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Basidiopycnides albertensis gen. et sp. nov., a new anamorphic fungus with phylogenetic affinities in the *Atractiellales (Basidiomycota)*

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Abstract — A new anamorphic genus, *Basidiopycnides*, and its type species, *Basidiopycnides albertensis*, are described. Strains of *Basidiopycnides albertensis* produce what superficially appeared to be a *Graphium*-like conidial state with percurrently proliferating annellophores. Detailed morphological and molecular data analysis showed these isolates represent a new taxon that belongs to the *Atractiellales (Basidiomycota)*.

Key words — bark beetles, DAPI staining, molecular phylogeny

Introduction

In 1987 collections of bark beetle infested material from various coniferous hosts yielded isolates resembling synnematous species of either *Graphium* Corda or *Pesotum* J.L. Crane & Schokn. But since then, species of these and other similar genera have been studied extensively. Seifert & Okada (1993) proposed that *Graphilbum* H.P. Upadhyay & W.B. Kendr., *Pesotum*, *Hyalopesotum* H.P. Upadhyay & W.B. Kendr., *Phialographium* H.P. Upadhyay & W.B. Kendr., *Pachnodium* H.P. Upadhyay & W.B. Kendr., and *Graphiocladiella* H.P. Upadhyay were all synonymous with *Graphium*. However, later detailed morphological and molecular taxonomic studies showed that *Graphium* and *Pesotum* are distinct (Okada et al. 1998b, 2000), and that while *Graphium* species are linked phylogenetically to the *Microascales*, *Pesotum* species are linked to the *Ophiostomales* (Okada et al. 1998b, 2000; Jacobs et al. 2003). Therefore, *Ophiostoma* anamorphs formerly placed in *Graphilbum*, *Hyalopesotum*,

Phialographium, *Pachnodium,* and *Graphiocladiella* now should be treated under *Pesotum* J.L. Crane & Schokn. emend. G. Okada & Seifert (Okada et al. 1998b, p. 1503; 2000).

Although two variants of percurrently proliferated conidiogenous cells were recognized in *Graphium* species (Seifert & Okada 1993, Okada et al. 1998b, 2000), both are said to be enteroblastic annellidic: one form produces nodular annellations; the second a mixture of nodular and dense annellations. In contrast conidiogenesis in *Pesotum* is either holoblastic or enteroblastic phialidic (Okada et al. 1998b).

Five cultures isolated from material collected in Banff National Park, Alberta, Canada, exhibited serial percurrent formation and secession of holoblastic conidia that resulted in the formation of proliferated annellophores sensu Kiffer & Morelet (2000), with elongate intervals between the successive annellations that mark spore-production sites. However, the nodular annellations seen in some *Graphium* species were absent. The conidia ultimately aggregated into slimy masses, but the conidiophores were neither verticillate nor penicillate. Their macronematous conidiomata also differed markedly from synnemata produced by *Pesotum* and *Graphium* species, in that they developed from simple erect hyphal initials that produced basally constricted branches from just below the septa. These branches curved sharply upward immediately after initiation, and grew parallel to the parent element; each branch produced several new branches in a similar manner. At the apices of the ultimate branches, the annellophores were formed. There was a wide range of sizes noted amongst the conidiophores observed, and when conidiomata coalesced laterally, top-shaped structures were formed with globules of conidia at their apices. This developmental pattern is different from those of synnemata as described by Seifert (1985) and Seifert & Okada (1990).

As the conidiogenesis and conidiophore branching of our strains were similar to certain basidiomycete species, e.g., *Stilbotulasnella conidiophora* Bandoni & Oberw. (1982), *Gloeosynnema ochroleucum* (Penz. & Sacc.) Seifert & G. Okada (1988), *Gloeosynnema roseum* Matsush. (1995), *Filosporella annelidica* (Shearer & J.L. Crane) J.L. Crane & Shearer (1977), and species of the ascomycete genus *Pyxidiophora* Bref. & Tavel (Blackwell & Malloch 1989), we used sequence analyses of the 18S ribosomal RNA gene (18S rDNA) and rDNA internal transcribed sequences (ITS), and incident-light fluorescence microscopy to determine their phylogenetic relationship and nuclear status.

Materials and methods

Strains studied and morphology. Five isolates, whose collection details are given under the new species description, were obtained from host material from which beetles had been removed at the collection site according to

Hutchison & Reid (1988) and Eyjólfsdóttir (1990). These have been maintained at 4 °C in darkness on slants of malt extract agar (Difco, MI; Fisher Scientific Fair Lawn, NJ) plus yeast extract (MEYE, Hausner et al. 2003) and culture descriptions on agar were also prepared according to Hausner et al. (2003). Isolates were also grown on autoclaved *Pinus mugo* Turra and *Pinus sylvestris* L. twigs embedded aseptically in both 2.0 % water agar or MEYE in Petri dishes at 20 °C to see if different forms of fruiting occurred. The twig culture technique was repeated with our strains using three separate sets of twig-plates inoculated in combination with one of three strains of *Clonostachys rosea* (Link) Schroers et al. (1999); the latter had successfully induced perithecial formation in strains of *Ceratocystiopsis falcata* (E.F.Wright & Cain) H.P. Upadhyay [≡ *Cornuvesica falcata* (E.F. Wright & Cain) Viljoen et al.] (Hutchison & Reid 1988, Kawchuk et al. 1993). Inoculated twig plates lacking the *Clonostachys* strains served as controls. Portions of both living and dried specimens have been deposited in the University of Alberta Microfungus Collection and Herbarium (UAMH), Devonian Botanic Garden, and are also held in the Biological Sciences Department [WIN(M)] of the University of Manitoba.

Morphological structures used to identify isolates were mounted on slides in either 85 % lactic acid or 85 % lactic acid/water solutions (1:15 by volume), and cover slips were ringed with nail varnish prior to observation. But the pure 85 % lactic acid mounts were placed on a slide warmer at 40 °C for 24 hours before ringing, while the lactic acid/water mounts were ringed and studied immediately; the latter yielded clearer preparations (Eyjólfsdóttir 1990). At least 50 measurements of spores were recorded for statistical analysis, and either a Leitz Ortholux II or a Zeiss Photomicroscope II was used for observations, drawings and photography. We used Munsell Soil Color charts (Kolmorgen Corp., Baltimore, MD) to categorize for colours, and Gams (1971) for descriptions of colony texture and conidiophore organization.

Nuclear staining and incident-light fluorescence microscopy. Aseptically, in a laminar flow chamber, sterile slides were dipped in sterile molten malt extract (2 g malt extract, 20 g agar, 1 l tap water). One side was wiped clean, and the slide placed agar side up on a sterile bent glass rod in a sterile Petri dish moist-chamber lined with several layers of moistened sterile paper toweling. Slides were inoculated aseptically at the centre with a spore mass from a fresh culture of the selected test strain, and the Petri dishes were incubated at 20 °C for 7 days. If at that time growth had occurred, slides were fixed in 100 % methanol and stained with DAPI (4', 6-diamadino-2-phenylindole) according to Hamada & Fujita (1983) as described by Okada et al. (1998a). But we replaced the 2-mercapto-ethylamine in the staining solution with 2-mercaptoethanol (Aldrich Chemicals, Milwaukee, WI); the latter had been shown to be highly effective in fungi (M. Young, personal communication).

When maintained on ice in a refrigerator at 4 °C, these slides yielded good results for up to 5 days after preparation. The nuclei were visualised using a Zeiss Axio Imager and the images collected digitally with an AxioCam Mrm camera.

DNA extraction and amplification protocols. DNA extraction, purification, and agarose electrophoresis protocols were those of Hausner et al. (1992). Whole cell DNA served as the template for amplifying DNA fragments of interest using the Invitrogen-BRL PCR System (Buffer and Taq polymerase, Invitrogen, Fredrick, MD). Primers SSUZ and LSU4 (Hausner et al. 2005) were used to amplify the ITS regions, and primers SSJ and SST were employed to recover the 18S rDNA. The PCR primer sequences, amplification conditions, sizes of the expected PCR products, and preparation of sequencing templates for ITS, and 18S rDNA fragments have been described previously (Hausner & Reid 2004, Hausner & Wang 2005, Hausner et al. 2005). DNA sequencing templates were prepared with the aid of the Promega Wizard SV Gel and PCR clean-up system (Promega, Madison, WI). Purified double-stranded PCR products were sequenced in both directions using the cycle-sequencing protocols performed according to the manufacturers' recommendations (Perkin Elmer Applied Biosystems, Foster City, CA), and automated Fluorescent DNA sequence analysis was performed using an ABI Prism 310 Genetic Analyzer system (PEAB at the University of Calgary, DNA sequencing facility, Calgary, AB).

Analyses of DNA sequence data. Forty-seven 18S rDNA sequences were aligned with CLUSTAL-X (Thomson et al. 1997) and, when appropriate, modified with the alignment editor program GeneDoc v2.5.010 (Nicholas et al. 1997, http://www.psc.edu/biomed/genedoc). The alignment (EMBL-Align database accession: ALIGN_001145) covered 1742 positions and the 18S rDNA sequence of *Saccharomyces cerevisiae* Meyen ex E.C. Hansen var. *cerevisia*e served as the outgroup in phylogenetic analysis. First, programs contained within PHYLIP (Felsenstein 2006, Version 3.66; http://evolution.genetics.washington.edu/phylip/getme.html) were used to resolve phylogenetic relationships among the tested sequences, and the data set was then analyzed with DNAPARS (maximum parsimony) and DNADIST (F84 setting). From the latter, the distance matrix generated was utilized in the NEIGHBOR program (NJ setting) for inferring a phylogenetic tree. The phylogenetic estimates were evaluated using the bootstrap procedure (SEQBOOT 1000 replicates; and CONSENSE) in PHYLIP. The 18S rDNA data was also analyzed with the Tree-Puzzle (TP) program [maximum likelihood (ML) phylogenetic analysis using quartets and parallel computing (Schmidt et al. 2002)]. The settings for the quartet puzzling algorithm were as follows: 10000 puzzling steps, transition/transversion parameter estimated from data set, HKY evolutionary model

(Hasegawa et al. 1985). However tree topologies were essentially identical to those of parsimony and NJ analysis. Finally the MrBayes (version 3.1) program (Ronquist & Huelsenbeck 2003; Ronquist 2004) was used for Bayesian analysis. The NEXUS file format necessary for the alignment (input) file was generated with the file converting option available within the DAMBE (Xia 2001). The DNA substitution model setting for Bayesian analysis was: GRT, gamma distribution with 4 gamma rate parameters. The model of DNA substitution was chosen based on evaluating the 18S rDNA alignment with the Modeltest 3.0 program (Posada & Crandall 1998). The Bayesian inference of phylogenies was initiated from a random starting tree, and four chains were run simultaneously for 250000 generations; trees were sampled every 100 generations. The first 25 % of trees generated were discarded ("burn-in") and the remaining trees were used to construct both a 50 % majority rule consensus tree and compute the posterior probability values.

Results

Our fungus morphologically resembled anamorphs of both some ascomycetous and basidiomycetous taxa, but the nuclear staining and fluorescent microscopy clearly showed that individual hyphal cells, conidiogenous cells, and conidia were very rarely other than binucleate (see under Taxonomy). This dikaryotic condition suggests these isolates are the anamorph of a basidiomycete.

This staining technique produced surprisingly good nuclear staining and, unexpectedly, it also showed the proliferated annellophores extremely well.

To investigate the molecular phylogeny of our fungus, the 18S rDNA sequences were determined for two of our isolates [UAMH 10782 and 10785], these were identical (GenBank EF406118), and were used as queries in a Blastn (Altschul et al. 1997) NCBI database search. The results showed they were closely related to the holotypes of *Basidiopycnis hyalina* Oberw. et al. and *Proceropycnis pinicola* M. (Oberwinkler et al. 2006), both monotypic genera. At least partially by comparing 18S rDNA sequences of their two new species with a variety of basidiomycete sequences, six of which were from species of the *Atractiellales*, they placed their new species within the order *Atractiellales*, class *Atractiellomycetes*, subphylum *Pucciniomycotina*, division *Basidiomycota* (Bauer et al. 2006).

Because our strains closely resembled *Basidiopycnis hyalina* in some features, 18S rDNA sequences of the following species were included in the phylogenetic analysis: *Basidiopycnis hyalina*, *Proceropycnis pinicola, Phleogena faginea* (Fr.) Link, *Helicogloea lagerheimii* Pat., and *Atractiella solani* (Cohn & J. Schröt.) Oberw. & Bandoni (Fig. 1A). Various ascomycete taxa that morphologically resembled our isolates were also included, e.g. *Ophiostoma ulmi* (Buisman) Nannf.(has a *Pesotum* anamorph) and several *Graphium* species. Phylogenetic analysis of the 18S rDNA data using four different (NJ, Parsimony, Bayesian,

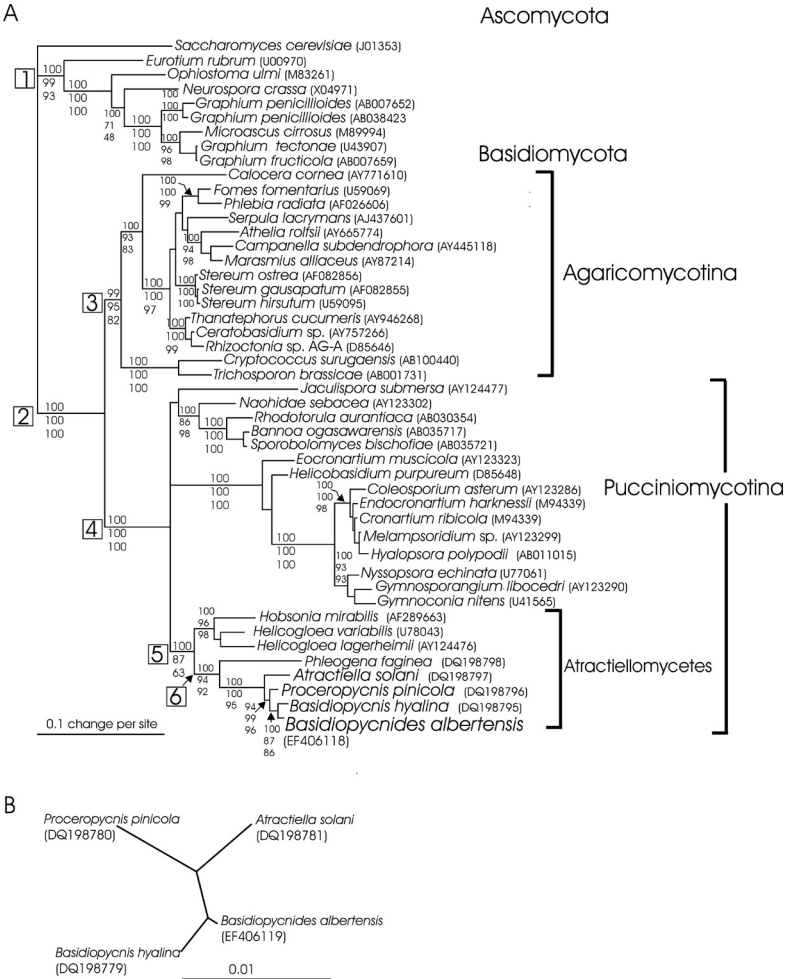

Fig. 1: Phylogenetic analysis of the 18S rDNA sequence data. A. Phylogenetic tree for *Basidiopycnides albertensis* within the *Atractiellales*. Tree is based on a 50 % majority rule consensus tree obtained from Bayesian inference. The first number at the nodes above the line represents the posterior probability values obtained from Bayesian analysis. The second and third number (below the lines) at the nodes indicate the level of support based on bootstrap analysis (Felsenstein 1985; % based on 1000 bootstrap replicates) in combination with NJ and PARS analysis respectively. The 18S rDNA alignment can be retrieved from the EMBL-Align database under the following accession: ALIGN_001145. B. The unrooted Neighbour-Joining (PHYLIP, NJ, DNADIST: setting K84) phylogenetic network showing the relatedness among partial ITS region sequences for four species of the *Atractiellales* including *Basidiopycnides albertensis*. For those sequences that were obtained from NCBI the GenBank accession numbers are provided for in brackets following the species names. The phylogenetic tree (A) and dendrogram (B) were drawn with the TreeView program (Page 1996) using the PHYLIP tree outfiles, and annotations were added to the figures with the aid of Corel Draw (Corel Corporation and Corel Corporation Limited, Ottawa, Canada).

Maximum-likelihood as implemented in TreePuzzle) methods for inferring evolutionary relationships yielded trees with essentially similar topologies (Fig. 1A).

This 18S rDNA data set also separated members of the *Ascomycota* from those of the *Basidiomycota* and, as expected from previously proposed phylogenies (Sikaroodi et al. 2001, Swann et al. 2001, Bauer et al. 2006, Blackwell et al. 2006), placed members of the *Agaricomycotina* and *Pucciniomycotina* of the *Basidiomycota* into specific clades. The 18S rDNA sequences of UAMH 10782 and 10785 grouped within a clade that included species of the class *Atractiellomycetes* (see Fig 1A, node 5). More precisely, our strains, under the name of *Basidiopycnides albertensis* (see Taxonomy) , were placed within the *Atractiellales* in a monophyletic clade that contains *Basidiopycnis hyalina, Proceropycnis pinicola, A. solani,* and *Phleogena faginea* (Fig. 1A, node 6). Most notably, the18S rDNA sequences of these strains differed by only 3 substitutions from the 18S rDNA sequence of *B. hyalina,* but there were 5 and 6 nucleotide differences between the UAMH strains and the sequences of *A. solani* and *Proceropycnis pinicola*, respectively.

The rDNA ITS regions (ITS1, 5.8S rDNA, ITS2) for all of our five strains (UAMH 10782 to UAMH 10786) were sequenced (GenBank EF406119), and these ITS regions of 551 bp, were identical. Partial ITS region sequences (405 bps) from *Basidiopycnis hyalina* (DQ198779), *P. pinicola* (DQ198780) and *A. solani* (DQ198781) were compared with those of our strains (Fig. 1B) and, excluding gaps, there were 16 nucleotide differences between the ITS sequences of the UAMH strains and that of *B. hyalina*, compared to 46 and 52 nucleotide differences between our strains and the ITS sequences of *P. pinicola* and *A. solani* respectively.

These results show that our isolates are distinct from related species, and we propose a new species in a new anamorphic basidiomycete genus.

Taxonomy

Basidiopycnides J. Reid, Eyjólfsd. & G. Hausner, **gen. nov.**

MycoBank MB510902

Hyphomycetus. Hyphae hyalinae, leaves, septatae sine fibulis, cellulae binucleatae. Conidiophora ab initio ex hypha singulare erecta constantia; postea regulatim multo ramificantia, sic habitu mononemata vel macronemata. Conidiophora singula suis lateribus adhaerescentia, habitu saepe turbinata. Cellula conidiogena terminalis, annellophorum percurrens, primo ad apicem conidium singulare formans posteaque, primum conidium secessum, successive conidia additicia holoblastica formans. Conidia in guttas mucosas aggregata, unicellularia, hyalina, laevigata; oblonga vel breviter clavata apicibus obtusis, sed basibus truncatis cum fimbriis curtis basilaribus. Conidia plerumque tempore germinationis nova annellophora emittunt.

Etymology: From the generic name *Basidiopycnis*, and -ides (Latin, = like, connected with)

Type species:

Basidiopycnides albertensis J. Reid, Eyjólfsd. & G. Hausner, **sp. nov.**

MycoBank MB 510903 Figs. 2 - 6.

Hyphomycetus. Coloniae in agaro cum extracto malti fermentique ad 20 °C aut 16-32 mm diametro post 12 dies aut 26-80 mm post 21 dies in obscuritate, aut 13-17 mm post 12 dies in luce obscuritateque alternanti; albae vel dilutissime brunneae; demum crassis muscosis massis conidiorum obtectae. Hyphae 1.5-3.0 µm diametro, hyalinae, laeves sine fibulis. Cellulae binucleatae. Conidiophora 220-400 µm longa, mononemata vel macronemata. Rami proxime infra septa orientes, saepe in origine attenuati, autem cito sursum curvati dilatatique. Cellulae conidiogenae terminales, holoblasticae, cylindricae vel basi leniter contractae, annellophorae percurrens 25-90 (100) x 2.5-3.4 (4.0) µm. Situs conidiogeni successivi 0.5-7.0 µm distantes. Conidia 6.5-16(-18.5)(sd=11.45 ± 2.78) X 2.5-4.0 (-4.5)(sd=3.45 ± 0.65) µm in guttas mucosas aggregata, unicellularia, hyalina, laevigata, oblonga vel breviter clavata; basibus truncatis cum fimbriis; per hyphas vel annellophora secundaria germinantia.

Holotype: Canada, Alberta; Taylor Lake Hiking Trail, Banff National Park. Dried colonies on sterile pine twigs embedded in malt extract/yeast extract agar (MEYE) isolated from bark beetle galleries in *Pinus contorta* var. *latifolia* Engelm. bark, collected 23 September 1987, J. and B. Reid, derived from UAMH 10782.

Isotype: WIN(M)1397

Etymology: From the provincial name, Alberta.

Colonies on MEYE white to very pale brown (10YR8/2 to 8/3) with even margins in alternating light and dark at 20 °C, margins white (10YR8/2) to translucent. Dark grown colony margins often irregular due to faster growing hyphae developing short, lateral dendroid branches around the margins, but these are less pronounced in alternating light. White (10YR8/2) beneath areas of denser mycelium, but colourless elsewhere. Odour lacking or indistinct. Attaining 16-32 mm diam in 12 days or 26-80 mm in 21days in darkness, and 13-17 mm in 12 days in alternating light and dark, all at 20 °C. Hyphae hyaline, smooth-walled, 1.5-3.6 µm wide, cells binucleate, aerial hyphae collapsing in older cultures. Clamps and chlamydospores lacking. Conidiophores erect, initially arising from single hyphal elements, phalacrogenous, mononematous, semi-macronematous or macronemetous, the latter often coalescent laterally and then resembling top-shaped conidiomata with glistening apices (Figs. 2,3).

Macronematous conidiophores 220-400 µm long, with each branch narrowed at its point of emergence from immediately below a septum in the originating hypha, curving upward sharply and widening to normal size; the vast majority have a basal septum above the curve, and all are terminated by percurrently proliferating annellophores (Figs. 3,4,5); producing slimy conidial masses; medium-embedded hyphae may produce crescent-shaped conidial masses. Conidiogenous cells holoblastic, terminal, integrated, percurrent, forming conspicuous annellophores 25-150 X 2.5-3.5 (-4.0) µm; these are at

Fig. 2: Conidiomata of *Basidiopycnides albertensis* (strain: UAMH 10784 = WIN(M) 720) in various stages of development, growing on an autoclaved pine twig in agar. Bar = 10 mm.

first parallel-sided but taper gradually towards the apex, and are occasionally either slightly constricted or form slightly swollen nodular annellations at their base. Annellations occur at intervals of 0.5-7.0 µm, with the longer intervals formed first. Conidia (Figs. 3,6) unicellular, hyaline, binucleate, smooth-walled, oblong to occasionally short clavate, with an obtuse apex and truncate base that may be slightly narrowed, but with a short but definite frill, 6.5-16(-18.5) (sd=11.45 ± 2.78) X 2.5-4.0(-4.5) (sd=3.45 ±0.65) µm, narrowed bases 2.5 ± 0.8 µm, produced singly, but aggregating in slimy masses in culture, germinating by germ tubes or directly produced annellophores.

Teleomorph: Not observed.

> Additional isolates examined (paratypes). Same site and same date as the holotype: UAMH 10783 = WIN(M) 719 from the same tree as holotype; UAMH 10784 = WIN(M) 720, UAMH 10785 = WIN(M) 1399, and UAMH 10786 = WIN(M) 1425 were from three different trees.

Individual dark grown isolates varied slightly in growth rates and colony morphologies, but in alternating light and dark growth they were very similar. And in all cases secondary conidium production greatly increased the size of the slimy conidial masses on the surface of a colony. Importantly, the nature of the conidiomata formed in twig cultures was the same as on agar plates, e.g., size variation, branching pattern, conidium size, etc.

Occasionally conidia remain attached at the conidiogenous locus; this caused bending in the annellation zone (Fig. 3). However, this is different from the situation where seceded conidia sometimes remain adherent after secession; presumably because of the presence of mucilaginous material on either walls of the conidia or conidiogenous cell. When viewed with phase contrast optics, both conidium initials and most conidia have a halo surrounding them (Fig. 6); that may be a mucilaginous sheath.

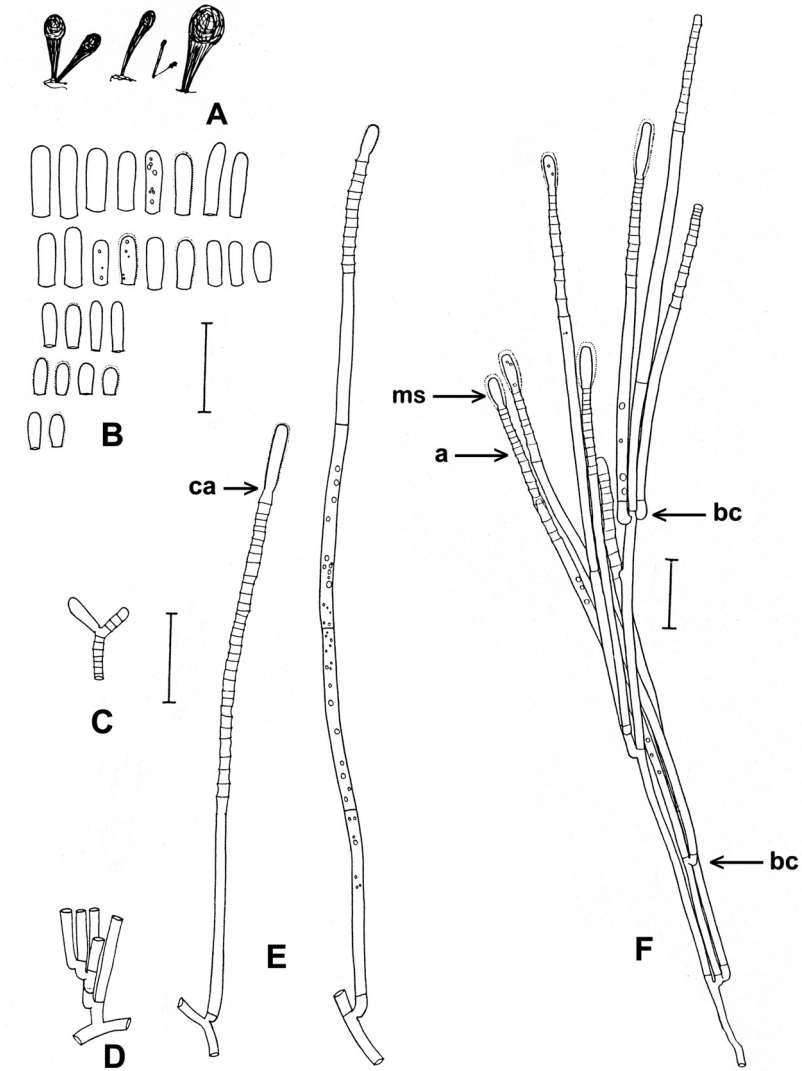

Fig. 3: Line drawings of *Basidiopycnides albertensis* (strain: UAMH 10786 = WIN(M) 1397). A. Habit sketch of individual conidiophores aggregations; not to scale. B. Conidial size variation. Some conidia have guttules and / or sheaths. Bar = 20 µm. C. An annellation zone from which a conidium was displaced in a sympodular manner. D. Basal branching of a complex conidiophore. E. Two mononematous conidiophores (annellophores), one highly guttulate. When a conidium is formed initially, it is often subtended by a slight constriction in the originating conidiogenous cells (ca). Bar for C, D, and E = 20 µm. F. Young conidioma with branched conidiophores arising from a single element; conidium with mucilaginous sheath (ms); annellation (a); branch constriction (bc). Bar = 20 µm.

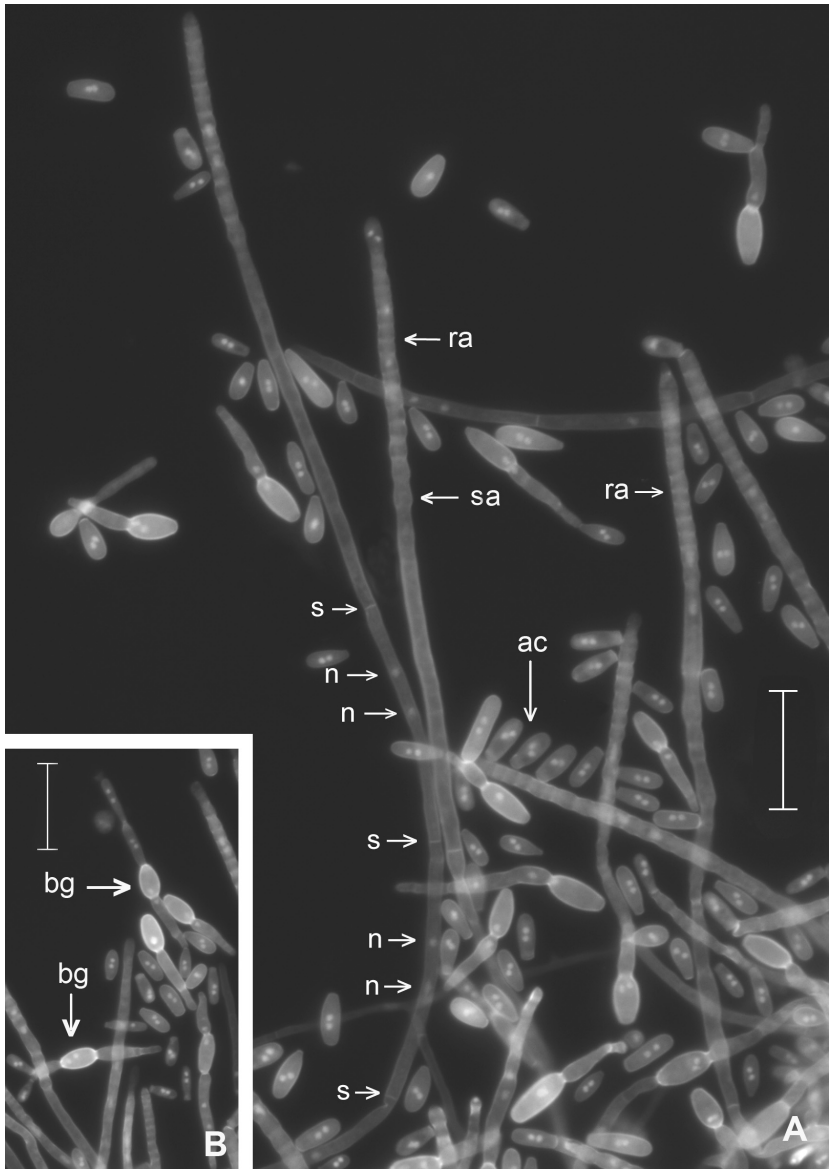

Fig. 4: An incident-light fluorescent micrograph of a DAPI-stained slide culture (strain: UAMH 10784 = WIN(M) 720]. A. Binucleate hyphal cells; septa (s) and nuclei (n). Swellings on the annellophore base (sa). Laterally displaced seceded conidia (ac) still adhering to a proliferated annellophore. Repetitively produced annellations (ra). Bar = 20 μm. B. Two bipolar germinating conidia (bg). Bar = 20 μm. In both A and B, the conidia are regularly binucleate and, on germinating, successive conidiogenous sites are often narrower.

Discussion

None of the strains of *Basidiopycnides albertensis* ever produced basidiomata in culture, and their conidiomata superficially resemble synnemata as characterized by Seifert (1985) and Seifert & Okada (1990). However, our DAPI nuclear staining results that showed the hyphal cells and the conidia are consistently binucleate, and the DNA sequence phylogeny data combined, provide strong evidence that this species is a member of the Basidiomycota closely allied to, but not identical with, *Basidiopycnis hyalina* of the *Atractiellales*. And while morphological similarities were evident in these two species, e.g., the manner of conidiophore branching, size and shape of conidia and the latter's aggregation into slimy masses. Neither significant annellations nor pronounced annellation intervals were described or figured for *Basidiopycnis hyalina* – herein we use annellation to denote the sites where single conidia are produced, and annellation interval(s) the distance(s) between two such successive sites – but it was said to produce annellides.

In some features *Basidiopycnides albertensis* also resembles the basidiomycete *Stilbotulasnella conidiophora*. However, Bandoni & Oberwinkler (1982) state the annellations in *S. conidiophora* are inconspicuous and only clearly visible when stained, but its annellation intervals as figured are significant. In *Basidiopycnides albertensis* the annellations are seen easily in both water or KOH mounts (Fig. 5). Thus we assume that significant annellation intervals are lacking in *Basidiopycnis hyalina*, since Bandoni & Oberwinkler (1982) illustrated such for *S. conidiophora* where visualization was markedly improved in stained mounts, while Oberwinkler et al. (2006) who also used stained preparations did not record these features in *Basidiopycnis hyalina*. Nor did they record conidial germination by direct production of annellophores in *Basidiopycnis hyalina* as we found in *Basidiopycnides albertensis*; they only reported germination by production of hyphae. Indeed, the conidia of *Basidiopycnides albertensis* typically germinate by the production of either uni- or bipolar secondary annellophores. These can, and do, produce multiple secondary conidia (Figs. 4A, B). Finally, *Basidiopycnides albertensis* is only known from a single location from several *Pinus contorta* var. *latifolia* trees at a single location, while *Basidiopycnis hyalina* occurs in galleries of multiple species of bark-beetles infesting both *Picea abies* (L.) H. Karst. and *Pinus sylvestris* in Germany, Italy and Switzerland (Oberwinkler et al. 2006).

The unusually percurrently proliferating annellophores produced by *Basidiopycnides albertensis* are not unique. Similar annellophores that develop as extensions of conidiophores and conidiophore branches are reported in *Filosporella* [= *Rogersia annelidica* Shearer & J.L. Crane (1976)], but this has very different conidia. Blackwell & Malloch (1989) described the hyaline imperfect state of a *Pyxidiophora* sp. as having 200-300 µm long conidiophores

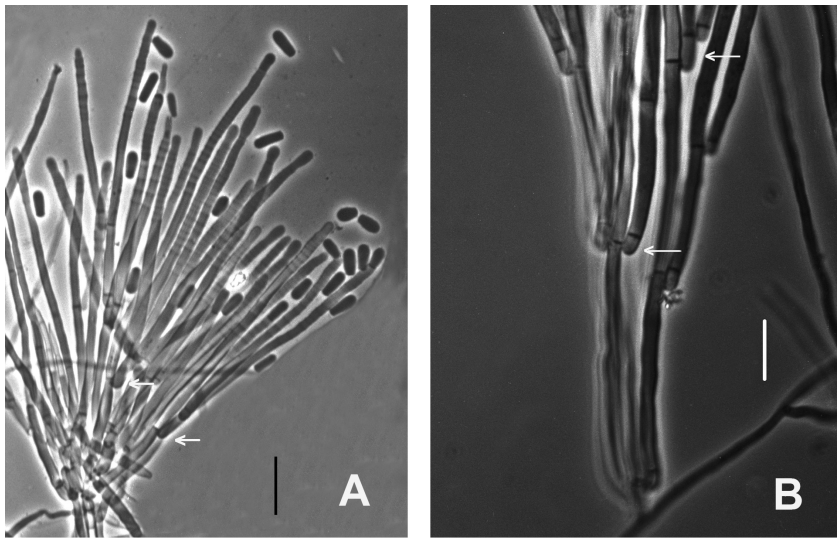

Fig. 5: Phase contrast micrographs of conidiomata (strain UAMH 10786 = WIN(M) 1397). A. An apparent aggregation of at least three conidiomata arising from at least three separate hyphal elements (see base). Note the constricted branch bases (arrows), and the very regular intervals between successive annellations, Bar = 20 µm. B. The base of a conidioma arising from a parent hyphal element shown in two different focal planes. The constricted bases of branching elements can be clearly seen, even in successive branches (arrows). Note on the right the "three level branching pattern of the conidiophore" sensu Jacobs et al. (2003, Fig 12); on the left the conidioma is derived from a single branch arising from a vegetative hyphal element, Bar = 10 µm.

and percurrently proliferating conidiogenous cells; this too was ruled out after examination of a prepared slide loaned by Dr. Malloch. Indeed, such annellophores have been reported in a wide variety of fungi (Glawe 1989), sometimes being produced concurrently with sympodially proliferated conidiogenous cells e.g. in *Diatrype albopruinosa* (Schwein.) Cooke (Glawe & Rogers 1982).

Basidiopycnides albertensis strains resemble superficially species of *Graphium* and *Pesotum* (Seifert & Okada 1993, Okada et al. 1998b) but differ in the branching nature in the conidiomata of *Basidiopycnides albertensis*; lengths of both annellophores and conidiation site intervals; the presence of only slight swelling of some of the basal annellation intervals in the annellophores (Fig. 5), rather than the significant nodulation found in *Graphium* spp.; and the conidiogenous cells that are markedly different to those found in *Pesotum* spp. But the basal branching in *Basidiopycnides albertensis* is strikingly similar to the "Three-level branching pattern of the conidiophore" that illustrates how conidiophores are produced in the capitulum of *Graphium laricis* K. Jacobs et

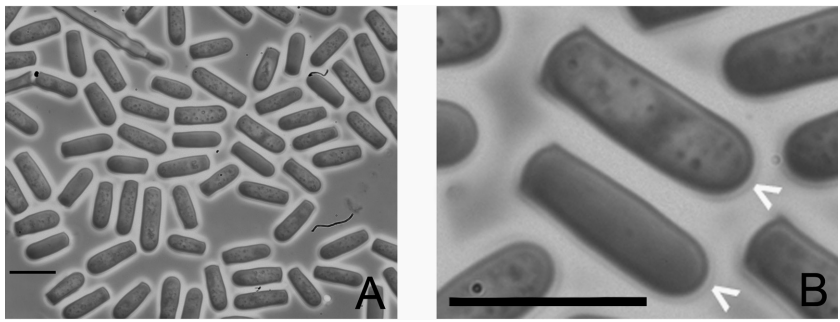

Fig. 6: Phase contrast micrographs of conidia (strain UAMH 10786 = WIN(M)1397). A. Oblong conidia with truncated basal frill [compare with Jacobs et al. 2003, Fig 10], Bar = 10 µm. B. Conidia. The presumed sheath is indicated by arrows. Bar = 10 µm.

al. (2003, Fig. 12), except in the latter the branches do not have constricted bases at their points of origin.

When first reported in the literature (Bauer et al. 2006, pp. 43, 57-58, Fig. 1), *Basidiopycnis hyalina* was treated as one of two new *Atractiellomycetes* with "pycnidial basidiocarps", and only designated by a herbarium number in the discussion in that paper. Later, only *Basidiopycnis hyalina* was confirmed to produce a "pycnidial basidiocarp", i.e., a structure resembling a pycnidium in which basidia and basidiospores are produced; the second species, *Proceropycnis pinicola*, was treated as a new anamorphic fungus (Oberwinkler et al. 2006). However, the latter authors reported that the basidiomata of *Basidiopycnis hyalina* were only produced when strains were paired with other fungi, but not in collections from host material.

We never found basidiomata of *Basidiopycnides albertensis* either on host material or under any culture conditions.

Oberwinkler et al. (2006, p. 644) did not propose an anamorph genus for their asexual phase of *Basidiopycnis hyalina*, stating "We were not able to find morphological characteristics sufficient for separating these basidiomycetes anamorphs on a generic level and, therefore, do not propose a new genus for the anamorph of *B. hyalina*." In support of this they refer specifically to S*tilbotulasnella* Oberw. & Bandoni, a monotypic genus based on *S. conidiophora*, and two species of *Pistillaria* Fr. (Koski & Perrin 1971); the latter two are now considered species of *Typhula*: *T. micans* (Pers.) Berthier and *T. setipes* (Grev.) Berthier (Berthier, 1976). But an anamorph genus is required because criteria other than conidiomatal structure separate organisms such as *S. conidiophora* and *T. micans* from *B. hyalina*. For example, *Basidiopycnis hyalina* has simple septate pores surrounded by atractosomes (Oberwinkler et al. 2006), while *S. conidiophora* has dolipore septa, lacking parenthosomes. We did not examine

pore structure in *Basidiopycnides albertensis*. And both *T. setipes* and *T. micans* have clamp connections (Koski & Perrin 1971), but *Basidiopycnis hyalina* (Oberwinkler et al. 2006), *S. conidiophora* and *Basidiopycnides albertensis* do not.

These factors, plus the fact that *Basidiopycnides albertensis* did not produce a teleomorph state, made it difficult to determine the various relationships. But it suggests strongly that relationships should not be assumed based simply on the production of presumptive "enteroblastic annelloconidia". It is doubtful *Graphium* spp. sensu Okada et al. (2000) are closely related to the organisms discussed above, but the method of conidial production in species such as *Graphium laricis* (Jacobs et al. 2003), *Graphium penicillioides* Corda (Okada et al. 2000) or *Remersonia thermophila* (Fergus) Seifert & Samson (Seifert et al. 1997) are superficially strikingly similar to that of *Basidiopycnis hyalina*, *Basidiopycnides albertensis* and *S. conidiophora*. It is also highly unlikely that *Graphium* spp. consistently produce binucleate conidia as observed in *Basidiopycnides albertensis*. Probably, the superficial similarity in conidiogenesis observed in the genera/species listed above simply reflects convergent evolution of a similar, but not identical, characteristic that has been selected for in different groups of unrelated fungi. The production of large masses of sticky spores on upward pointing conidiophores would facilitate their being brushed onto the bodies of insects, and thus favor their dissemination by insects. With the inclusion of sequence data along with morphological criteria, *Basidiopycnides albertensis* can be distinguished from "*Graphium*-like" anamorphs that evolved within both ascomycetous and basidiomycetous lineages. In order to facilitate a better understanding of the biology of these organisms we need to designate a phylogenetic classification. It is likely that more "*Graphium*-like" fungi will be recovered from bark beetle galleries and these organisms should be placed into appropriate genera.

Throughout we have used the term annellophore in reference to conidiogenesis in *Basidiopycnides albertensis*, but this is contrary to the recommendation by Kendrick (1971, p. 261) that the term annellophore should be replaced with annellide to mean annellated conidiogenous cell. However, annellated was defined in that same Proceedings chapter (Kendrick 1971, p. 254) as "condition of a conidiogenous cell which has undergone a number of **very short** percurrent vegetative proliferations, each of which terminated in the production of a single holoblastic conidium. Each --- a **narrow band** of wall material encircling the conidiogenous cell, is called an annellation." (The bold usage is ours). Although subsequently many mycologists have followed this recommendation, we do not feel the term annellide, as defined above, is appropriate for the conidiogenous structure seen in *Basidiopycnides albertensis*. And problems with this general usage of the term annellide have been noted before.

Hammill (1972), in one of a series of papers on conidiogenesis in fungi based on transmission electron microscopy studies, drew attention to an unusual form of such in *Monotosporella sphaerocephala* (Berk. & Broome) S. Hughes [≡ *Acrogenospora sphaerocephala* (Berk. & Broome) M.B. Ellis (1971)]. Here, the subsequent unusual pattern of conidium maturation aside, initial development involved percurrent growth of intraconidiophore hyphae through a previously formed conidium-delimiting septum.

Cole & Samson (1979) using scanning electron microscopy confirmed, and expanded on, Hammill's work and concluded, page 78, "--- it is probable that several genera of annellated Deuteromycetes develop in this manner". But what is significant, from our point of view, is that the annellations on the conidiophore are produced at significant intervals, not very short percurrent vegetative proliferations (see above).

The term annellide was defined by Sutton (1980, p. 642) as "a holoblastic percurrently proliferating conidiogenous cell", a more succinct but nonetheless accurate rendering of Kendrick's (1971, p. 258) definition. And an example of the confusion that existed at that time is to be seen in Bandoni & Oberwinkler (1982, p. 1879) who were unsure as to the true nature of the conidiogenous cells in *S. conidiophora* and wrote "Although we have used the term annellide in reference to the conidiogenous cells, it remains to be demonstrated they are homologs of structures so designated in ascomycetes and hyphomycetes". Then, in a progressive step, Sutton and his colleagues (Minter et al. 1983, pp. 117-118) argued that at least two sets of developmental processes were being defined by the term annellide. This view was supported by both Wang (1990) and Kiffer & Morelet (2000), who both resurrected and defined their concept of an annellophore and redefined an annellide. We concur an annellophore is really an annellated conidiophore; see Wang (1990, pp. 53-56) for a precise description of the postulated process, and that annellophore is the appropriate term to describe conidiogenesis in *Basidiopycnides albertensis*, and quite possibly fungi such as *S. conidiophora* as well. It is probable that transmission electron microscopic pictures will show that the two latter species develop annellophores in a manner consistent with Wang's (1990) theory and, as a consequence, the term annellophore should be reinstated.

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