

## Rediscovery of *Albosynnema elegans* and *Solheimia costaspora*

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*Albosynnema elegans* and *Solheimia costaspora*, synnematos hyphomycetes, previously only known from descriptions based on type specimens collected in Panama, have been found in the southern Costa Rica. Cultural studies of these fungi are presented and neotype specimens for both species are designated. Relationships between these genera and to anamorphic states of the *Nectria ralfsii* group, *Virgatospora* and *Myrothecium*, are postulated. New host plants for *S. costaspora*, *A. elegans*, *Virgatospora echinofibrosa*, and *Myrothecium cinctum* are reported.

Keywords: Hypocreales, *Nectria*, *Myrothecium*, *Virgatospora*, synnemata, Costa Rica.

During investigations of fungi of decomposing leaf litter (Bills & Polishook, 1994) and endophytic fungi from rain forests in southern Costa Rica, two unusual synnematos hyphomycetes were isolated which were subsequently identified as *Albosynnema elegans* Morris and *Solheimia costaspora* Morris. A search of the literature revealed no reports of additional collections of these fungi since their original descriptions from collections made in the Panama Canal Zone (Morris, 1967). Furthermore, the type specimens of these fungi were not in the collections of University of Iowa, now at Iowa State University (Lois Tiffany, pers. comm.), or of the U. S. National Fungus Collections as indicated in the original descriptions, or at Western Illinois University (Robert Gessner, pers. comm.). A culture of *S. costaspora* was not deposited in the American Type Culture Collection as indicated by Morris (S. C. Jong, pers. comm.). Lyophilized cultures of *S. costaspora*, accessioned to the U.S. Army Quartermaster Collection in 1967 (QM8849) and later housed at the NRRL, were no longer viable (Kerry O'Donnell, pers. comm.).

Because little is known about these unusual but distinctive anamorphic fungi, we redescribe them from new collections. Also when we grew these fungi in culture, we recognized that they bore a

strong morphological resemblance to each other, to *Virgatospora echinofibrosa* and to *Myrothecium* spp.

### Material and methods

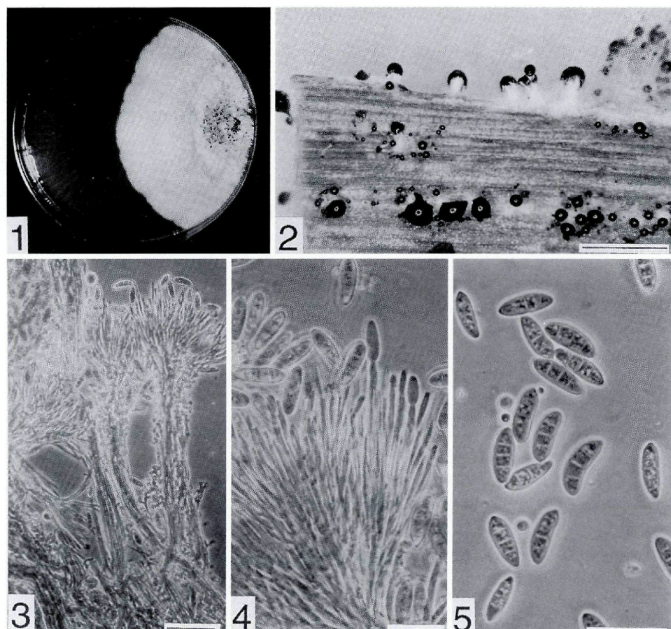
The following descriptions are based on cultures grown at 25° C, 12 hr photoperiod, under a combination of fluorescent and near-ultraviolet light for 21 days. The media were oatmeal, cornmeal and malt-yeast extract agars commercially available from Difco. To stimulate more natural development of synnemata and sporodochia, isolates were subcultured onto cornmeal agar on which was placed a small piece (1–2 cm<sup>2</sup>) of banana leaf (Matsushima, 1971; 1975). Leaves were obtained frozen from Oriente, Karexx International, Inc. and then sterilized by autoclaving. Measurements of micromorphological features were made from synnemata grown on banana leaves. Capitalized color names are from Ridgway (1912). Methods for isolation of *S. costaspora* were described in Bills & Polishook (1994). Methods for isolation of *A. elegans* and *V. echinofibrosa* from living twigs were described in Petrini (1986). Cultures are maintained in the Merck Microbial Resources Culture Collection. Dried cultures and slides are deposited at the U. S. National Fungus Collections (BPI).

For scanning electron microscopy (SEM), small pieces from banana leaf cultures were flooded with OsO<sub>4</sub> for 20 h, rinsed with phosphate buffer, flooded with glutaraldehyde for 24 h, rinsed with phosphate buffer and dehydrated through an ethanol series. After critical point drying, the pieces were attached to aluminum stubs, coated with gold-palladium using a sputter coater, and examined with an AMRAY 1200B scanning electron microscope.

### Descriptions of taxa

***Albosynnema elegans*** Morris, Mycopathol. Mycol. Appl. 32: 179. 1967. – Figs. 1–9.

**Synnemata** 180–650 µm tall, 15–120 µm wide at midpoint, arising from a cottony, white mass of basal hyphae, caespitose or gregarious, occasionally solitary, determinate, composed of loosely aggregated, parallel hyphae, with as few as 10 parallel strands, but usually many more, straight to slightly flexuous, unbranched or basally branched, with surface translucent to shiny, smooth to longitudinally striate, translucent to white when young, pale pink in age, unchanging in 3% KOH or lactic acid. – **Hyphae** of the stipe 2–4 µm in diam, thin-walled, smooth to minutely incrustated, with scattered protruding marginal hyphae, with marginal hyphae



Figs. 1-5. *Albosynnema elegans* (GB 3101, Neotype). - 1. Three-week old culture mat on malt-yeast extract agar, note irregular synnemata. - 2. Synnemata from culture on banana leaf. Bar = 1 mm. - 3. Synnemata, conidiophores and conidia, note ornamented stipe hyphae. Bar = 50  $\mu\text{m}$ . - 4. Conidiophores with conidia. Bar = 20  $\mu\text{m}$ . - 5. Conidia. Bar = 20  $\mu\text{m}$ .

flexuous, contorted, irregularly lobed or knobby, with lobes protruding up to 2  $\mu\text{m}$ . - Conidiophores continuous with hyphae of stipe apex, mono- or biverticillate, with metulae supporting a whorl of 2-4 conidigenous cells, metulae when present 19-40  $\mu\text{m}$  long, 1-3  $\mu\text{m}$  wide, hyaline. - Conidigenous cells determinate, enteroblastic, phialidic, cylindrical to subulate, straight, 20-45  $\mu\text{m}$  long, 1-3  $\mu\text{m}$  wide, terminating in a shallow, broad, cylindrical to slightly flared collarette, with collarette 1-2  $\mu\text{m}$  in diam, hyaline, thin-walled, smooth. - Conidia broadly elliptical, curved in side view, with proximal end sometimes slightly truncate, 3-septate, with guttulate and refractive cytoplasm, smooth, slightly thickened at the septa, (17.5-)19-23.5  $\times$  6.5-8  $\mu\text{m}$ , dull greenish gray in lactophenol, discoloring yellowish brown in 3% KOH.

### Colony characters

On oatmeal agar attaining diam of 40–45 mm, cottony or woolly at first, becoming plumose with suberect to erect mycelial tufts and mounds towards center, with abundant sterile and fertile synnemata over the inner older half, with aerial hyphae and synnemata up to 7 mm above the agar surface, the margin even to undulating, white to pale cream or pinkish cream, Pale Pinkish Buff, with synnematal heads grayish green to blackish green, Dull Greenish Black, Blackish Green-Gray, with reverse colorless to pale grayish rose. Exudates of clear to pale straw-colored liquid on surface. Odor absent.

On malt-yeast extract agar (Fig. 1) attaining 47–48 mm, essentially identical to morphology on oatmeal agar, with margin slightly submerged, reverse pale yellow, Cream Color, to yellow, Naples Yellow, Mustard Yellow, Warm Buff, or rosy yellow, Apricot Yellow, Light Ochraceous-Salmon. Fewer and smaller drops of exudates than on oatmeal agar. Odor absent. No growth observed at 37° C on malt-yeast extract agar.

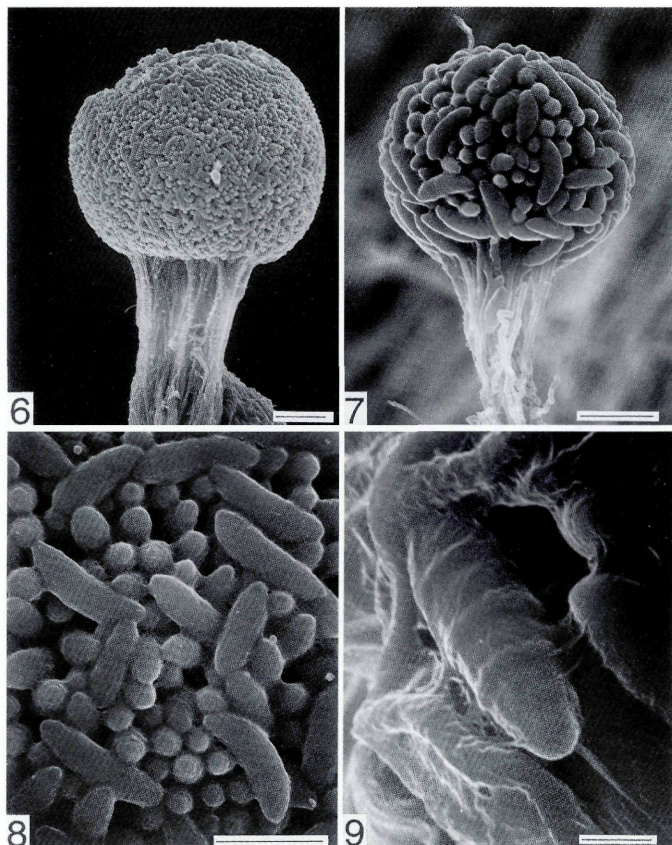
On cornmeal agar attaining 50–51 mm, appressed, with hyphae radiating outward from the center in a fan-like pattern, translucent, with margin slightly raised, undulating, with scattered caespitose to single synnemata over inner one-third, reverse translucent. Exudates and odor absent.

Specimen examined. – COSTA RICA. PUNTARENAS: Reserva Forestal Golfo Dulce, Finca de Antonio Vargas, 8° 41' 30" N, 83° 31' 00" W, 100 m elevation, conidial isolate from synnemata emerging from surface-sterilized living twig of *Poulsenia armata* (Miq.) Standl. (Moraceae), GB3101, 18 Nov 1992, (BPI, NEOTYPE of *Albosynnema elegans* designated herein).

Morris (1967) first described this fungus from bark of an unidentified dead angiosperm branch. Our recovery of an isolate from a rigorously surface-sterilized living twig indicates the species is capable of endophytic infection. Although no authentic material of *A. elegans* is available, comparison with the protologue leaves little doubt that this is the same fungus that Morris described. Therefore, we designate a dried culture mat of the isolate (GB 3101) as a neotype. Although synnemata form on agar, they form more rapidly and are more consistent in form and size when cultures are grown on the surfaces of banana leaves supported by agar medium.

***Solheimia costaspora*** Morris, Mycopathol. Mycol. Appl. 32: 181. 1967. – Figs. 10–19.

Synnemata 300–900 µm tall, 35–90 µm wide at midpoint, arising from compact, rhizoidal mycelial base embedded in



Figs. 6-9. *Albosynnema elegans*. SEM. (GB 3101, Neotype). - 6. Synnematal head. Bar = 50 µm. - 7. Synnematal head. Bar = 25 µm. - 8. Conidia. Bar = 20 µm. - 9. Conidium. Bar = 5 µm.

substratum, gregarious to caespitose, determinate, subulate-capitate to cylindrical-capitate, at first translucent greenish gray, soon blackish green to nearly black, paler towards the apex, leaching a dull vinaceous gray or vinaceous brown pigment in 3% KOH, no change in lactic acid, with surface longitudinally fibrous or twisted fibrous, aborted synnemata forming submerged in the agar. - Conidial

heads blackish green to black, hemispherical to subglobose, coalescing when densely gregarious, shiny, moist to watery when fresh, granular when old. - Hyphae of the stipe 1-3  $\mu$ m wide, septate, straight, mostly unbranched, with walls thickened, finely verruculose, dark green to blackish green. - Conidiophores a continuous extension of stipe hyphae, unbranched or branched 1-2 times. - Conidiogenous cells determinate, enteroblastic, phialidic, cylindrical to narrowly clavate, 15-23  $\mu$ m long, 2-4  $\mu$ m wide, rounded at apices, terminating in a minute pore or minute tubular to slightly flared collarete, minutely verruculose, with verrucules decreasing in density towards apex, intermingled with sterile terminal cells of stipe hyphae. - Conidia elliptical, (10-)10.5-13.5  $\times$  (2.8-)3.5-5  $\mu$ m, slightly thickened and flattened at proximal end, smooth, with walls slightly thickened, with 8-10, longitudinal, or longitudinally spiral costae, with intercostae 0.5-1  $\mu$ m deep, greenish brown in lactophenol.

#### Colony characters

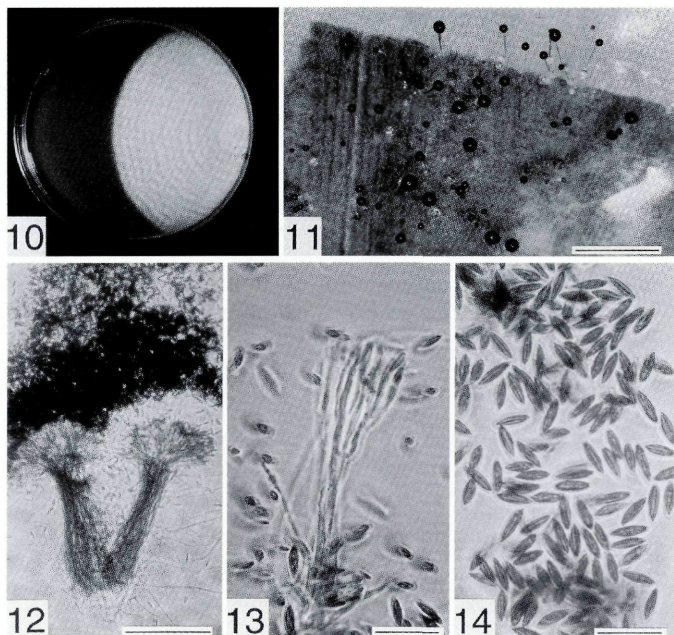
On oatmeal agar attaining 45-48 mm, felty to woolly, radially rugulose or plicate, white to pale pinkish buff, Pale Cinnamon-Pink, Pale Pinkish Cinnamon, Pinkish Buff, at center, with scattered synnemata near inoculation point, with margin appressed, even, pale grayish buff in reverse, medium buckling in reverse. Odor and exudates absent.

On malt-yeast extract agar (Fig. 10) attaining 50-52 mm, felty, cottony to woolly, conspicuously zonate, colors the same as on oatmeal agar, with a few diminutive synnemata at inoculation point, with margin submerged, even, reverse faintly zonate, yellow, Cream-Buff, Chamois, Deep Colonial Buff. Odor and exudates absent. No growth observed at 37° C on malt-yeast extract agar.

On cornmeal agar attaining 54-56 mm, appressed, with distant radial hyphae, or with scant white, downy aerial mycelium at inoculation point, translucent, reverse translucent. Odor and exudates absent.

Specimens examined. - COSTA RICA. PUNTARENAS: Reserva Forestal Golfo Dulce, Finca de Antonio Vargas, 8° 41' 30" N, 83° 31' 00" W, from mixed leaf litter of primary forest, GB3165 (BPI, NEOTYPE of *Solheimia costaspora* designated herein), GB3290, GB3291, GB3292, 18 Nov 1992. LIMÓN: Reserva Biológica Hitoy Cerere, near ranger station, 8° 42' 20" N, 83° 31' 30" W, 100 m elevation, decomposing leaves of *Heliconia mariae* J. P. Hook. (Heliconiaceae), GB 3804 and four additional isolates, 14 Sep 1993.

From only a few preliminary isolation experiments with leaf litter from the Pacific and Caribbean sides of Costa Rica, we were able to obtain several isolates of this fungus, indicating that it may be locally abundant in litter of lowland rain forests of southern Central



Figs. 10–14. *Solheimia costaspora* (GB 3165, Neotype). – 10. Three-week old culture mat on malt-yeast extract agar, dark spots below inoculation point are synnemata. – 11. Synnemata from culture on banana leaf. Bar = 1 mm. – 12. Synnemata with conidial mass. Bar = 200  $\mu$ m. – 13. Conidiophores with conidia. Bar = 20  $\mu$ m. – 14. Conidia. Bar = 20  $\mu$ m.

America. Because authentic specimens collected by Morris are no longer available, we designate a dried culture mat (GB 3165) as a neotype. As in *A. elegans*, synnemata form on agar, but they develop more rapidly and are more consistent in form and size when cultures are grown on surfaces of banana leaves.

#### Other fungi examined

*Virgatospora echinofibrosa* Finley, Mycologia 59: 538. 1967.

Specimen examined. – COSTA RICA. PUNTARENAS: Reserva Forestal Golfo Dulce, Sendero Los Guapinoles, above Centro Boscosa, conidial isolate from synnemata emerging from surface-sterilized living twigs of *Inga*

*goldmanii* Pittier (Mimosaceae), 8° 42' 20" N, 83° 31' 30" W, 100 m elevation, GB 3158, 19 Nov 1992 (BPI).

This appears to be the first report that *V. echinofibrosa* can form latent endophytic infections. As with the other synnematosus fungi above, synnematal formation was enhanced by culturing on banana leaves. *Virgatospora echinofibrosa* was also first described from specimens collected in the Panama Canal Zone by Morris and colleagues (Finley, 1967). It has been collected previously in Guanacaste Province, Costa Rica on bamboo and woody branches (Morris, 1972).

***Myrothecium cinctum*** (Corda) Sacc., Sylloge Fungorum 4: 751. 1886.

Specimen examined. – COSTA RICA. LIMÓN: near ranger station, Reserva Biológica Hitoy Cerere, 8° 42' 20" N, 83° 31' 30" W, isolated from decomposing leaves of *Heliconia mariae*, GB 3810, 14 Sep 1993 (BPI).

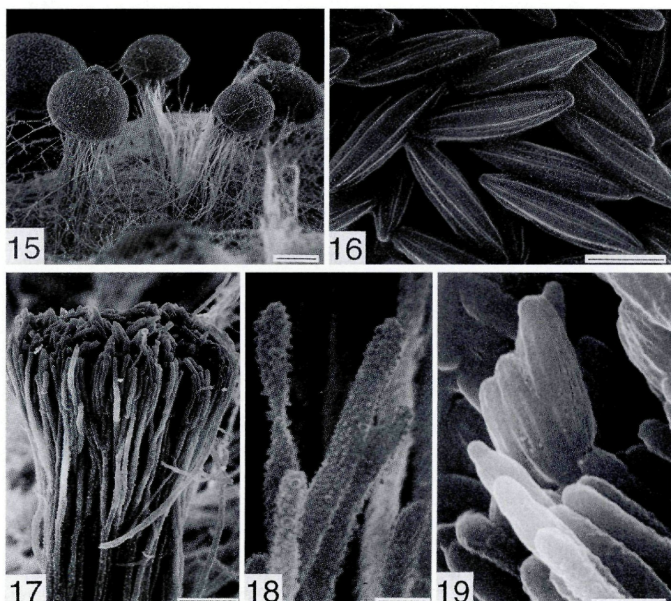
This is the first report of this species in Costa Rica (Covington, 1980). Conidia from sporodochia on banana leaves measured 6–8.5 × 2.5–3.5 µm. Matsushima (1980) divided populations of *M. cinctum* into different short-spored and long-spored groups, based on conidial dimensions. The conidial dimensions of the latter group would overlap with those of *S. costaspora*. Sporodochia developed more uniformly and densely on banana leaves than on agar.

## Discussion

The genera *Albosynnema* and *Solheimia* are synnematosus hyphomycetes with large, slimy, dark green, elongate conidia having either a smooth or distinctly ribbed surface. Both genera can be diagnosed by means of the “key to genera of slimy-spored phialidic hyphomycetes producing determinate synnemata” provided by Seifert (1985). These fungi are morphologically similar to *Virgatospora echinofibrosa* from which the latter varies in having striate conidia with truncate ends borne on phialides developing on penicillately branching conidiophores that are aggregated to form synnemata. Seifert (1990) recognized the relationship of *V. echinofibrosa* with *Myrothecium* based on phialide and conidial characteristics shared with the two synnematosus fungi described above. Thus, we contend that *A. elegans* and *S. costaspora* are anamorphic Hypocreales.

The teleomorph of *V. echinofibrosa* is *Nectria spirostriata* Rossman having 3-septate, striate ascospores. This teleomorph belongs to the *Nectria ralfsii* Berk. & Broome-group as defined by Samuels & Rossman (Booth, 1959; Rossman, 1983; Samuels, 1976).





Figs. 15–19. *Solheimia costaspora*. SEM. (GB3165, Neotype). – 15. Synnemata. Bar = 200 µm. – 16. Conidia. Bar = 5 µm. – 17. Young conidiophores at apex of synnemata, note incrusting hyphae. Bar = 50 µm. – 18. Incrusting hyphal ends from stipe apex. Bar = 5 µm. – 19. Conidiogenous cells with young conidia. Bar = 5 µm.

Members of the *N. ralfsii*-group are united by similarities in ascocarp wall structure, anamorph morphology, and habit. The group also includes *N. chlorogloea* Samuels and *N. pityrodes* (Montagne) Montagne which have *Myrothecium* anamorphs (Samuels & al., 1990; Samuels, 1976, 1988; Tulloch, 1972) and *N. septomyrotheciae* Samuels with a *Septomyrothecium* anamorph (Samuels, 1988). Samuels & Seifert (1987) suggested that this group has affinities with the *N. ochroleuca* (Schwein.) Berk.-group and Samuels & al. (1990) included both in the *N. ochroleuca*-group. In a recent comparison of ribosomal DNA sequences among hypocrealean fungi, *N. pityrodes* clustered with other pallid species of *Nectria* but on a different clade from the other pallid *Nectria*-like fungi that have *Dendrodochium-Clonostachys* anamorphs (Rehner & Samuels, 1994).

Species of *Myrothecium* usually have conidiophores aggregated in sporodochia or synnemata (Tulloch, 1972). For example, *M.*

*mucunae* Castañeda Ruiz & Kendrick (1991) is a recently described synnematosus species, while the *Myrothecium* state of *N. pithyroides* is described as being synnematosus or sporodochial. Often these conidiomata have marginal or stipe hyphae that are ornamented or incrustated as are the stipe hyphae of *Virgatospora*, *Albosynnema*, and *Solheimia* (Figs. 3, 7, 17, 18). A particularly striking similarity among the three synnematosus genera and *Myrothecium* spp. are their colony morphologies in agar culture (Figs. 1, 10). On nutritionally rich media, e.g. malt-yeast extract agar or oatmeal agar, all three synnematosus genera and *Myrothecium* spp. grow moderately fast, form white to pale pink, cottony or lanose aerial mycelium, and form dark greenish black, moist to slimy conidiomata. Young colonies of *S. costaspora*, *A. elegans*, or *V. echinofibrosa* in which sporulation has started prior to elongation of synnematal stipes, could be mistaken for *Myrothecium* spp. The pale green ornamented hyphae of the sporodochia of *M. cinctum* are very similar in morphology to the stipe hyphae of *S. costaspora* (Figs. 17, 18). When grown side-by-side on autoclaved banana leaves, the young conidiomata of *M. cinctum* and *S. costaspora* are nearly indistinguishable. The spore shape and ornamentation of these two fungi were nearly identical, but conidia of *S. costaspora* differed from those of *M. cinctum* by their 40–50% larger size. Considering that some populations of *M. cinctum* have conidia of approximately the same dimensions of *S. costaspora* (Matsushima, 1980), there seems to be no characters that consistently separate *S. costaspora* from *Myrothecium* spp., and thus *S. costaspora* could be accommodated within the genus *Myrothecium*.

Based on examination of published descriptions, *Phialoarthrobotryum triseptatum* Matsushima (1975) and *Phialoarthrobotryum brasiliense* Upadhyay (1981) are additional synnematosus hyphomycetes with phialidic, penicillately arranged conidiophores, and moist, multiseptate conidia that have many similarities with the group of anamorphic genera outlined above. Also the generic placement of the second species of *Albosynnema*, *A. filicola* Sherwood (1974), should be reexamined. Sherwood described the conidia as “borne on the tips of unspecialized hyphae” and she suggested that they were “blastic”, but in fact they are phialidic (K. Seifert, pers. comm.). The conidia were illustrated as being “distoseptate”, whereas the conidia of *A. elegans* are simple septate.

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