostrata*.

*Phoma omnivirens* Aveskamp, Verkley & Gruyter, sp. nov. MycoBank MB512570 Figs. 3j–l, 7

Conidia subcyindrical usque ellipsoidea, hyalina, continua, (3.5–)4.5–5.5(–7) × (1.5–)2–2.5(–3) μm. Guttulis polaribus 1–2. Chlamydosporae unicellulares oblongae, plurumque in catenis longas posite, 7–14(–20) × (4–)4.5–8.5(–18) μm. Pluriguttulae. Chlamydosporae multicellulares irregulares, dictyosporae, botryoideae, intercalares, in agaro immersae, (12–)15–52.5(–70) μm diam.

*Pycnidia* solitary or confluent, immersed or on the agar surface, globose to slightly subglobose, with many hyphal outgrowths, dark brown to black, 100–260(–350) × (90–)100–240(–300) μm, uni-ostiolate, nonpapillate, papillate or sometimes with a broad, elongated neck, giving the pycnidium a somewhat
ovoid appearance. Pycnidial wall pseudoparenchymatous, composed of isodiametric to elongated cells, 2–6 layers, 10.5–16.5(–17.5) μm thick. *Microycnidia* if present, generally darker than the regular pycnidia, solitary or confluent, globose, obpyriform or elongated, (40–)65–120 × (40–)60–100 μm. Conidiogenous cells phialidic, hyaline, simple, smooth, globose to flask-shaped, ca. (4.5–)5–6 × 4.5–5.5 μm. Conidia from both pycnidial types indistinguishable, subcylindrical to ellipsoidal, thin-walled, smooth, globose to aseptate, (3.5–)4–5.5(–7) × (1.5–)2–2.5(–3) μm, with (1–)2 small to medium-sized polar guttules; conidial matrix buff. Submerged hyphae smooth, hyaline, thin-walled, but often becoming pigmented and swollen, attaining a width of up to 9.5 μm. *Unicellular chlamydospores* oblong, brownish, often in long chains, 7–14(20) × (4–)4.5–8.5(–18) μm, with many guttules in each cell. *Multicellular chlamydospores* consisting of agglomerates of unicellular chlamydospores, irregularly shaped, dictyosporous, botryoid, brownish, intercalary, submerged in the agar, (12–)15–52.5(–70) μm diam.

Colony on OA 35–60 mm diam, with entire, smooth, sharp margin. Aerial mycelium tufted, floccose to compact, locally well developed, white to (pale-)olivaceous gray, sometimes greenish olivaceous near margin. Immersed mycelium dark mouse-gray to leaden-black, toward the colony margin the color fades away to dull-green and white. Reverse concolorous or greenish black. Often an amber, primrose or buff diffusible pigment can be observed on the agar. Colony on MEA 28–52 mm diam; margins entire, smooth, sharp, or lobate to crenate. Aerial mycelium, floccose, wooly or compact, white or with various shades of gray (pale mouse-gray, olivaceous-gray, iron-gray). Reverse olivaceous-gray to leaden-black. After application of NaOH the agar color changes to bright green. Colony CHA as on MEA, but aerial mycelium less well developed.

**Etymology:** Name refers to the omnipresence of this species, which has been isolated from a wide range of hosts and geographical locations.

**Specimens examined:** BELGIUM, Gembloux. From *Phaeseolus vulgaris*, 1968, L. Obando (HOLOTYPE CBS H-20151) (culture CBS 341.86); INDIA, Japalbur. From an unknown substrate, 1977, D.P. Tiwari, (CBS H-20152) (culture CBS 654.77); PAPUA NEW GUINEA, Varirata National Park. From soil, Aug 1995, A. Aptroot, (CBS H-20153) (culture CBS 991.95); Varirata National Park. From...
Chrysanthemum indicum

This species has been isolated from a wide variety of substrates and from geographically distinct locations. Isolates have been identified erroneously as *P. cyanea* due to the similarity in shape of the chlamydospores, but this species is distinguishable by the absence of pink or reddish pigments in the colony.

**Phoma pomorum var. circinata** (Kusnezowa) Aveskamp, Gruyter & Verkley, comb. nov. MycoBank MB512571.


= *Phoma jolyana* var. circinata (Kusnezowa) Boerema, Dorenb. & Kesteren, in Kew Bull. 31:535. 1977 [1976].


For detailed descriptions see Boerema et al (1977), Boerema (1993) and Morgan-Jones and Burch (1987).


*Notes.* This taxon, which was seen as a variety of *P. jolyana*, differs by only one nucleotide in the ITS sequence from *P. pomorum var. pomorum* (CBS 539.66), whereas both ACT and TUB sequences do not show any consistent differences. Nevertheless this taxon is distinct morphologically. *Phoma pomorum var. circinata* has somewhat larger conidia, (3.5–)5–9×2–3.5 μm than the type var., (4–)5–7×1.5–2.5–3 μm (Boerema 1993). Furthermore unicellular chlamydospores are absent in *P. pomorum var. circinata*. Thus far strains have been reported only from Novosibirsk, Russia.

**Phoma pomorum var. cyanea** (Jooste & Papendorf)

Aveskamp, Gruyter & Verkley, comb. nov. MycoBank MB512572.


For detailed descriptions see Jooste and Papendorf (1981) and Boerema (1993).

*Specimen examined:* SOUTH AFRICA, Heilbron. From straw of *Triticum* sp., 1972, W.J. Jooste, holotype PREM 45736, (culture CBS 388.80).

*Notes.* *Phoma pomorum var. cyanea* is a species that thus far has been reported only from South Africa. It is easily distinguishable from *P. pomorum var. pomorum* by the production of a bluish pigment in the hyphae, pycnidia and chlamydospores. The remaining morphological characters however fit within the scope of *P. pomorum*. Furthermore the sequence analyses in the present study show a 100% similarity on ITS, ACT and TUB between the two taxa. It is concluded therefore that *P. cyanea* should be reduced to a variety of the older *P. pomorum*, as *P. pomorum var. cyanea*.

**Phoma sancta** Aveskamp, Gruyter & Verkley, sp. nov. MycoBank MB512573

Figs. 3m–o, 8.

Conidia ovoidea, hyalina, continua, 5–7(–7.5) × 2.5–4(–4.5) μm, guttulis polaribus 3–9(–12). Chlamydosporae multicellulares alternariodeae, phragmosporae vel dictyosporae, (11–)16–26(–30) × (6.5–)7.5–11(–13.5) μm, solitariae, terminales, in hyphis aeris brevibus formatae.

*Pycnidia* solitary or confluent, globose, glabrous or covered with short hyphal outgrowth, superficially on the agar and in aerial mycelium, (80–)125–260 μm diam, conspicuously papillate; ostioles 1(–2), 20–40 (–60) μm diam. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 3–7 layers, relatively thick, 21–43(–51) μm thick. *Microspycnidia* formed in the aerial mycelium, generally paler than regular pycnidia or even hyaline, solitary, (sub)globose, (40–)60–80(–110) μm diam. *Conidiogenous cells* phialidic, hyaline, simple, smooth, globose to flask-shaped, (5–)6–7×(5–)5.5–6.5 μm. *Conidia* ovoid, thin-walled, smooth, hyaline, aseptate, 5–7(–7.5) × 2.5–4(–4.5) μm, with 3–9(–12) polar guttules. *Conidial matrix salmon*.

*Chlamydosporae* multicellular, alternariae, phragmosporae or dictyosporae, (11–)16–26(–30) × (6.5–)7.5–11(–13.5) μm, dark brown, terminal on erect aerial hyphae, solitary.

Colonies on OA 45–60 mm diam, with entire, smooth, sharp margins. Aerial mycelium sparse or absent, tufted, gray to white. Immersed mycelium fawn, but fading away to gray-olivaceous, becoming hyaline near margin; reverse concolorous. After application of NaOH the agar near the hyphae becomes inconspicuously reddish brown. Colonies on MEA 52–57 mm diam, with entire, smooth, sharp margins. Aerial mycelium greenish olivaceous to white, floccose and abundant near center, toward the margin less well developed. Immersed mycelium iron-black with or without vinaceous sectors. Reverse concolorous. Colonies on CHA similar to MEA, but aerial mycelium less well developed.

*Etymology:* Named because of its association with the hosts *Gleditsia triacantha* (Christusdoorn in Dutch, meaning Christ’s thorn) and *Ailanthus altissima*, tree of heaven.

*Specimen examined:* SOUTH AFRICA. From dead

*Notes.* *Phoma sancta* appears to be widespread, and clusters within a group in which among others *P. glomerata*, *P. pomorum* and *P. jolyana* are accommodated. This species is recognizable by the high percentage of phragmospores that are formed in culture. The latter feature might have been the reason for the previous identification as *P. jolyana*. The latter species produces its chlamydospores mainly in the agar and in the aerial mycelium on a wide range of media, whereas the multicellular chlamydospores of *P. sancta* are formed mainly on OA, and are terminally located on short, erect hyphae emerging from the agar surface.

*Phoma schachtii* Aveskamp, Gruyter & Verkley, sp. nov. MycoBank MB512574 Figs. 3p–r, 9

Conidia ellipsoidea, hyalina, continua, (4–)4.5–5.5(–6) × (1.5–)2–2.5 μm, eguttulata, vel guttulis polaribus 2(–3). Chlamydosporae multicellulares dictyosporae, alternarioideae vel botryoidae, (15.5–)31–81.5(–101.5) × (9.5–)19–50.5(–63) μm diam, viridulae, terminales, solitariae vel in catenas breves positae, in culturis vestioribus confluent aggregatae et pseudosclerotioideae.


*Pycnidia* solitary or confluent, globose, completely covered with hyphal outgrowths, submerged in the agar, (180–)220–600(–650) μm diam, papillate, or with an elongated neck, and a single inconspicuous ostiole. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric to oblong cells, 4–9 layers, (22.5–)26.5–37(–41.5) μm thick. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped, ca. 5–7 × 4–6 μm. *Conidia* ellipsoidal, thin-walled, smooth, hyaline, aseptate, (4–)4.5–5.5(–6) × (1.5–)2–2.5 μm, eguttulate or with 2(–3) polar guttules. Conidial matrix cream white. *Multicellular chlamydospores* developing after several weeks, dictyosporous, alternarioid or botryoid, abundant in the aerial mycelium, (15.5–)31–81.5(–101.5) × (9.5–)19–50.5(–63) μm diam, greenish, terminal, single or in short chains with up to three elements, in older cultures aggregating into pseudosclerotioideae.

Colonies on OA 26–32 mm diam, with entire, smooth, sharp margins; aerial mycelium felted, mostly olivaceous-gray, near center iron-gray and smoke-gray near margins. Reverse olivaceous-gray with some olivaceous zones. Colonies on MEA 20–24 mm diam, with entire, smooth, sharp margins. Aerial mycelium...
felted or floccose to tufted, greenish gray or pale olivaceous-gray. Reverse olivaceous-black to dark slate-blue, near margin somewhat brown-vinaceous. Colonies on CHA 28–32 mm diam, with entire, smooth, sharp margins, covered by a compact or felted mycelial mat, olivaceous-gray to fuscous, near the center mouse-gray. Reverse concolorous.

Etymology: Named after the host species on which the fungus was found, a cyst of the nematode Heterodera schachtii.


Notes. At least nine Phoma species, of which most are capable of producing chlamydospores, have been isolated from the cysts of Heterodera spp. (Chen et al 1996). Phoma schachtii, which has been found parasitizing a cyst nematode, has many characters in common with P. chrysanthemicola, which explains why it has not been recognized previously as a separate taxon. The two species can be distinguished by the clear alternarioid-botryoid chlamydosporas that are present in fresh cultures. In later stages these will aggregate and form long pseudoscleroid masses. Those masses are generally smoother than in P. chrysanthemicola, which has more warty chlamydospore walls.

DISCUSSION

In their final publication after more than 40 y of morphological studies on genus Phoma, Boerema and co-workers listed the 225 specific and infraspecific taxa that they recognized (Boerema et al 2004). Since the publication of this identification manual several studies on Phoma species have been conducted with DNA sequence phylogenies, revealing Phoma to be a more complicated genus than previously considered (Reddy et al 1998; Torres et al 2005a, b). In addition to the unclear generic definition (Aveskamp et al 2008) the current morphology-based subdivision of Phoma appears not to be in congruence with its molecular phylogeny.

Two species that are regarded members of section Peyronellaea (viz. P. chrysanthemicola and P. violicola) and the newly described species P. schachtii do not group with the majority of the Peyronellaea species in clade 1 but are found among the basal lineages,
together with the type species of Phoma sections Heterospora, Paraphoma and Plenodomus (Fig. 1). Characters that are considered to be typical for these sections, namely pluriform conidia, setose pycnidia or scleroplectenchyma respectively, were never observed in P. chrysanthemica, P. violicola or P. schachttii. These species all are characterized by the formation of chlamydospores in so-called pseudosclerotoid masses.

Most dictyochlamydospore-producing taxa cluster together with P. herbarum, the type species of genus Phoma and as a consequence also of section Phoma in clade 1. Further these taxa cluster with the type species of two other sections, namely P. exigua var. exigua (sect. Phyllostictoides) and P. zeae-maydis (sect. Macrophora) (Fig. 2). Chlamydospores produced by the taxa in this cluster represent the botryoid and alternariaoid types, except for those of the novel species P. coffeeae-arabicae, which are pseudoscleroticoid. Based on these results the subdivision of genus Phoma (Boerema 1997) therefore can be questioned. This observation is in congruence with the study of Torres et al (2005a), who found major inconsistencies between the system of Boerema (1997) and their molecular data and advocated that the current taxonomy of the genus Phoma needs to be thoroughly revised.

The Phoma anamorph state is found in multiple Pleosporalean teleomorphs, including Didymella, Leptosphaeria and Pleospora (Aveskamp et al 2008). The backbone structure (Fig. 1) can be explained largely by the clustering of Peyronellaea species with the different teleomorph groups. Most species studied cluster with P. zeae-maydis (teleomorph D. zeae-maydis) in clade 1, indicating that Didymella would be the most likely teleomorph for those species if a sexual state were encountered. Phoma violicola and P. schachtii are found in clade 2a, in which P. lingam also is accommodated, which has a teleomorph in Leptosphaeria. Clade 2b represents the Pleospora-associated clade. In clade 3 three species are grouped for which thus far no teleomorph has been recorded. Species in this clade represent sections Peyronellae (P. chrysanthemica), Heterospora (P. samavorum) and Paraphoma (P. radicina, type species of its section). Clades found in this study resemble some of the groups found in Schoch et al (2006) in the Pleosporales. The phylogenetic distances between clades 1 and 2 were observed by Reddy et al (1998) and Torres et al (2005b) and forced these authors to advocate the reinstatement of the anamorph genus name Plenodomus for the Leptosphaeria-associated species. However, such a taxonomic recombination requires further evaluation of all Phoma species and associated genera.

Phoma identification is problematic and gives rise to many misidentifications (Bridge et al 2003), but most strains studied here have been classified properly. Strains that could not be identified upon collection due to overlapping species characters now can be delimited and defined with molecular characterization tools. In the present study we recognize five novel dictyochlamydospore-forming species that were preserved in culture collections under incorrect names or as unidentified species. New combinations in a further five taxa were made to ensure consistency with the DNA data obtained in the present study. The species concepts defined in the past appear to be still valid for P. americana (Morgan-Jones and White 1983, Boerema 1993), P. epicoccina (Boerema 1993, Arenal et al 2000), P. glomerata, P. pomorum var. pomorum (Boerema et al 1965, Boerema 1993), P. chrysanthemica, P. pimprina, P. subglomerata, P. violicola and P. zantedeschiae (Boerema 1993). Also P. sorghina (Boerema et al 1968, White and Morgan-Jones 1983) appears to be properly described and represents a monophyletic clade, although a high level of infraspecific genetic variation has been observed. Only two strains (CBS 991.95 and CBS 992.95) were morphologically and genetically clearly distinct and are reclassified in the novel species P. omnivirens here. The remaining 13 species clustered together in a P. sorghina superclade, in which no less than nine different, often well supported subclades are recognized (Fig. 2). The morphological variation was sparse however, and all strains fitted within the scope of the species as described by Boerema et al (1973) and White and Morgan-Jones (1983). Also the host association and the origin are too diverse to provide further information on a possible further classification. The high genetic variety in comparison to P. glomerata, for example, might indicate a high recombination rate. Sexual recombination, although a teleomorph has never been observed, might be one of the reasons for this phenomenon.

Much confusion still surrounds the identity of P. jolyana. A relatively wide species concept had been applied to this taxon (Boerema et al 1965, Morgan-Jones and Burch 1987), which gave rise to many incorrectly identified isolates. At least two new taxa were encountered among the strains that initially were stored in the CBS and PD culture collections as P. jolyana and are renamed P. coffeeae-arabicae and P. sancta in this study. Three varieties previously were recognized within this species, of which the type variety was widespread, whereas var. circinata and sahariensis had been collected on only a few occasions from isolated places (Boerema et al 2004). In this study both varieties have been recombined: var. sahariensis is elevated to species level as P. calidophila, whereas var. circinata has been recombined to a variety of P. pomorum. Given the isolated origins of
these isolates we expect that many more dictyochlamydospore-producing taxa will be encountered once these origins are sampled more.

This study also addresses the problem that single morphological characters cannot always be discriminative between taxa. A good example is the genetic similarity of *P. pomorum* and *P. cyanea*. Although *P. cyanea* was easily distinguishable due to the obvious production of a bluish pigment in its hyphae, pycnidia and chlamydospores, sequence analysis proved it to be highly similar to *P. pomorum*. Because several other morphological characters showed high similarity between the two taxa it was concluded that *P. cyanea* should be reduced to a variety of the older *P. pomorum*.

The taxa that clustered in section *Peyronellaea* resemble a genetically heterogenous group. The ability to produce dictyochlamydospores probably has been lost and gained multiple times in the evolution of the *Pleosporales*. This character is also easily lost in culture, as has been reported in literature (Boerema et al. 1965, Dorenbosch 1970). Chlamydospore production in fungi is generally considered to be a survival strategy due to harsh conditions by perennation (Kirk et al. 2008). Although the strains used in this study were collected from a wide variety of environments, a relatively high number was retrieved from plant material belonging to the Gramineae. Also many of the chlamydospore-forming species have been found in association with cyst nematodes (Heteroderidae, Chen et al. 1996). It is tempting to link the similarity in hosts with the capability to produce chlamydospores. Therefore it very well might be that production of such thick-walled spores serves ecological purposes other than long-term survival. Further research should be conducted on the functioning of these structures.

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LITERATURE CITED


———, Bollen GJ. 1975. Conidiogenesis and conidial septation ads differentiating criteria between *Phoma* and *Ascochyta*. Persoonia 8:111–144.


de Gruyter J, Noordeloos ME, Boerema GH. 1993. Contributions toward a monograph of *Phoma* (Coelomycetes) I–2. Section *Phoma*: additional taxa with very
small conidia and taxa with conidia up to 7 μm long. Persoonia 15:369–400.
de Hoog GS, Gerrits van den Ende AHG. 1998. Molecular
diagnostics of clinical strains of filamentous Basidio-
Dorenbosch MMJ. 1970. Key to nine ubiquitous soil-borne
Ellis JB, Everhart BM. 1888. New species of fungi from
Gams W, Verkley GJM, Crous PW. 2007. CBS Course of
Mycology. 5th ed. Utrecht, the Netherlands: Centraal-
bureau voor Schimmecultures.
Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian
inference of phylogenetic trees. Bioinformatics 17:
754–755.
Jooste WJ, Papendorf MC. 1981. Phoma cyanea sp. nov. from
Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008.
Ainsworth & Bisby’s Dictionary of the Fungi. 10th ed.
Luedemann GM. 1961. The dictyochlamydospore of
Peyronellaea glomerata (Corda) Goidanich ex Togliani
contrasted with the dictyospore of Alternaria tenuis
Phoma IX. Concerning Phoma jolyana. Mycotaxon 30:
239–246.
Phoma americana sp. nov. Mycotaxon 16:403–413.
Noordeloos ME, de Gruyter J, van Eijk GW, Roeijmans HJ.
1993. Production of dendritic crystals in pure cultures
of Phoma and Ascochyta and its value as a taxonomic
character relative to morphology, pathology and
Nylander JAA. 2004. MrModeltest 2.2. Program distributed
by the author. Uppsala, Sweden: Evolutionary Biology
Centre, Uppsala University.
Page RDM. 1996. Treeview: an application to display
phylogenetic trees on personal computers. Bioinfor-
matics 12:357–358.
Rayner RW. 1970. A mycological colour chart. Kew, UK:
Commonwealth Mycological Institute and British My-
cological Society.
Reddy PV, Patel R, White JF. 1998. Phylogenetic and
developmental evidence supporting reclassification of
cruciferous pathogens Phoma lingam and Phoma
Saccas AM. 1981. Etude de la flore cryptogamique des
cafeiers en Afrique Centrale. Paris, France: Institut
Français du Café et du Cacao.
Schoch CL, Shoemaker RA, Seifert KA, Hambleton S,
Spatafora JW, Crous PW. 2006. A multigene phylogeny
of the Dothideomycetes using four nuclear loci. Mycolog-
ia 98:1041–1052.
Shimodaira H, Hasegawa M. 1999. Multiple comparisons of
log-likelihoods with applications to phylogenetic infer-
bootstrap algorithm for the RAxML Web-Servers. Syst
Biol 75:758–771.
Swofford DL. 2003. PAUP*: phylogenetic analysis using
Torres MS, White JF, Cazares G, Bergen M, Bischoff JF,
placement in the Didymella/Phoma complex (Phaeo-
———, Bergen M, Singh S, Bischoff JF, Sullivan RF, White
JF. 2005b. Plenodomus morganjonesii sp. nov. and a
discussion of the genus Plenodomus. Mycotaxon 93:
333–343.
vander Aa HA, Noordeloos ME, de Gruyter J. 1990. Species
concepts in some larger genera of the Coelomycetes.
Stud Mycol 32:3–19.
Paraconiothyrium, a new genus to accommodate the
mycoparasite Coniothyrium mimitans, anamorphs of
Paraphaeosphaeria, and four new species. Stud Mycol
50:323–335.
Phoma II. Concerning Phoma sorgi. Mycotaxon 18:
5–13.
Concerning Phoma macrostoma. Mycotaxon 20:197–
204.
and direct sequencing of fungal ribosomal RNA genes for
phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ,
White TJ, eds. PCR Protocols: a guide to methods and