5-1-2006

Vittatispora, a New Melanosporaceous Genus From Indian Soil

Preeti Chaudhary
Biocom Ltd.

Jinx Campbell
University of Southern Mississippi

David L. Hawksworth
MycoNova

Kedarnath N. Sastry
Biocon Ltd.

Mycologia, 98(3), 2006, pp. 460–467.

Vittatispora, a new melanosporaceous genus from Indian soil

Preeti Chaudhary

Research & Development, Biocon Ltd, 20th km, Hosur Road, Electronic City P.O., Bangalore 560 100, India

Jinx Campbell

Department of Coastal Sciences, University of Southern Mississippi, 703 East Beach Drive, Ocean Springs, Mississippi 39564

David L. Hawksworth

MycoNova, The Yellow House, Calle Aguila 12, Colonia la Maliciosa, Mataelpino, Madrid 28492, Spain

Kedarnath N. Sastry[1]

Research & Development, Biocon Ltd, 20th km, Hosur Road, Electronic City P.O., Bangalore 560 100, India

Abstract: *Vittatispora coorgii* gen. sp. nov., isolated from soil in India, is described and illustrated. The fungus has morphological characteristics of the genera *Melanospora*, *Sphaerodes* and *Syspastospora*. The most striking feature is the presence of a thick hyaline ridge along the vertical axis of the lemon-shaped ascospores wall. Perithecia also have a long neck composed of adhering hyphae, similar to that of *Syspatospora*. Phylogenetic studies on the 28S rDNA indicate it is closely related to *Melanospora* and *Sphaerodes* and belongs in the Ceratostomataceae. The new genus is based on the distinctive morphology and phylogenetic analyses. The fungus grew in culture only conjointly with a sterile fungus which a BLAST analysis suggested was close to *Tetracladium marchalianum*.

Key words: Ascomycota, Ceratostomataceae, *Melanospora*, perithecia, *Sphaerodes*, *Syspastospora*, 28S rDNA

INTRODUCTION

During cellulolytic myxobacteria isolations, perithecia of an unknown fungus were observed on filter paper, and this fungus was subjected to further studies. The fungus is characterized by perithecia with a bare, long neck composed of aggregated hyphae like *Syspastospora* P.F. Cannon & D. Hawksw., lemon-shaped, smooth-walled ascospores with apiculate germ pores like *Melanospora* Corda and a raised rim surrounding the germ pore as seen in *Sphaerodes* Clem. (Cannon and Hawksworth 1982, Stchigel et al 1999, Garcia et al 2002, Zhang and Blackwell 2002). The fungus is unlike any of the known species of *Melanospora* or *Sphaerodes* in having a thick hyaline ridge running the vertical length of the ascospore between the germ pores. Due to the differences in morphology its inclusion in any of these genera was problematic. We therefore used molecular analyses to objectively assess the phylogenetic importance of the distinctive morphological characters in the fungus. These data, together with the morphological features, showed that the fungus was best accommodated in a separate genus, and here we describe it as a new genus and species.

Accepted for publication 13 Apr 2006.

[1] Corresponding author. E-mail: kedarnath.sastry@biocon.com

MATERIAL AND METHODS

Sampling and isolation.—Soil samples were collected from the Coorg district in southern India at about 1500 m elevation on the plateau of the western Ghats. The terrain is rocky, hilly, thickly forested, interspersed with tea, coffee, honey, cardamom, pepper and orange estates. Coorg receives an annual rainfall of 2717 mm with temperatures of 15–35 C. The natural vegetation of the region is a jungle comprising diverse kinds of trees including *Micelia*, *Mesua*, *Diospyros*, *Chickrassia tubularis*, *Calophyllum angustifolium* and *Canarium strictum*.

Samples were collected from the top 5 cm of the soil. Collections were concentrated on areas rich in decaying plant matter and other organic debris. Samples were placed in fresh zip-lock plastic bags, returned to the laboratory, transferred to sterile Petri dishes, allowed to air dry, then stored in plastic bottles at room temperature (22–25 C) 7–15 d before isolations were made.

Isolation procedures were targeted at cellulolytic myxobacteria and employed a mineral salts medium (Stan 21 agar) with filter paper (Reichenbach 1994). Soil was mixed thoroughly with the help of an isopropyl alcohol (IPA) sterilized spatula and sprinkled on the paper. Because cellulolytic myxobacteria are slow growing, the plates are incubated more than a month (4–6 wk) at 30 C in the dark, therefore very little soil was sprinkled into each plate to reduce contamination by nematodes and protozoans. After 1 mo perithecia were observed and picked up with the help of a flame-sterilized needle under a stereo-zoom microscope (Olympus SZ×12) and subcultured on filter paper (Whatman No. 1, Maidstone, UK) on Stan 21 agar. After two subcultures the filter paper was replaced by 1% microcrystalline cellulose powder (MCC Sancel-W; NB Entrepreneurs, Nagpur, India). The perithecia were subcultured to Stan 21 agar, malt-extract agar (MEA; HiMedia, Mumbai, India), cornmeal agar (CMA; HiMedia), potato-carrot agar (PCA; HiMedia), oatmeal agar (OMA; Difco, Sparks, Maryland) and V8 juice agar (HiMedia). Plates were incubated at 16, 24, 26, 28, 30 and 37 C.

Sporulation studies.—Because Stan 21 agar is a synthetic medium we set up an experiment to see which components of the medium triggered sporulation. Media Petri dishes were made with all but one component of Stan 21 agar (pH 7–8). Media plates of Stan 21 agar with different substrates—cellulose, wheat bran, vitacel, millet flour, grits, ground barley, oat bran, dextran, xylan and glucose— also were set up to see the effect of substrates on sporulation. Perithecia were subcultured on the media plates and incubated at 28 C.

Morphological study.—Observation and measurements of fungal structures were carried out in water and lactophenol cotton-blue mounted material. A compound microscope (Olympus BH2-UMA) was used for microscopy. SEM pictures were taken with JSM 5600 LV (Jeol). The culture has been cryopreserved (Biocon culture collection, BICC 7817).

DNA extraction from ascospores, PCR amplification and sequencing.—Perithecia were crushed in sterile saline. The spore suspension was filtered repeatedly (5 times) through layers of sterilized Miracloth (Calbiochem, La Jolla, California) to remove mycelial and perithecial debris completely. The absence of mycelial contamination was confirmed by inoculating the ascospore suspension in potato-dextrose broth (HiMedia) and incubating it on a shaker at 28 C, 5 d; no growth was observed. The spore suspension was centrifuged to concentrate the ascospores. The pellet was resuspended in 500 microliters of 50 mM Tris-HCI, ph 7.5; 10 mM EDTA; 1% SDS and DNA was extracted by disrupting the cell walls with the aid of glass beads (0.3–0.4 microns; Sigma, St Louis, Missouri) as described by Cassago et al (2002). D1, D2, D3 domains of 28S rDNA (LSU) fragment were amplified with universal primer set LROR/LR6 (Vilgalys Laboratory, Duke University: http:/www.biology.duke.edu/fungi/mycolab/primers.htm) by polymerase chain reaction (PCR). PCR was carried out in 0.2 mL tubes in 50 µL volume containing 60 ng template DNA, 1× *Taq* buffer, 2 mM dNTPs, 4 pM of each primer, and 0.5 units *Taq* polymerase (Bangalore Genie, Bangalore, India).

PCR was carried out in a thermal cycler (PTC-100, MJ Research, Waltham, Massachusetts) with this protocol: 94 C, 4 min; 30 cycles of 94 C, 45 s; 58 C, 45 s; 72 C, 80 s; and 72 C for 10 min final extension. The PCR product was purified with a QIAquick gel extraction kit (QIAGEN, Hilden, Germany) and sequenced with primers LROR and LR6. The sequencing reactions were run on an automated ABI Prism 3700 DNA sequencer (Applied Biosystems, Foster City, California). The sequences were assembled and corrected using Contig express, VectorNTI (InforMax, Frederick, Maryland)

Phylogenetic analyses.—Sequences were aligned with published sequence data using Clustal X (Thompson et al 1997) and refined manually in Se-Al (Rambaut 1996). Cladistic analyses were performed in PAUP* 4.0b 10 (Swofford 2002) with maximum parsimony and weighted parsimony criteria. Maximum parsimony analyses were performed with heuristic searches employing random starting trees, random stepwise addition on 100 replicates, a tree-bisection-reconnection branch-swapping algorithm and gaps treated as missing data. Weighted parsimony analyses were performed with a step matrix to weight nucleotide transformations based on the reciprocal of the observed transition: transversion (ti/tv) ratio (Spatafora et al 1998) and heuristic searches as described above. Parsimony tree scores for the consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated in PAUP* for each tree generated. Alternative tree topologies were tested with Kishino-Hasegawa (K-H) (Kishino and Hasegawa 1989) and Shimodaira-Hasegawa (S-H) (Shimodaira and Hasegawa 1999) maximum likelihood examinations. Bootstrap values (Felsenstein 1985) were calculated from 1000 replications with a heuristic search on 10 replicates with random starting trees, random stepwise addition and MULTREES on. Decay indices (Bremer 1988, 1994) were calculated in AutoDecay (Eriksson 1998).

RESULTS

Cultural characteristics.—On Stan 21 agar, colonies were thin, flat, round, spreading, pale white and translucent, attaining approximately 3 cm diam in 10 d at 26, 28 and 30 C. No aerial mycelia were observed. The perithecia started appearing on the 10th d of incubation, protruding a long neck over the surface of the agar which by the 15th d appeared as hairy projections. Perithecia covered the entire plate by the 20th d. The best sporulation on Stan 21 agar was observed at 26 and 28 C. No sporulation was observed at 16 C.

On MEA the colonies were nonsporulating, round, nonspreading and creamish, with a raised central convex region and ridged peripheral region, attaining 1–2 cm diam in 7 d at 26, 28 and 30 C. Colonies on other mycological media also did not sporulate. No growth was observed at 37 C.

Sporulation.—Perithecia formed on all substrates except wheat bran, ground barley and glucose. In the absence of K_2HPO_4, KNO_3 and $CaCl_2$ sporulation was initiated, but perithecia remained sparse and stunted. Sporulation was enhanced when these salts were incorporated into the media. These media trials indicated that K_2HPO_4, KNO_3 and $CaCl_2$ significantly enhanced sporulation and perithecia formed on a wide range of complex substrates.

TAXONOMY

Vittatispora P. Chaudhary, J. Campb., D. Hawksw. & K.N. Sastry, gen. nov.

Etymology: Latin, *vittatus* = with a longitudinal ridge, referring to the ridge running the vertical axis of the spore.

MycoBank No. MB500705

Ascomata perithecia, solitaria aut gregaria, vel in superficie vel submersa, globosa, ostiolata, perlucida, collum longum cylindricum, quod ex hyphis in ordinibus aequalibus collatis consistit. Peridium membranosum, ex cellis fuscis, perlucidis, Paraphyses absentes. Asci unitunicati, octo spori, clavati-evanescentes. Ascosporae hyalinae-fuscae vel opacae fuscae, ovata, fastigiferens, nonseptatae, maceriis glabris cum duobus poris fastigiosis germinis. Foramen germinis a erecto crassoque dorso circumdatum, quod per totam germinis longitudinem percurrit. Conidia ignota.

Typus. Vittatispora coorgii P. Chaudhary, J. Campb., D. Hawksw. & K.N. Sastry.

Ascomata perithecial, solitary or gregarious, superficial to submerged, globose, ostiolate, light to dark brown, translucent, appearing black when full of ascospores, with a long cylindrical neck protruding above the surface of the agar; neck cylindrical, light brown, composed of parallel aggregated hyphae. *Perithecia* sparsely setose; setae generally on the apex under the base of the neck, straight to curvaceous, pale brownish, nonseptate, thick-walled. *Peridium* membranous, composed of brownish, translucent, polygonal to irregular cells forming *textura angularis*. *Paraphyses* absent. *Asci* unitunicate, clavate, thin-walled, evanescent, 8-spored. *Ascospores* ellipsoidal to citriform, apliculate, single-celled, smooth-walled, brown to dark brown on maturity, with two apical germ pores; germ pores surrounded by a raised rim; a thick hyaline ridge running vertical length of the ascospore between the germ pores. Conidia unknown.

Vittatispora coorgii P. Chaudhary, J. Campb., D. Hawksw. & K.N. Sastry, sp. nov. FIGS. 1–12

MycoBank No. MB500706

Etymology. After Coorg in India, where the soil was collected.

Ascomata globosa, 100–175 µm diam, ostiolata, leviter vel opace fulva, perlucida, cum collum cylindricum, leviter fulvum, 625–1000 × 27–37 µm, quod ex hyphis in ordinibus aequalibus collatis consistit; rare setosa; setae rectae vel curvatae, 35–60 × 2.5–3 µm. Peridium membranosum, ex cellis fuscis, perlucidis 8–10 µm diam. Asci 13–15 × 5–7 µm. Ascosporae 6–7 × 3–3.5 µm.

Typus. India: Western Ghats; Coorg District, Kakkabe, 12.17–12.50°N, 75.22–76.0°E, alt. 1500 m, isolated from soil, 23 Mar 2003, *P. Chaudhary* (BICC 7817– holotypus).

Perithecia solitary or gregarious, superficial to submerged, globose, ostiolate, 100–175 µm diam, light brown to dark brown, translucent, appearing black when full of ascospores, with a long cylindrical neck protruding above the surface of the agar; neck cylindrical, light brown, 625–1000 × 27–37 µm long, composed of parallel aggregated hyphae, individual hyphae 0.9–1.2 µm wide. *Perithecia* sparsely setose; setae generally on the apex of the body of the perithecium and under the base of the neck, straight to slightly curved, 35–60 × 2.5–3 µm, pale brownish, nonseptate, thick-walled. *Peridium* membranous, composed of brownish, translucent, polygonal to irregular pseudoparenchymatous cells, 8–10 µm diam. *Paraphyses* absent. *Asci* unitunicate, clavate, thin-walled, evanescent, deliquescing within the perithecia at the primordial stages of development, 13–15 × 5–7 µm, 8-spored. *Ascospores* irregularly arranged inside the asci, hyaline at first but becoming brown to dark brown on maturity, ellipsoidal, apiculate, single celled, smooth-walled, with two apical germ pores, 6–7 × 3–3.5 µm; germ pores surrounded by a raised rim; a 0.25 µm thick hyaline ridge running the entire vertical length of the ascospore between the germ pores; mature ascospores released from the perithecia through the neck and forming a cirrhus. Conidia not seen.

28S rDNA analysis.—28S rDNA sequence was submitted to GenBank (accession No. DQ017375). Of 878 aligned characters, 257 were parsimony informative (~30%). Maximum parsimony analysis generated 14 most parsimonious trees; weighted parsimony analysis (ti/tv = 2.49) generated one tree. All trees were of length 984, with a consistency index (CI) of 0.47, retention index (RI) of 0.72 and rescaled consistency index (RC) of 0.34. The best tree (as determined by K-H and S-H tests) was one of the unweighted parsimony trees and is

←

FIGS. 1–11. *Vittatispora corgii.* 1. Perithecia on Stan21 agar (50×). 2. Translucent globose perithecia, full of mature ascospores, protruding a long neck (50×). 3. Immature perithecia (200×). 4. Straight to curved, thick walled, nonseptate setae especially seen near the base of the neck (1000×). 5. Peridium composed of polygonal to irregular cells (200×). 6. Ascospores moving through the neck (1000×). 7. Asci with immature ascospores (1000×). 8. Lemon-shaped ascospores (6000×). 9. Long neck composed of parallel aggregated hyphae (3700×). 10. Ascospore showing the germ pore surrounded by raised rim (25 000×). 11. Ascospore showing the thick hyaline ridge running between the germ pores (20 000×).

presented (FIG. 12). Support measured by bootstrapping (>50%) and decay indices (d) is given at the corresponding branches. *Melanospora* and *Sphaerodes* species form a monophyletic clade with 100% bootstrap and a decay index of more than 15. Included in this clade is our isolate BICC 7817. Although some differences in tree topology among the 14 unweighted parsimony trees were observed, this did not relate to the position of our isolate in the melanosporaceous clade but rather to the terminal position of some of the Hypocreales. The *Melanospora-Sphaerodes* clade was monophyletic in all tree topologies. The weighted parsimony tree was identical in tree topology to the best unweighted parsimony tree.

DISCUSSION

Melanospora first was described by Corda in 1837 to accommodate two pyrenomycete species with membranous, translucent, light colored, ostiolate, globose perithecia with a neck clothed with hyaline setae around the tip or without a neck in which case the ostiole is fringed by hyaline setae ascospores that are dark brown or black (Petch 1938). The type species is *M. zamiae* Corda. Since 1837 *Melanospora* has been shuttled between various families and orders due to its morphological heterogeneity (Zhang and Blackwell 2002). It was reassessed based mainly on ascospore morphology by Cannon and Hawksworth (1982), who placed species with smooth-walled, lemon-shaped to ellipsoidal ascospores with sunken germ pores in a recircumscribed *Melanospora,* while species usually with raised reticulate ridges on the walls of the ascospores and with raised tubercle-like germ pores were placed in *Sphaerodes* Clem. The genus *Syspastospora* P.F. Cannon & D. Hawksw. was established to accommodate *Melanospora parasitica* (Tul.) Tul. & C. Tul., differing from other *Melanospora* species in having a long neck (400–3000 µm) composed of parallel aggregated hyphae and slightly narrower (2–2.5 µm) cylindrical to doliform ascospores with truncate ends and large germ pores. In *Melanospora* the neck is short, made of pseudoparenchymatous cells, and fringed with hyaline setae at the tip, whereas in *Syspastospora* there are no setae at the tip of the neck (Cannon and Hawksworth 1982, Harveson and Kimbrough 2001, Zhang and Blackwell 2002, Garcia et al 2002).

Vittatispora coorgii possesses morphological features which were among the chief criteria to define and differentiate between the three genera *Syspastospora, Melanospora* and *Sphaerodes.* The perithecium morphology is similar to that of *Syspastospora parasitica* in that the globose ascoma have a long neck (1000 µm) composed of adhering hyphae and the tip of the neck is devoid of hyaline setae. On the other hand, the ascospores are lemon-shaped and smooth-walled like *Melanospora* with apiculate germ pores surrounded by a raised tuberculate rim similar to that of *Sphaerodes* species. In addition to *Syspastospora parasitica,* two more species have been published in this genus, both having similar doliform to cylindrical truncate ascospores with large germ pores; *S. tropicalis* D. Garcia, Stchigel & Guarro is described as having perithecia with a short neck, and *S. boninensis* Horie, Udagawa & P.F. Cannon characterized by nonostiolate perithecia (Garcia et al 2002). Based on these morphological features our isolate cannot be placed with any of the species described in *Syspastospora.* Further, in our molecular analyses, the type species of *Syspatospora* falls among the main hypocrealean clade and is remote from the *Melanospora-Sphaerodes* clade, as reported by Zhang and Blackwell (2002). *Sphaerodes* is characterized by cleistothecial or ostiolate perithecia with a short neck fringed with hyaline setae and generally coarsely reticulate ellipsoidal ascospores with tuberculate germ pores (Cannon and Hawksworth 1982). The raised rim around the germ pore is a feature of *Sphaerodes* and not *Melanospora,* with the exception of *M. pascuensis* Stchigel & Guarro, *M. collipora* Stchigel & Guarro, *M. zobelii* (Corda) Fuckel, *M. singaporensis* Morinaga, Minoura & Udagawa, *Microthecium ellipsosporum* Takada, and *Microthecium levitum* Udagawa & Cain (Stchigel et al 1999, Cannon and Hawksworth 1982). Although these species have "*Sphaerodes*-type" germ pores, they were retained in *Melanospora* mainly due to the smooth-walled ascospores. In the phylogenetic study of Zhang and Blackwell (2002), *Melanospora* and *Sphaerodes* cluster together, indicating that in Ceratostomataceae ascospore shape and germ pore aperture are more informative characters than wall ornamentation. Thereafter the presence of tuberculate germ pores has been accepted as the fundamental feature of *Sphaerodes.* This was substantiated further by the presence of both reticulate and smooth-walled ascospores within *S. perplexa* D. Garcia, Stchigel & Guarro (Garcia et al 2004). Our fungus differs from these "intermediate species" by its "*Syspastospora*-type" neck and the distinct hyaline ridge that runs between the germ pores.

Doguet (1955) listed three species in *Melanospora* with long-necked perithecia and lemon-shaped ascospores: *M. arenaria* Fischer & Mont., *M. caprina* (Fr.) Sacc., and *M. lagenaria* (Pers.) Fuckel. *Melanospora arenaria* had been placed earlier in a separate genus, *Rhynchomelas* Clem., which was treated as a synonym of *Melanospora* by von Arx and Müller (1954). These species differ from the new fungus in having fringe of

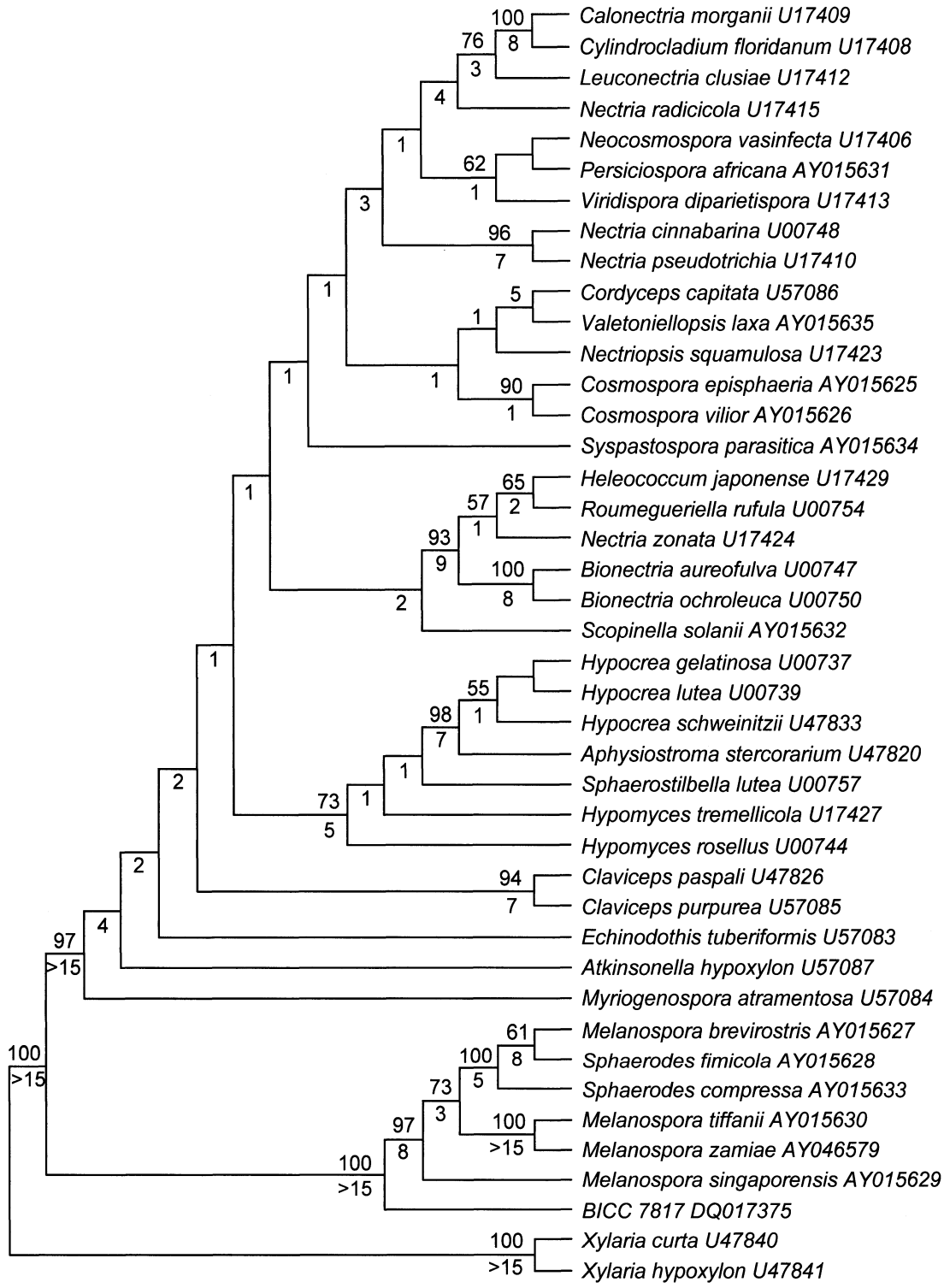

FIG. 12. Cladogram of the best tree (as determined by K-H and S-H tests); generated with unweighted parsimony and of length 984, CI of 0.47, RI of 0.72 and RC of 0.34. Support measured by bootstrapping (>50%) and decay indices are given respectively above and below the corresponding branches.

hyaline setae at the tip of the long neck and strongly tomentose perithecia. Further the lemon-shaped ascospores are not described as having a rim around the germ pores or a single vertical ridge on the ascospores (Doguet 1955, Cannon and Hawksworth 1982), which are distinctive features of *V. coorgii*.

Unlike most species in *Melanospora* the perithecia of *V. coorgii* are produced abundantly on filter paper

placed on Stan 21 agar. Media trials indicated that K_2HPO_4, KNO_3 and $CaCl_2$ greatly enhanced sporulation. Hawker (1947) had incorporated KNO_3 and KH_2PO_4 in the basal medium for sporulation studies on *M. destruens*, but we did not come across any reference to the sporulation of *Melanospora* on cellulose.

The fungus occurred in a mixed culture, and we were unable to get the *Vittatispora* into axenic culture. The BLAST analysis of the 18S rDNA fragment of the second fungus showed a sequence similarity to *Tetracladium marchalianum*, an aquatic hyphomycete. It therefore might be the case that the new fungus requires another fungus for growth, but it was not obviously mycoparasitic, as are many species of *Melanospora* (Cannon and Hawksworth 1982); the nature of the relationship merits further investigation.

Vittatispora coorgii was placed with 100% bootstrap support as a sister taxon of all other species of *Melanospora* and *Sphaerodes* included in the analysis. Our molecular phylogeny indicates that *Sphaerodes* is congeneric with *Melanospora*, which also is in agreement with the results of Zhang and Blackwell (2002). It therefore follows that the raised ridge surrounding the germ pore is not as phylogenetically informative as had been assumed. Our analyses also indicate that the neck structure may not be phylogenetically informative in that a very similar neck structure is seen in *Syspastospora parasitica*, which falls into the main hypocrealean clade. The necks of species that have these in the *Melanospora-Sphaerodes* clade on which data is available, however, do appear to have a cellular rather than a hyphal structure. We therefore establish the new genus *Vittatispora* in view of the sister relationship of *V. coorgii* to the other species examined, the structure of the neck and the unique vertical ridge on the ascospores.

ACKNOWLEDGMENTS

We are indebted to Shrikumar Suryanarayanan for his constant encouragement which made it possible to pursue this study. We gratefully acknowledge Dr Meredith Blackwell, Dr R.M. Harveson, Dr Fernando Vega, and Dr Jeffrey K. Stone for their invaluable advice. Our sincere thanks to the Biodiversity group, R&D Biocon; Bindu for her help with DNA extraction and PCR; and Surekha, Raju and Sheetal for their assistance throughout the course of this work. Thanks also to Dr K.R. Kannan and Mr K. Satyanarayanan of Indians Institute of Science, Bangalore, for assistance with SEM. The first author acknowledges Sanath, Sonali and Gautam for accompanying her on the sample collection trip. This work was undertaken while D.L.H. was supported by the Programa Ramón y Cajal of the Ministry of Science and Technology of Spain held in the Departamento de Biologìa Vegetal II of the Facultad de Farmacia of the Universidad Complutense de Madrid.

LITERATURE CITED

Bremer K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42: 795–803.

———. 1994. Branch support and tree stability. Cladistics 10:295–304.

Cannon PF, Hawksworth DL. 1982. A re-evaluation of *Melanospora* Corda and similar pyrenomycetes, with a revision of the British species. Bot J Linn Soc 84:115–160.

Cassago A, Panepucci RA, Baiao AMT, Henrique-Silva F. 2002. Cellophane based mini-prep method for DNA extraction from the filamentous fungus *Trichoderma reesei*. BMC Microbiol 2:14.

Doguet G. 1955. Le genre *Melanospora*: biologie, morphologie, développement, systématique. Botaniste 39: 1–313.

Eriksson T. 1998. AutoDecay. Version 4.0.2. Stockholm: Department of Botany, Stockholm University.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

Garcia D, Stchigel AM, Guarro J. 2002. A new species of *Syspastospora* from tropical soils. Mycologia 94:862–865.

———, ———, ———. 2004. Two new species of *Sphaerodes* from Spanish soils. Studies in Mycology 50:63–68.

Harveson RM, Kimbrough JW. 2000. First report of *Persiciospora moreaui*, a parasite of *Fusarium oxysporum*, in the western hemisphere. Mycotaxon 76:361–365.

———, ———. 2001. Parasitism and measurement of damage to *Fusarium oxysporum* by species of *Melanospora, Sphaerodes* and *Persiciospora*. Mycologia 93: 249–257.

———, ———. 2001. The identification of *Melanospora* and its allies from field isolations of *Fusarium oxysporum*. Int J Plant Sci 162:403–410.

Hawker LE. 1947. Further experiments on growth and fruiting of *Melanospora destruens* Shear in the presence of various carbohydrates, with special reference to the effects of glucose and sucrose. Ann Botany 11:245–255.

Hawksworth DL, Kirk PM, Sutton BC, Pegler DN. 1995. Ainsworth & Bisby's dictionary of the fungi. 8th ed. Wallingford, UK: CAB International.

Kishino H, Hasegawa M. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order of *Hominoidea*. J Mol Evol 29:170–179.

Posada F, Vega FE, Rehner SA, Blackwell M, Weber D, Suh S-O, Humber RA. 2004. *Syspastospora parasitica*, a mycoparasite of the fungus *Beauveria bassiana* attacking the Colorado potato beetle *Leptinotarsa decemlineata*: a tritrophic association. J Insect Sci 4:24. (Available online: insectscience.org/4.24)

Petch T. 1938. British Hypocreales. Trans Br Mycol Soc 21: 249–301.

Rambaut A. 2001. Se-Al: sequence alignment editor. Version 2.0a9. Oxford: University of Oxford.

Reichenbach H, Hofle G. 1994. Discovery of a new antifungal mechanism of action: Soraphen—an almost success story. GBF Sci Ann Rep 1994:5–22.

Shimodaira H, Hasegawa M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol Biol Evol 16:1114–1116.

Spatafora JW, Blackwell M. 1993. Molecular systematics of unitunicate perithecial ascomycetes: the Clavicipitales-Hypocreales connection. Mycologia 85:912–922.

———, Volkmann-Kohlmeyer B, Kohlmeyer J. 1998. Independent terrestrial origins of the Halosphaeriales (marine Ascomycota). Am J Bot 85:1569–1580.

Stchigel AM, Cano J, Guarro J. 1999. A new species of *Melanospora* from Easter Island. Mycol Res 103:1305–1308.

———, Guarro J, Figueras MJ. 1996. A new species of *Melanospora* from India. Mycol Res 101:446–448.

Swofford DL. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland, MA: Sinauer Associates.

Thompson JD, Gibson TJ, Plewniak F Jeanmougin F, Higgins DG. 1997. The Clustal-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25: 4876–4882.

von Arx JA, Müller E. 1954. Die Gattungen der amerosporen Pyrenomycete. Beiträge der Kryptogamenflora der Schweiz 11(1):1–434.

Zhang N, Blackwell M. 2002. Molecular phylogeny of *Melanospora* and similar pyrenomycetes fungi. Mycol Res 106:148–155.