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Freshwater ascomycetes: *Alascospora evergladensis*, a new genus and species from the Florida Everglades

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Abstract: *Alascospora evergladensis*, a freshwater ascomycete collected from submerged dead petioles of *Nymphaea odorata* during a survey of aquatic fungi along a phosphorus gradient in the Florida Everglades, is described and illustrated as a new genus and species in the Pleosporales (Pleosporomycetidae, Dothideomycetes). The new fungus is unique among genera in the Pleosporales based on a combination of morphological characters that include light brown, translucent, membranous, ostiolate ascomata with dark, amorphous material irregularly deposited on the peridium, especially around the ostiole; globose, fissitunicate, thick-walled asci; septate pseudoparaphyses; and 1-septate ascospores that are hyaline when young, and surrounded by a hyaline gelatinous sheath that is wing-shaped in outline on each side of the ascospore. The sheath is distinctive in that it first expands in water and is translucent, then condenses and darkens around older ascospores, giving them a dark brown, verruculose appearance.

Key words: aquatic fungi, herbaceous, *Nymphaea odorata*, saprophyte, systematics

INTRODUCTION

Fungi have been recognized as important to nutrient cycling in freshwater systems (see review by Gessner et al 2007) and might be affected by phosphorus (P) enrichment (Hackney et al 2000, Gulis and Suberkropp 2003). For these reasons a study was initiated to determine what fungi are present on decaying plant material in the Everglades and whether their frequency and distribution differ along a P gradient. During this study a new ascomycete was found on the bases of submerged petioles of *Nymphaea odorata* Aiton at two sites (U3 and F4) of the Water Conservation Area (WCA-2A) of the South Florida Water Management District.

Morphological characteristics such as light brown, perithecial ascomata with an ostiole; cellular pseudoparaphyses; fissitunicate asci; and hyaline to dark brown, 1-septate, ascospores with a gelatinous sheath indicate the new fungus belongs to the Pleosporales (Pleosporomycetidae, Dothideomycetes). None of the genera included in the Pleosporales however have a combination of globose to subglobose, light brown, membranous, translucent, ostiolate ascomata, and hyaline, multiguttulate, 1-septate ascospores equipped with a large gelatinous sheath taking the form of two wings. The sheath of young ascospores expands in water and is translucent but appears condensed and darkens on older, brown ascospores to give a dark brown, verruculose appearance to the ascospore wall. This fungus therefore is described and illustrated herein as a new genus and species and is compared with morphologically similar genera.

MATERIALS AND METHODS

Methods for collection, characterization and illustration of freshwater ascomycetes are described in Fallah and Shearer (2001), Shearer et al (2004), Raja and Shearer (2008) and Raja et al (2009).

TAXONOMY

Alascospora Raja, Violi et Shearer gen. nov.
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Ascomata superficialia, partim immersa, globosa ad subglobosa, membranacea, ostiolata. Peridium pseudoparenchymaticum. Pseudoparaphyses hyalinum, one-septatum, ad septatum constrictum. Asci bitunicati, globosi vel subglobosi, cum muris crassis. Ascosporae ellipsoideae, uniseptatae, hyalinae, serius brunneae, verruculosae, multiguttulatae, vagina gelatinosa extensa.

Etymology. al = L. for ala, the diminutive for winged and ascospora = L. for ascospore, referring to the winged ascospores.

Ascomata superficial to partly immersed, scattered, globose, subglobose or hemispherical and flattened at the base, membranous, ostiolate, light brown, translucent when young, darkening with age. Peridium composed of hyaline to brown pseudoparenchymatic cells with dark amorphous material irregularly depos-

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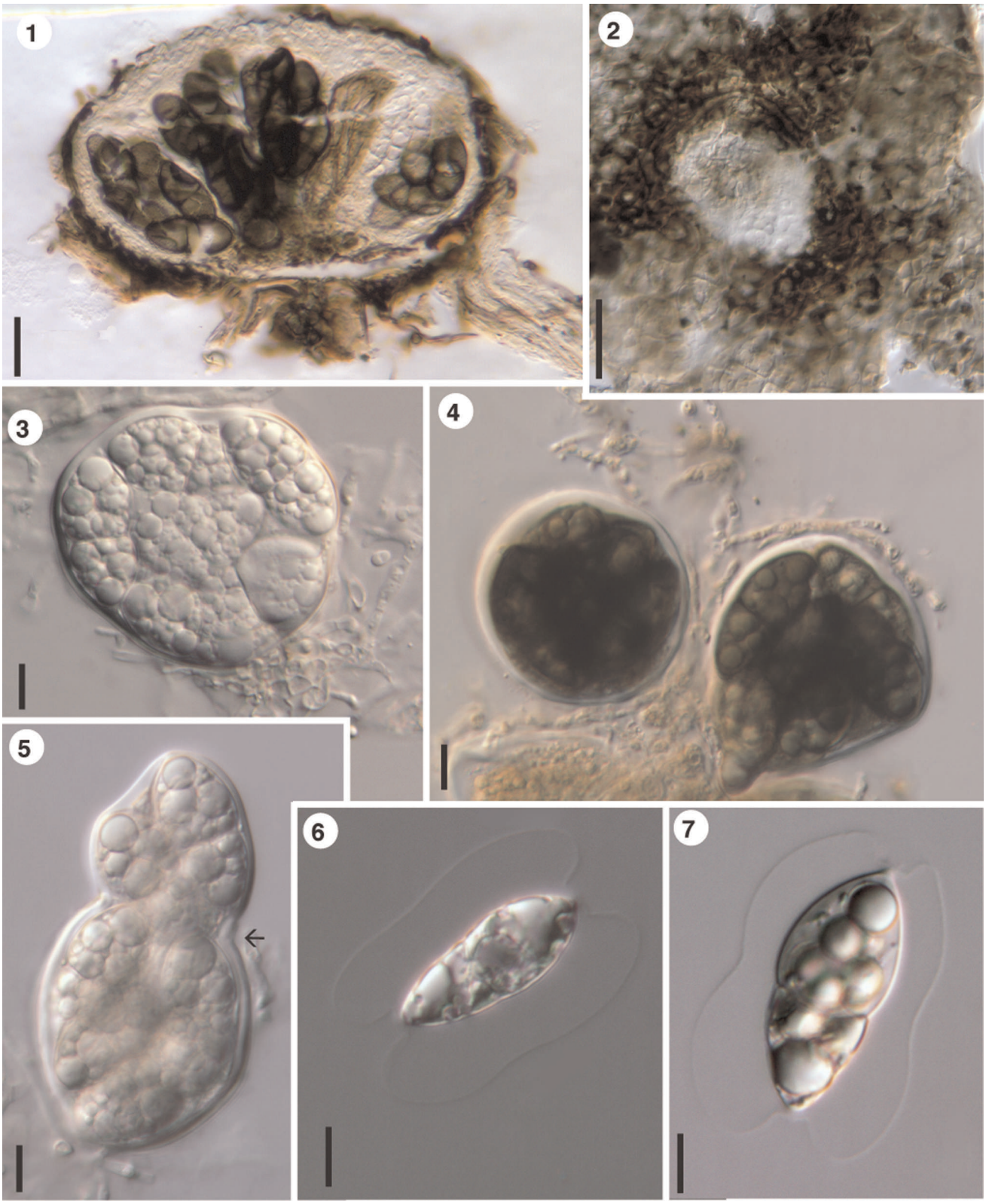
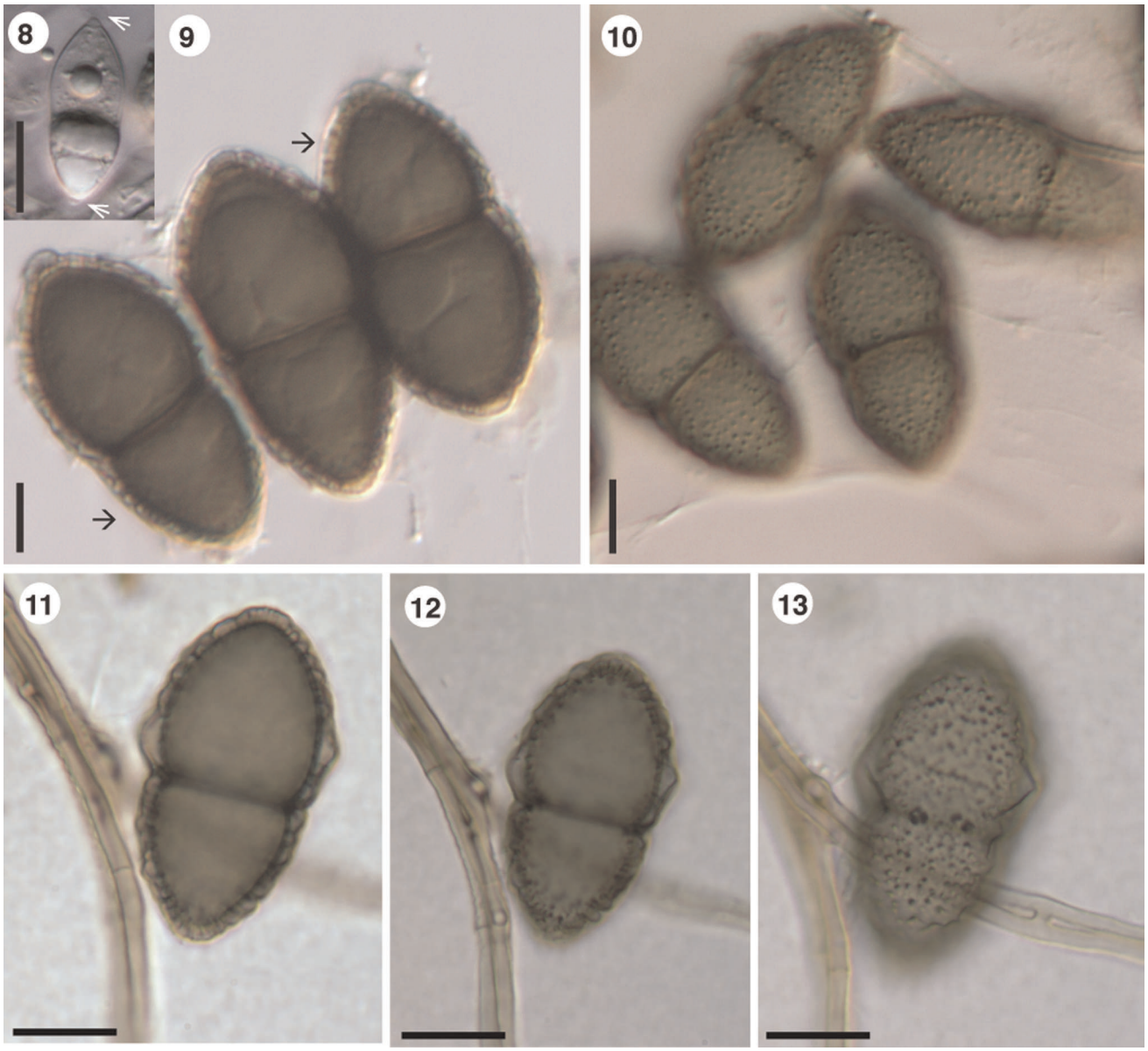


FIGS. 1–13. *Alascospora evergladensis* from the holotype (SFWMD-1). 1. Longitudinal section through the ascoma. 2. Squash mount of an ascoma showing ostiole (note dark amorphous material around the ostiole). 3. Young, globose, thick-walled ascus. 4. Globose to subglobose ascus with mature ascospores. 5. Fissitunicate ascus; arrow indicates area where endoascus extends from ectoascus. 6, 7. Young, multiguttulate ascospores with wing-shape gelatinous sheath. 8. Young ascospore with hyaline



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apical papillae indicated by arrows. 9. Mature ascospores; arrows indicate appressed sheath. 10. Verruculose ascospores (note the wide upper cell and narrow lower cell of the ascospores). 11–13. Series of mature ascospores at different focal levels using oil immersion (100×) to reveal appressed sheath. (Note how sheath is verruculose on the surface thus giving a verruculose appearance to the mature ascospore wall.) 11. Below the upper surface, showing the condensed, ruffle-like sheath in outline. 12. Between the spore interior and spore surface. 13. Spore surface showing appressed sheath that appears verruculose due to condensed sheath. Bars: 1, 2 = 50 μm; 3–7, 9, 10 = 10 μm; 8 = 20 μm; 11–13 = 20 μm.

ited on the surface, especially dense around the ostiole. Pseudoparaphyses filamentous, sparse, hyaline, septate. Asci bitunicate, thick-walled, globose to subglobose, pedicellate, containing eight irregularly arranged ascospores. Ascospores ellipsoidal, 1-septate, slightly constricted at septum, septum becoming thicker and darker in older ascospores, upper cell longer and wider than lower cell, multiguttulate, hyaline when young, brown at maturity with minute, hyaline apical papillae, with a gelatinous sheath appearing wing-shaped on each side of the ascospore; sheath expands in water, translucent at first but condenses on older darkened ascospores to give a dark brown verruculose appearance to the ascospore wall.

TABLE I. Comparison of selected characters of *Alascospora evergladensis* with morphologically similar genera in the Pleosporales

	Alascospora evergladensis	*Caryospora putaminum*[a]	*Zopfia rhizophila*[b]
Habitat	Freshwater	Terrestrial	Terrestrial
Substrate	*Nymphaea odorata* Aiton petiole	*Prunus persica* (L.) Batsch endocarps on the ground	*Asparagus officinalis* L. rhizomes and roots
Ascomata	Light brown, translucent, ostiolate	Black, carbonaceous; ostiolate	Dark, nonostiolate
Peridium	Membranous, thin-walled, not cephalothecoid; with amorphous dark deposits around the ostiole	Reddish brown with numerous layers of compressed cells	Cephalothecoid
Asci	Thick-walled, globose to subglobose, 8-spored	Thin-walled oblong, ellipsoid or clavate, 2–3-spored	Thick-walled, globose, 4–8-spored
Ascospores	One-septate, ellipsoidal, hyaline when young, brown and rough walled when mature with hyaline, apical papillae	One-septate, broadly ellipsoidal or biconic, smooth or roughened; with hyaline apical papillae	One-septate, dark brown to black, walls crenately roughened; apical papillae absent
Sheath	Large, translucent gelatinous sheath appearing wing like in shape; in older ascospores sheath condensing and darkening to form a dark, appressed, verruculose ascospore wall covering	Gelatinous sheath about 8–12 μm wide	Absent

Type species. Alascospora evergladensis

Alascospora evergladensis Raja, Violi et Shearer sp. nov. FIGS. 1–13

MycoBank MB 514032

Ascomata 125–232 × 135–236 μm, superficialia, partim immersa, globosa ad subglobosa, membranacea, ostiolata. Peridium 5–10 μm latum, pseudoparenchymaticum. Pseudoparaphyses 20–40 × 2–4 μm, hyalinum, 1-septatum, ad septatum constrictum. Asci 32–74 × 34–58 μm, bitunicati, globosi vel subglobosi, cum muris crassis. Ascosporae 30–40 × 12–16 μm, ellipsoideae, uniseptatae, hyalinae, serius brunneae, verruculosae, multiguttulatae, vagina gelatinosa extensa.

Ascomata 125–232 × 135–236 μm, superficial to partly immersed, scattered, globose, subglobose or hemispherical and flattened at the base (FIG. 1), membranaceous, ostiolate, light brown, translucent when young, darkening with age. Peridium ca. 5–10 μm wide, composed of hyaline to brown pseudoparenchymatic cells, with dark brown amorphous material deposited irregularly on the peridial surface, especially dense around the ostiole (FIG. 2). Pseudoparaphyses sparse, hyaline, septate, filamentous, sometimes irregular, constricted or not at the septa, 20–40 × 2–4 μm. Asci 32–74 × 34–58 μm, (mean and SD = 60 ± 7 × 43 ± 6 μm, n = 25), bitunicate, thick-walled, globose to subglobose (FIGS. 3, 4), short, pedicellate (FIG. 5), containing eight irregularly arranged ascospores. Ascospores 30–40 × 12–16 μm (mean and SD = 36 ± 2.3 × 14 ± 1.3 μm, n = 45), ellipsoidal, 1-septate, septum thin and hyaline at first becoming thicker and darker in older ascospores, upper cell longer and wider than lower cell, hyaline when young, younger ascospores with minute hyaline, apical papillae (FIG. 8), multiguttulate, becoming dark brown with age, surrounded by a translucent gelatinous sheath that appears as lateral wings in outline; each side of the sheath ca. 40–45 × 9–10 μm (FIGS. 6, 7); in older ascospores sheath condensing and darkening to form a dark, appressed, verruculose ascospore wall covering (FIGS. 9–13).

Etymology. Referring to the type locality, the Everglades

TYPE. USA. FLORIDA: Everglades Water Management District, Water Conservation Area 2A, phosphorus unenriched site U3, 26°17′15.070″N, 80°24′41.08″W, water temperature 19 C, pH 7, on submerged *Nymphaea odorata* petiole, 17 Nov 2008, *Huzefa A. Raja* and *Helen Violi*, *SFWMD 1-1.* (HOLOTYPE, ILL40789).

Additional specimen examined. Everglades Water Management District, Water Conservation Area 2A, phosphorus enriched site F4, on submerged *Nymphaea odorata* petiole, Jan 2009, *Helen Violi*, *SFWMD 1-2*; phosphorus unenriched site U3, (U3 NYOD No. 3) on submerged *Nymphaea odorata* petiole, 26 Mar 2009, *Jessica Fay*, *SFWMD 1-3*.

TABLE I. Continued

Pontoporeia biturbinata[c]	*Zopfiofoveola punctata*[d]	*Testudina terretris*[e]	*Verruculina enalia*[f]
Marine	Terrestrial	Terrestrial	Mangrove
Posidonia oceanica Delile rhizome	Earthworm excrement	Wood of *Abies* and *Pinus*; leaf litter of *Taxus*	Woody debris
Dark, nonostiolate	Dark, carbonaceous, nonostiolate	Dark, carbonaceous, nonostiolate	Dark, ostiolate, carbonaceous, with clypeus
Irregularly cephalothecoid	Distinctly cephalothecoid	Distinctly cephalothecoid	Dark, irregular, flattened cells
Thick-walled, obovate to clavate, 8-spored	Very thick-walled, obovate, 8-spored	Thick-walled, broadly clavate to subglobose, 8-spored	Thick-walled, cylindrical, 8-spored
One-septate brown, with hyaline apical papillae	One-septate, ellipsoidal, brown to dark brown with hyaline apical papillae	One-septate, ellipsoidal, with rounded end cells; apical papillae absent	One-septate, dark brown, ellipsoidal, verrucose to verruculose; apical papillae absent
Absent	Absent	Conspicuous, hyaline, reticulate sheath	Absent

[a] Jeffers 1940, Barr 1979.
[b] Hawksworth and Booth 1974.
[c] Kohlmeyer 1963, Hawksworth and Booth 1974, Jones et al 2009.
[d,e] Hawksworth and Booth 1974, Hawksworth 1979.
[f] Kohlmeyer and Volkman Kohlmeyer 1990.

Known distribution. USA (FL)

Comments. Alascospora evergladensis shares many characters with taxa in the Pleosporales. These include perithecial ascomata with an ostiole, cellular pseudoparaphyses, fissitunicate asci, and hyaline to dark brown, 1-septate ascospores with a gelatinous sheath (Kirk et al 2008). However the relationship of *Alascospora* to known families within the Pleosporales is not clear.

Lumbsch and Huhndorf (2007) currently recognize 19 families within the Pleosporales, Pleosporomycetidae, while Kirk et al (2008) recognize 23. The 1-septate, brown ascospores with verruculose ornamentation on the ascospore wall (FIGS. 9–13) resemble brown, 1-septate ascospores of genera such as *Zopfia* Rabenh, *Pontoporeia* (Durieu & Mont.) Kohlm., and *Zopfiofoveola* D. Hawksw. in family Zopfiaceae, as well as *Testudina* Bizz. and *Verruculina* Kohlm. & Volkm.-Kohlm. in family Testudinaceae. *Alascospora* however differs from taxa in both the Zopfiaceae and the Testudinaceae because it has a light brown, translucent, membranous, ostiolate ascomata (FIGS. 1–2, TABLE I) instead of cleistothecial and often cephalothecoid ascomata characteristic of the two families (Cannon and Kirk 2007). *Alascospora* also differs from the aforementioned taxa in habitat, substrate type, ascus and ascospore morphology and the presence of a distinctive gelatinous sheath (see TABLE I).

Alascospora also should be compared with *Caryospora* de Not., placed in the Zopfiaceae (Kirk et al 2008, Lumbsch and Huhndorf 2007), in that both taxa have brown 1-septate ascospores, with pale end cells, and a gelatinous sheath as in (*C. putaminum* (Schw. Ex. Fr.) de Not.) (Jeffers 1940, Barr 1979). The two taxa however differ in habitat, substrate, ascomata and ascus morphology (TABLE I).

We establish *Alascospora* as a new genus in the Pleosporales *incert. sed.* pending molecular work on this genus. Thus far attempts to isolate this fungus in pure culture have not been successful due to failure of ascospores to germinate. In our current study we have examined more than 75 dead, decaying rhizomes and petioles of *N. odorata* but have found *A. evergladensis* only three times. This suggests that *A. evergladensis* might be a rare species or that the range of environmental conditions within which it reproduces might be quite narrow.

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