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Neophaeosphaeria and *Phaeosphaeriopsis*, segregates of *Paraphaeosphaeria*

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Two new genera, *Neophaeosphaeria* and *Phaeosphaeriopsis*, are described to accommodate species of *Paraphaeosphaeria* that are not congeneric based on morphological characters and results of 18S rDNA sequence analyses. *Paraphaeosphaeria s. str.* is restricted to species with two-septate ascospores and anamorphs that produce non-septate, smooth, pale brown conidia enteroblastically from phialides which have some periclinal thickening. Species in *Neophaeosphaeria* have 3–4-septate ascospores and anamorphs that produce ovoid to ellipsoid, non-septate, brown, verrucose or punctate conidia from percurrently proliferating conidiogenous cells. *Paraphaeosphaeria barrii*, *P. conglomerata*, *P. filamentosa* and *P. quadriseptata* are transferred to *Neophaeosphaeria*. At present all species in *Neophaeosphaeria* occur on *Yucca* (*Agavaceae*). *Phaeosphaeriopsis* is described for species that produce 4–5-septate ascospores. Known anamorphs produce cylindrical, 0–3-septate, brown, punctate conidia from percurrently proliferating conidiogenous cells or bacillar conidia from simple phialides. *P. agavensis*, *P. glauco-punctata*, *P. nolinae* and *P. obtusispora* are transferred to *Phaeosphaeriopsis*. *P. amblyspora* is described as a new species.

INTRODUCTION

Paraphaeosphaeria O. E. Erikss. 1967 was established to accommodate species similar to *Phaeosphaeria* Miyake 1909 but producing ascospores with an inflated cell immediately above the A1 septum, a longer more highly septate proximal than distal hemispore, and a '*Coniothyrium* type (conidia brown, non-septate)' anamorph (Eriksson 1967). Although all known species occur on monocots, taxa differ in ascomatal structure, ascospore septation and size, and host. Described species of *Paraphaeosphaeria* also differ in anamorph characteristics, including conidiomatal structure, type of conidiogenesis, and conidial morphology. Some anamorphs are *Coniothyrium*-like whereas others are more typical of *Microsphaeropsis* Höhn. 1917 (Sutton 1980).

Previous work demonstrated that *Paraphaeosphaeria* is polyphyletic (Câmara *et al*. 2001, Checa *et al*. 2002) and the ten species studied grouped in at least three clades. Clade A (Câmara *et al*. 2001) included the type species *Para. michotii* (West.) O. E. Erikss. 1967 and *Para. pilleata* Kohlm., Volkm.-Kohlm. & O. E. Erikss. 1995, both of which produce 2-septate ascospores in thin-walled ascomata. The anamorphs of both species produce smooth-walled, pale brown conidia from inconspicuous phialides with some periclinal thickening, typical of *Microsphaeropsis*. They are phylogenetically distant and morphologically different from the eight other species studied (Câmara *et al*. 2001, Checa *et al*. 2002).

In this study, phylogenetic analyses of small subunit (SSU) nuclear ribosomal DNA (nrDNA) were used to confirm the placement of Clades B and C (Câmara *et al*. 2001) outside of *Paraphaeosphaeria*. Pairwise sequence comparisons of the complete internal transcribed spacer (ITS) 1 and 2 regions, including the 5.8S rDNA, in combination with morphological data were used to infer generic affiliations of eight other taxa previously placed in *Paraphaeosphaeria*.

MATERIALS AND METHODS

Morphometric studies

Results of morphological studies of the ten previously described species of *Paraphaeosphaeria* that are treated

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in this paper were reported elsewhere (Câmara *et al.* 2001, Checa *et al.* 2002). Cultures of the newly described *Phaeosphaeriopsis amblyspora* were obtained by excising an ascoma from host material, opening it in a drop of sterile tap water, isolating released asci, washing individual asci three times in sterile tap water, and placing them on the surface of Difco Potato Dextrose Agar (PDA) in Petri dishes. Conidia from a squashed conidioma from ascospore-derived cultures were suspended in a drop of sterile tap water on PDA and the suspension spread over the surface of PDA with a sterile glass rod. After 48 h, single germinating conidia were removed and placed on PDA.

Measurements were made from hand sections or squashes of material placed in a drop of tap water. Growth rates and cultural characteristics were determined from cultures growing at room temperature (RT) on PDA in Petri plates sealed with Parafilm. Cultures have been deposited in the Centraalbureau voor Schimmelcultures, Utrecht (CBS).

DNA extraction, purification and amplification

Fungal DNA was extracted with the DNeasy Plant Mini kit (Qiagen, Chatsworth, CA) according to the manufacturer's instructions using approximately 15 mg dried mycelium. The ITS regions were amplified from *P. amblyspora* with ITS5/ITS4 (White *et al.* 1990) in 50 µl reactions on a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA) under the following reaction conditions: approximately 10 ng of genomic DNA, 200 µM each dNTP, 2.5 units AmpliTaq Gold (Applied Biosystems), 25 pmol of each primer, 1.5 mM $MgCl_2$ and the supplied PCR buffer. The thermal cycler programme was as follows: 10 min at 95 °C followed by 40 cycles of 30 s at 94 °, 30 s at 55 °, and 1 min at 72 °, with a final extension period of 10 min at 72 °. The SSU genes were amplified under the same conditions using the primer combination NS1/NS8 (White *et al.* 1990).

After amplification, the PCR products were purified with QIAquick columns (Qiagen) according to the manufacturer's instructions. Amplified products were sequenced in each direction with the BigDye version 2.0 kit (Applied Biosystems) on ABI 3100 or ABI 377 automated DNA sequencers. The ITS products were sequenced using the ITS5 and ITS4 PCR primers and the SSU genes were sequenced using the NS1 through NS8 primers (White *et al.* 1990).

The ITS sequences of the ten other previously described species of *Paraphaeosphaeria* that are treated in this paper were obtained in previous studies (Câmara *et al.* 2001, Checa *et al.* 2002).

Sequence editing, alignment and analysis

Raw sequences were edited using Sequencher version 4.05 for Windows (Gene Codes, Ann Arbor, MI). Individual pairs of ITS sequences were aligned by hand, minimizing gapped positions, in GeneDoc version 2.6.001 (Nicholas, Nicholas & Deerfield 1997). To determine sequence differences and infer generic affiliations of taxa previously placed in *Paraphaeosphaeria*, pairwise base differences across the ITS1, 5.8S nrDNA and ITS2 regions were calculated with PAUP* 4.0b10 (Swofford 2000). Due to the difficulty of aligning ITS sequences across the divergent genera and families included in this study, phylogenetic analysis of ITS sequence data was not considered appropriate.

Small subunit nrDNA sequences were aligned manually in GeneDoc version 2.6.001 (Nicholas *et al.* 1997). Only *Paraphaeosphaeria filamentosa* and *Para. glauco-punctata* were included in the SSU nrDNA sequence analysis because they grouped in two different clades in previous studies (Clades B and C, respectively in Câmara *et al.* 2001, Checa *et al.* 2002) and they are designated the type species for the two new genera. The SSU alignment consisted of 35 taxa and 1069 positions of the 5′ end of the SSU nrDNA, with 94 parsimony informative characters. Small subunit nrDNA gene trees were inferred by neighbour-joining (NJ) using the Kimura 2-parameter distance as implemented in PAUP* 4.0b10 (Swofford 2000) and by maximum parsimony (MP) using the heuristic search option with the random addition sequence (10^3 replications) with the MULTREES setting in effect, unlimited MAXTREES and branch swapping (tree bisection–reconnection). All molecular characters were unordered and given equal weight during the analysis. Gaps were treated as missing data.

Relative support for branches was estimated with 10^3 bootstrap replications (Felsenstein 1985) with MULTREES off, unlimited MAXTREES and 10 random sequence additions for MP bootstraps. Accession numbers for SSU sequences are listed in Fig. 1. The ITS sequence for *Phaeosphaeriopsis amblyspora* generated in this study was deposited in GenBank as AY188993. The SSU sequence alignment was deposited in TREEBase as S860.

RESULTS

Sequence analyses

Maximum parsimony sequence analysis of the SSU nrDNA for representative members of the *Phaeosphaeriaceae*/*Leptosphaeriaceae* and other loculoascomycetes including four taxa placed in *Paraphaeosphaeria*, resulted in 119 equally parsimonious trees with the following statistics: length = 230, CI = 0.704, RI = 0.874, RC = 0.616, and HI = 0.297. One arbitrarily chosen tree is shown (Fig. 1) with thickened lines indicating branches present in the strict consensus of all 119 trees. Both MP and NJ analyses placed *Para. filamentosa* and *Para. glauco-punctata* with members of the *Phaeosphaeriaceae*/*Leptosphaeriaceae*, whereas *Para. michotii* and *Para. pilleata* were placed with members of the *Montagnulaceae*/*Massariaceae* (Fig. 1). Major taxon groupings were consistent in both MP and NJ analyses

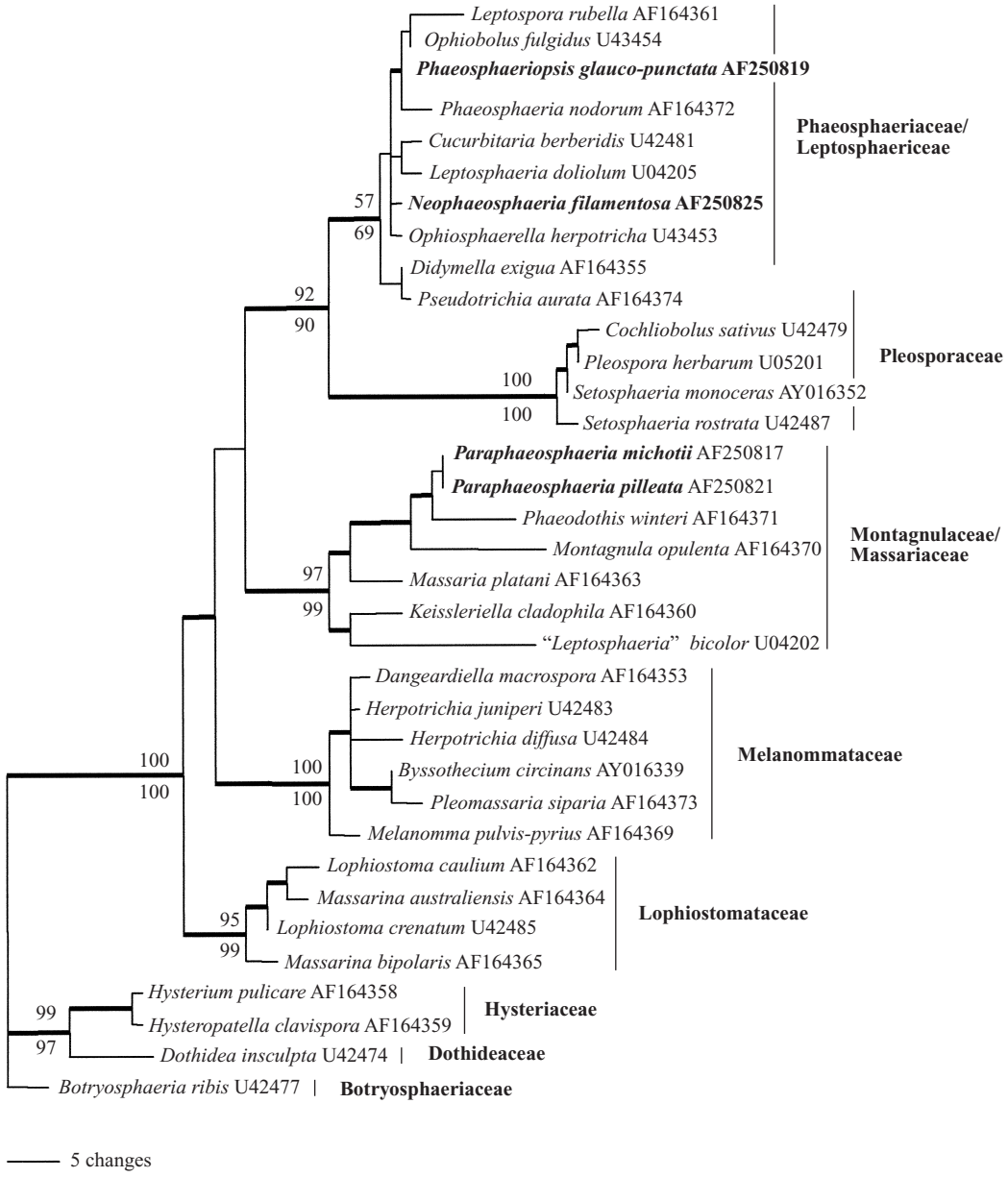


Fig. 1. One of 119 equally parsimonious trees resulting from analysis of 1069 bp of the 5′ small subunit nuclear rDNA of some loculoascomycetous fungi, including taxa previously placed in *Paraphaeosphaeria*. *Phaeosphaeriopsis glauco-punctata* and *Neophaeosphaeria filamentosa* are the type species of the newly described genera. Length = 230, CI = 0.704, RI = 0.874, RC = 0.616 and HI = 0.297. Numbers above and below branches indicate parsimony and neighbour-joining bootstrap supports, respectively, expressed as a percentage. Bootstrap percentages are included for family-level groups only. Thickened lines indicate branch was present in the strict consensus tree.

although minor differences in terminal branching of taxa were observed.

Para. filamentosa and *Para. glauco-punctata* were determined to differ in 21.4% (106/495) positions based on pairwise base differences in the ITS regions as calculated by PAUP* 4.0b10, which supported their placement into two different genera. Comparisons between *Para. glauco-punctata* and *Para. barrii* found 19.9% differing positions (100/502) indicating these two taxa are not congeneric. *Paraphaeosphaeria michotii* and *Para. pilleata* were not included in these comparisons because the SSU data clearly separate them from *Para. filamentosa* and *Para. glauco-punctata*.

Comparisons between *Para. filamentosa* and species that grouped with that species in Câmara *et al.* 2001 (Clade B) supported placement in the same genus. Base differences between *Para. filamentosa*, *Para. quadriseptata*, and *Para. conglomerata*, found 6.2% (31/504) and 3.6% (18/504) differences respectively. *Para. quadriseptata* and *Para. conglomerata* differed by 5.6% (28/504). Pairwise base comparisons between *Para. filamentosa* and *Para. barrii* found 10.5% positions differing (52/494). However, because *Para. barrii* contains a 21 bp insertion in the ITS1 not found in *Para. filamentosa* and not included in the calculation, this is a conservative estimate of the differences.

Comparisons between *Para. glauco-punctata* and four similar species (Clade C in Câmara *et al.* 2001), *Para. nolinae* AF250818, *Para. agavensis* AF250828, *Para. obtusispora* AF250822, and the undescribed taxon (*Phaeosphaeriopsis amblyspora*) found 9.1% (46/503), 12.2% (61/501), 7.3% (37/505) and 8.2% (41/503) differences, respectively. Comparisons among all combinations of taxa in this group ranged from 7.3 to 12.6% and sequences were easily alignable with one another. These results suggest that these species are congeneric.

TAXONOMY

Two new genera are described for species not congeneric with *Para. michotii* as determined in this study and others (Câmara *et al.* 2001, Checa *et al.* 2002).

Neophaeosphaeria Câmara, M. E. Palm & A. W. Ramaley, **gen. nov.**

Etym.: *neo* (Lat., new), refers to the similarity to *Phaeosphaeria*.

Similis *Paraphaeosphaeriae* sed ascosporae 3- vel 4-septatae, statu anamorpho similes *Coniothyrio*, cellulae conidiogenae percurrente prolificantes, conidia globosa, ovoidea, vel ellipsoidea, aseptata similitude ordinis DNA analysis connati.

Typus: *Neophaeosphaeria filamentosa* (Ellis & Everh.) Câmara, M. E. Palm & A. W. Ramaley 2003.

Ascomata immersed, subepidermal, usually erumpent at maturity and pushing up flaps of the epidermis, globose to sphaeroid to pyriform, often papillate, solitary or gregarious in a stroma, stroma of scleroplectenchyma or dark brown *textura angularis* or *textura prismatica*, often surrounded by septate, brown hyphae extending into the host tissues. *Asci* bitunicate, cylindric. *Ascospores* cylindric, broadly rounded at the apex and tapering to a more narrowly rounded base, 3- or 4-septate, first septum submedian, often constricted, brown, punctate to verrucose.

Anamorph: *Coniothyrium*-like. *Conidiomata* pseudoparenchymatous, sometimes stromatic. *Conidiogenous cells* lining entire locule, conidiogenesis holoblastic, proliferating percurrently, usually resulting in conspicuous annellations. *Conidia* globose, ovoid or ellipsoid, aseptate, yellowish brown often becoming brown at maturity, verrucose to punctate.

Neophaeosphaeria filamentosa (Ellis & Everh.) Câmara, M. E. Palm & A. W. Ramaley, **comb. nov.**

Basionym: *Leptosphaeria filamentosa* Ellis & Everh., *J. Mycol.* **4**: 76 (1888).

Paraphaeosphaeria filamentosa (Ellis & Everh.) M. E. Barr, *Mycotaxon* **43**: 392 (1992).

Neophaeosphaeria barrii (Checa) Checa, **comb. nov.**

Basionym: *Paraphaeosphaeria barrii* Checa, *Mycol. Res.* **106**: 377 (2002).

Neophaeosphaeria conglomerata (M. E. Barr) Câmara, M. E. Palm & A. W. Ramaley, **comb. nov.**

Basionym: *Paraphaeosphaeria conglomerata* M. E. Barr, *Mycotaxon* **46**: 508 (1993).

Neophaeosphaeria quadriseptata (M. E. Barr) Câmara, M. E. Palm & A. W. Ramaley, **comb. nov.**

Basionym: *Paraphaeosphaeria quadriseptata* M. E. Barr, *Mycotaxon* **43**: 394 (1992).

Phaeosphaeriopsis Câmara, M. E. Palm & A. W. Ramaley, **gen. nov.**

Etym.: *-opsis* (Greek, resembling), refers to a resemblance to *Phaeosphaeria*.

Similis *Paraphaeosphaeriae* sed ascosporae 4- vel 5-septatae, similitude ordinis DNA analysis connati.

Typus: *Phaeosphaeriopsis glauco-punctata* (Grev.) Câmara, M. E. Palm & A. W. Ramaley 2003.

Ascomata immersed, subepidermal, usually erumpent at maturity and pushing up flaps of the epidermis, globose to subglobose to pyriform, often papillate, solitary or gregarious in a stroma of scleroplectenchyma or dark brown *textura angularis* or *textura prismatica*, often surrounded by septate, brown hyphae extending into the host tissues. *Asci* bitunicate, cylindric. *Ascospores* cylindric, broadly rounded at apex, tapering to narrowly rounded base, 4–5-septate, first septum submedian, often constricted, brown, echinulate, punctate or verrucose.

Anamorph: *Coniothyrium*-like or *Phaeostagonospora* (Ramaley 1997). *Conidiomata* pseudoparenchymatous, sometimes of scleroplectenchyma. *Conidiogenous cells* lining locule; conidiogenesis holoblastic, proliferating percurrently resulting in inconspicuous annellations. *Conidia* cylindrical, often truncate at ends, 0–3-septate, yellowish brown, punctate. Sometimes only bacillar microconidia produced from simple, apparently non-proliferating phialides.

Phaeosphaeriopsis glauco-punctata (Grev.) Câmara, M. E. Palm & A. W. Ramaley, **comb. nov.**

Basionym: *Cryptosphaeria glauco-punctata* Grev., *Fl. Edin.*: 362 (1824).

Paraphaeosphaeria glauco-punctata (Grev.) Shoemaker & C. E. Babc., *Can. J. Bot.* **63**: 1286 (1985).

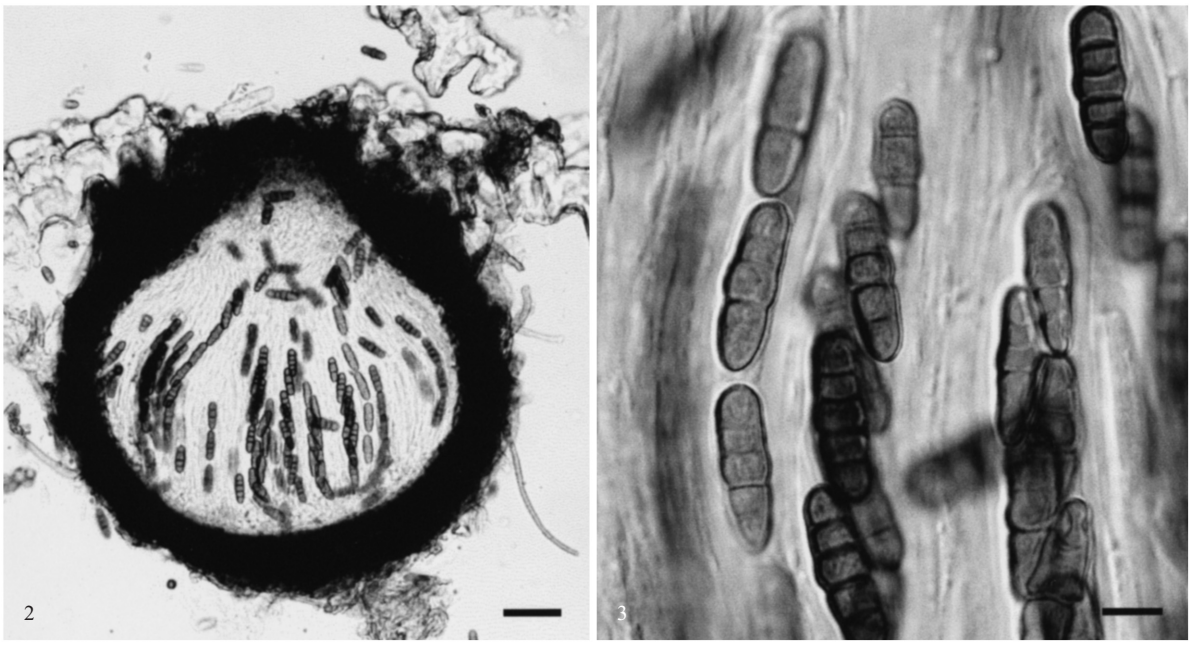
Sphaeria rusci Wallr., *Fl. Crypt. Germ.* **2**: 776 (1833).

Leptosphaeria rusci (Wallr.) Sacc., *Syll. Fung.* **2**: 74 (1883).

Paraphaeosphaeria rusci (Wallr.) O. E. Erikss., *Ark. Bot., ser. 2* **6**: 406 (1967).

Phaeosphaeriopsis agavensis (A. W. Ramaley, M. E. Palm & M. E. Barr) Câmara, M. E. Palm & A. W. Ramaley, **comb. nov.**

Basionym: *Paraphaeosphaeria agavensis* A. W. Ramaley, M. E. Palm & M. E. Barr, *Mycotaxon* **61**: 348 (1997).



Figs 2–3. *Phaeosphaeriopsis amblyspora* (holotype). **Fig. 2.** Cross-section of thick-walled, subepidermal ascoma. Bar = 35 μm. **Fig. 3.** Mostly 4-septate, brown, minutely roughened ascospores. Bar = 10 μm.

Phaeosphaeriopsis obtusispora (Speg.) Câmara, M. E. Palm & A. W. Ramaley, **comb. nov.**

Basionym: *Leptosphaeria obtusispora* Speg., Anal. Soc. Cient. Argentina **12**: 179 (1881).

Paraphaeosphaeria obtusispora (Speg.) O. E. Eriksss., *Ark. Bot., ser. 2* **6**: 406 (1967).

Phaeosphaeriopsis nolinae (A. W. Ramaley) Câmara, M. E. Palm & A. W. Ramaley, **comb. nov.**

Basionym: *Paraphaeosphaeria nolinae* A. W. Ramaley, *Mycotaxon* **61**: 351 (1997).

Phaeosphaeriopsis amblyspora A. W. Ramaley, **sp. nov.** (Figs 2–7)

Etym.: *ambly* (Greek, blunt or obtuse), referring to the spore shape.

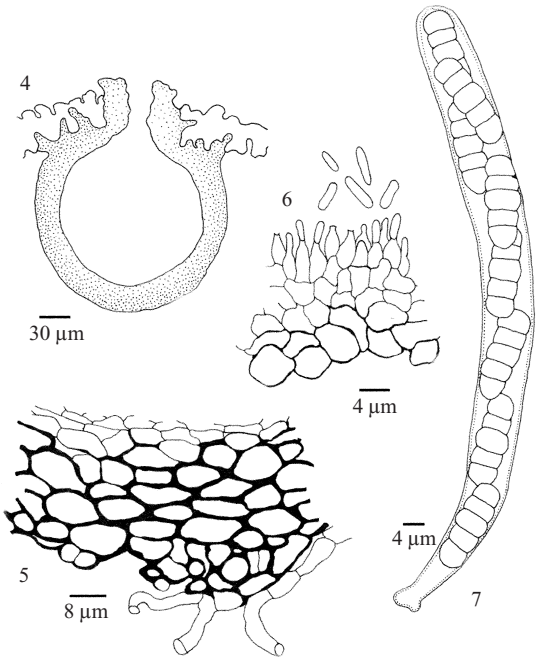
Ascomata immersa, subepidermalia, prope globosa vel sphaeroidea, papillata (150–)200–350 × (170–)250–385 μm, papilla brevi-emergentia per epidermidem, peridium 28–32 (–40) μm latum, ex 4–8 stratis texturae angularis constans. Asci cylindrici, (116–)120–160 × 12–16 μm. Pseudoparaphyses cellulosae, hyalinae, numerosae. Ascosporae anguste oblongae, cylindraceae, extremi ambobi obtuse-rotundati, 19.2–24(–27.2) × 6.4–8.8 μm, (3–)4(–5)-septatae, ordo septorum 2:3:1:4, echinulatae.

Typus: **USA**: *Colorado*: La Plata Co.: Durango, bike trail on Fort Lewis College mesa, in dead leaves of *Yucca baccata*, 7 May 2001, *A. W. Ramaley* 0133A (UC1726838 – holotypus; CBS 110131-cultura viva).

Ascomata immersed, subepidermal, nearly globose to subglobose, (150–)200–385 μm tall including the papilla, papilla *ca* 80 μm wide, 65–80 μm tall, at maturity epidermis broken and apex of papilla protruding, surrounded by septate, brown hyphae which extend into the host tissues, wall of 4–8 layers of dark brown *textura angularis*, thickest in upper portion of ascoma beneath host epidermis, 28–32(–40) μm diam. *Asci* bitunicate, cylindric, (116–)120–160 × 12–16 μm, ascospores uniseriate in the lower part of ascus, mostly biseriate closer to the apex. *Pseudoparaphyses* abundant, cellular, hyaline, 4–5 μm broad. *Ascospores* oblong-cylindric, both ends bluntly rounded, 19–24 (–27) × 6.5–9 μm, (3–)4(–5)-septate, first septum submedian, sequence of septation 2:3:1:4, any septum except the first may be absent, golden, becoming yellow-brown when more mature, finally brown, minutely echinulate, sheath present.

Anamorph: Known only from culture. *Colonies* 22 mm diam after 3 wk; pale gray or white, reverse light to dark gray, bright yellow pigment in droplets on surface of colony and in agar below, droplets at the periphery of a colony often colourless, increasingly yellow towards centre where often bright orange. *Conidiomata* pycnidioid, in groups in the aerial mycelium and at or below the agar surface; individual conidiomata at agar surface nearly globose, 65–120 μm diam, two to several conidiomatal initials often becoming confluent resulting in variable and irregular sizes and shapes of resultant conidiomata, sometimes forming composite structures up to 240 μm diam; wall of 1–2 layers of dark, thin-walled *textura angularis*. *Conidiogenous cells* developing from cells lining the interior of the conidioma, collapsing after conidia produced; *conidiogenesis* phialidic, no periclinal thickening observed. *Conidia* 4–7 × 1–1.5 μm, hyaline, more or less bacillar but sometimes inequilateral or swollen at one end, in white to pink droplets, germinating on PDA.

Phaeosphaeriopsis amblyspora is easily distinguished morphologically from other species with 4-septate



Figs 4–7. *Phaeosphaeriopsis amblyspora* (ex-holotype culture). **Fig. 4.** Subepidermal ascoma. **Fig. 5.** Cross-section of ascomatal wall. **Fig. 6.** Conidiogenous cells and conidia. **Fig. 7.** Ascus with eight uni- or bi-seriate ascospores.

ascospores. The sequence of septation for ascospores of *P. glauco-punctata* and *Paraphaeosphaeria vectis* is 3:2:3:1. The basal cell is not divided after formation of the A1 septum in the latter two species. The sequence of septation for ascospores of *P. amblyspora* is 2:3:1:4, the final septum dividing the basal cell. The ascospores and asci of *P. amblyspora* are larger than those of *P. glauco-punctata*, and the ascospores of the latter are reddish brown or yellowish brown and coarsely echinulate. The anamorph of *P. glauco-punctata* is *Coniothyrium*-like whereas *P. amblyspora* produces bacillar, hyaline conidia from non-proliferating, simple phialides. Ascospores of *Para. vectis* are narrowly fusiform whereas those of *P. amblyspora* typically have bluntly rounded ends. Neither *P. glauco-punctata* nor *Para. vectis* have been found on *Yucca*.

Neophaeosphaeria quadriseptata and *N. barrii* are found on *Yucca* and also have mostly 4-septate ascospores. They differ from *P. amblyspora* in that the anamorphs of both species are *Coniothyrium*-like with percurrently proliferating conidiogenous cells. In addition, the asci and ascospores of *N. quadriseptata* are smaller than those of *P. amblyspora*, whereas *N. barrii* has larger ascomata and ascospores and the ascospores are pale brown becoming dark brown or dark grey.

DISCUSSION

Câmara *et al.* (2001) reported that species of *Paraphaeosphaeria* grouped in three clades based on sequence analyses of the ITS region of the nrDNA, but no taxonomic decisions were made at that time. The same tree topology was maintained after the inclusion of new taxa by Checa *et al.* (2002). In this study, SSU nrDNA analyses confirm the separation of *Para. michotii* and *Para. pilleata* from *Neophaeosphaeria filamentosa* and *Phaeosphaeriopsis glauco-punctata*, type species in the newly described genera. Therefore, based on molecular and morphological data, we conclude that only *Para. michotii*, the type species, and *Para. pilleata* should be retained in *Paraphaeosphaeria*. Those two taxa group with members of the *Montagnulaceae*/*Massariaceae* (Fig. 1). Both species produce 2-septate ascospores and single, small, thin-walled ascomata. The anamorphs of both taxa produce smooth-walled, pale brown conidia from inconspicuous phialides with some periclinal thickening, typical of the genus *Microsphaeropsis* (Sutton 1980).

Using SSU nrDNA sequence data, *Neophaeosphaeria* and *Phaeosphaeriopsis* were included in a clade containing members of the *Phaeosphaeriaceae*/*Leptosphaeriaceae*. The two families could not be resolved from one another with this gene, suggesting that generic level relationships in these families would not be resolved either. Internal transcribed spacer region sequences vary widely across genera in these families making sequence alignment difficult and resulting in the exclusion of large numbers of positions due to uncertain homology. For this reason simple pairwise base differences were used to determine similarity of ITS sequences as an indication of congeneric status for morphologically similar taxa which had grouped together in earlier studies (Clades B and C in Câmara *et al.* 2001, Checa *et al.* 2002). Results of this study justify the description of new genera for those species (Câmara *et al.* 2001, Checa *et al.* 2002).

Neophaeosphaeria is described for species that produce 3–4-septate ascospores, relatively thick-walled pseudothecia, and a *Coniothyrium*-like anamorph with pigmented, aseptate conidia produced from holoblastic, percurrently proliferating conidiogenous cells with conspicuous annellations. The anamorphs of two of these species have not been found on host material. To date all occur on species of *Yucca* (*Agavaceae*). *Paraphaeospheria barrii* is included in *Neophaeosphaeria* even though it has a slightly larger proportion of differing base positions when compared to *N. filamentosa* than found among the other taxa placed in this new genus (3.6–6.2%), as well as a 21 bp insertion in the ITS1. It clearly is not related to *Paraphaeosphaeria s. str.*

Species in *Phaeosphaeriopsis* are more diverse than those in *Paraphaeosphaeria s. str.* or *Neophaeosphaeria*, but they share several morphological characters and are more similar to each other in ITS sequence than to those other genera. Most species occur on genera in the *Agavaceae*, however *P. glauco-punctata* is found on *Liliaceae* (*Ruscus*). Species produce uni- or multiloculate stromata and ascospores may be 4-septate as in *P. amblyspora* and *P. glauco-punctata*, or 5-septate as in *P. agavensis*, *P. obtusispora* and *P. nolinae*. The

anamorphs of *P. amblyspora* and *P. agavensis* produce hyaline, bacillar conidia from simple phialides. Anamorphs of *P. glauco-punctata*, *P. obtusispora*, and *P. nolinae* produce brown conidia from percurrently proliferating conidiogenous cells which are inconspicuously annellate. Conidia of *P. nolinae* become 3-septate and conidia of *P. glauco-punctata* often become 1-septate, while those of *P. obtusispora* remain non-septate.

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