
Phylogenetic evaluation and taxonomic revision of *Schizothecium* based on ribosomal DNA and protein coding genes

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Cai, L., Jeewon, R. and Hyde, K.D. (2005). Phylogenetic evaluation and taxonomic revision of *Schizothecium* based on ribosomal DNA and protein coding genes. *Fungal Diversity* 19: 1-21.

The taxonomy of *Schizothecium* and *Podospora* has been a subject of debate. Both of these genera have previously been treated as congeneric due to the lack of distinguishing morphological characters. This study focuses mainly on the phylogenetic relationships of *Schizothecium* and *Podospora*, and also a re-evaluation of the taxonomic significance of morphological characters. Multiple gene sequences (partial 28S rDNA, ITS/5.8S rDNA and partial β -tubulin) were analysed using maximum parsimony and Bayesian analyses. In all analyses, *Schizothecium* species characterised by perithecia adorned with swollen agglutinated hairs or prominent protruding peridial cells, grouped in a strongly supported monophyletic clade. *Schizothecium* should therefore, be given appropriate generic status and not treated as congeneric to *Podospora*. Phylogenetic analyses provide good support indicating that ascomatal morphologies are more phylogenetically informative than ascospore characters and host or habitat association. A synopsis of and key to *Schizothecium* species now recognised in the genus are given. *Podospora* was found to be a polyphyletic genus, consisting of a group of morphologically heterogeneous and phylogenetically distant species. Preliminary data indicate that a complete revision of *Podospora* and related genera is necessary.

Key words: *Lasiochaeriales*, molecular phylogeny, *Podospora*, rDNA, *Sordariales*, tubulin.

Introduction

Schizothecium was first established by Corda (1838) for a single species *S. fimicola*. Later *Schizothecium* and its type species were mistakenly synonymised to *Podospora* (Cesati, 1856). Despite a complicated history on the generic nomenclature (Lundqvist, 1972), the full legitimacy of *Schizothecium* as originally discussed by Corda (1838) has never changed (Donk, 1964; Lundqvist, 1972). Lundqvist (1972), therefore resurrected the genus *Schizothecium* and recognised 17 species. These species are characterised by

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perithecia adorned with swollen agglutinated hairs or prominent protruding peridial cells. Other characteristics of *Schizothecium* include the lack of interascal filiform paraphyses, ascospores becoming septate at an early stage of development, and pedicels being plasma-filled and persistent (Lundqvist, 1972). Other mycologists, however, have not accepted the separation of *Schizothecium* from *Podospora* and treated them as congeneric (Furuya and Udagawa, 1972; Krug and Khan, 1989; Bell and Mahoney, 1995; Stchigel *et al.*, 2002; Kirk *et al.*, 2001). Bell and Mahoney (1995) further pointed out that the absence of typical interascal paraphyses is not substantial for recognising a separate genus. Other characters such as early spore septum development and the persistent pedicel were thought to be too difficult to use in the taxonomy of this group of fungi (Bell and Mahoney, 1995).

Podospora is a widespread genus in the family *Lasio-sphaeriaceae* (*Ascomycota, Fungi*), and now includes approximately 78 species (Kirk *et al.*, 2001). *Podospora* species have considerable morphological variations especially in the structure of ascomatal wall and morphology of ascospore appendages (Mirza and Cain, 1969). Other lasiosphaeriaceous genera such as *Arnium*, *Cercophora*, *Strattonia*, *Tripterospora* and *Zopfiella* also possess morphological characters similar to *Podospora*, resulting in considerable confusion in the intergeneric relationships of these fungi.

In order to investigate the phylogenetic relationships of *Schizothecium* and *Podospora* and other allied fungi, a number of fungi which exhibit a broad range of ascomatal and ascospore morphologies were sampled. Sequence analyses were conducted based on partial nuclear 28S ribosomal DNA, nuclear ITS/5.8S ribosomal DNA and partial nuclear β -tubulin sequences using maximum parsimony and Bayesian analyses. The objectives of this study are: 1) to examine the phylogeny of *Schizothecium* and its affinity with *Podospora*; and 2) to provide an overview of the phylogenetic significance of morphologies in the delineation of *Podospora* and *Schizothecium* species.

Materials and methods

Fungal isolates and DNA extraction

Cultures were obtained from culture collections CBS (Netherlands) and IFO (Japan) (Table 1). Isolates were grown on potato dextrose agar (PDA) for 2-4 weeks and total genomic DNA was extracted from fresh mycelium using the protocol as outlined by Jeewon *et al.* (2003) and Lacap *et al.* (2003).

DNA amplification and sequencing

DNA amplification was performed by polymerase chain reaction (PCR). The partial 28S rDNA, complete ITS/5.8S rDNA and partial β -tubulin were amplified using fungal specific primers LROR and LR5 (Vilgalys and Hester, 1990), ITS4 and ITS5 (White *et al.*, 1990) and Bt2A and Bt2B (Glass and Donaldson, 1995) respectively. The amplification reaction was performed in a 50 μ l reaction volume as outlined by Jeewon *et al.* (2004). The PCR thermal cycle for all of the three regions were similar, consisting of 95°C for 3 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 52°C for 50 s and elongation at 72°C for 1 min, with a final extension step of 72°C for 10 min. PCR products were checked on 1% agarose electrophoresis gels stained with ethidium bromide.

PCR products were then purified using minicolumns, purification resin and buffer according to the manufacturer's protocols (Amersham product code: 27-9602-01). DNA sequencing was performed using the primers mentioned above in an Applied Biosystem 3730 DNA Analyzer at the Genome Research Centre, The University of Hong Kong.

Sequence alignment and phylogenetic analysis

For each fungal strain, sequences obtained from pair primers were aligned to obtain an assembled sequence using Bioedit (Hall, 1999). In total 3 datasets were analysed. They are 28S rDNA dataset, ITS/5.8S rDNA dataset and β -tubulin dataset. Novel sequences generated from this study were submitted to GenBank (Table 1). Sequences for each strain, together with reference sequences obtained from GenBank (Table 2), were aligned using Clustal X (Thomson *et al.*, 1997). Alignment was manually adjusted to allow maximum alignment and minimise gaps.

Phylogenetic analyses were performed by using PAUP* 4.0b10 (Swofford, 2002). Ambiguously aligned regions were excluded from all analyses. Unweighted parsimony (UP) and weighted parsimony (WP) analyses were performed with gaps treated as missing data. WP analyses were performed using a symmetric step matrix generated with the program STMatrix version 2.2 (François Lutzoni and Stefan Zoller, Department of biology, Duke University), by which the relative frequencies of nucleotide substitutions were calculated and converted into costs of changes. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed and all parsimonious trees were saved. Descriptive tree statistics such as tree length

Table 1. Sequences used in this study: taxon, isolate code, and GenBank accession number ^a.

Species	Isolate code ^a	Substrates	GenBank Nos.		
			28S rDNA	ITS rDNA	β -tubulin
<i>Cercophora ambigua</i>	CBS 215.60	Decorticated twig	AY999114	AY999137	AY999147
<i>Cercophora caudata</i>	CBS 606.72	Soil	AY999113	AY999135	AY999151
<i>Cercophora samala</i>	CBS 109.93	Dung of cow	---	---	AY999140
<i>Lasio-sphaeris hispida</i>	CBS 955.72	Decaying wood	AY681169	AY681203	AY681237
<i>Neurospora terricola</i>	CBS 298.63	Soil	AY681142	AY681176	AY681210
<i>Podospora appendiculata</i>	IFO 8549	Dung	AY999103	AY999126	AY999144
<i>Podospora austro-americanana</i>	CBS 724.68	Plant (<i>Carica papaya</i>)	AY999101	AY999124	AY999138
<i>Podospora cochleariformis</i>	CBS 247.9	Dung of <i>Redunca</i> sp.	AY999098	AY999123	AY999145
<i>Podospora cupiformis</i>	CBS 246.71	Dung of <i>Cobus defassa</i>	AY999102	AY999125	AY999149
<i>Podospora curvicolla</i>	IFO 8548	Dung of rabbit	AY999099	AY999122	AY999148
<i>Podospora didyma</i>	CBS 232.78	Dung of rabbit	AY999100	AY999127	AY999142
<i>Podospora intestinacea</i>	CBS 113106	Dung of horse	AY999104	AY999121	AY999152
<i>Schizothecium aloides</i>	CBS 879.72	Soil	AY999097	AY999120	AY999159
<i>Schizothecium carpinicola</i>	CBS 228.87	Plant (<i>Carpinus betulus</i>)	AY999095	AY999118	AY999158
<i>Schizothecium curvisporum</i>	CBS 507.50	Plant (<i>Daucus carota</i>)	AY999096	AY999119	AY999155
<i>Schizothecium fimbriatum</i>	CBS 144.54	Dung of horse	AY999092	AY999115	AY999156
<i>Schizothecium glutinans</i>	CBS 134.83	Plant (<i>Arctostaphylos uva-ursi</i>)	AY999093	AY999116	AY999157
<i>Schizothecium inaequalis</i>	CBS 226.87	Plant (<i>Carpinus betulus</i>)	AY999094	AY999117	AY999154
<i>Schizothecium conicum</i>		Dung of red deer		AY515356*	
<i>Schizothecium curvuloides</i>		Dung of goat		AY515357*	
<i>Schizothecium dakotense</i>		Dung of sheep		AY515358*	
<i>Schizothecium glutinans</i>		Plant <i>Arctostaphylos uva-ursi</i>		AY615207*	

Table 1 continued. Sequences used in this study: taxon, isolate code, and GenBank accession number ^a.

Species	Isolate code ^a	Substrates	GenBank Nos.		
			28S rDNA	ITS rDNA	β -tubulin
<i>Schizothecium glutinans</i>		Brushtail opossum dung		AY615208*	
<i>Schizothecium miniglutinans</i>		Dung of rabbit		AY515362*	
<i>Schizothecium vesticola</i>		----	AY780076*	AY515365*	
<i>Sordaria lappae</i>	CBS 154.97		AY681137	AY681171	AY681205
<i>Zopfiella karachiensis</i>	IFO 32902	Garden soil	---	---	AY999153
<i>Zopfiella tabulata</i>	CBS 230.78	Dung of porcupine	AY999105	AY999132	AY999143
<i>Zopfiella erostrata</i>	CBS 255.71	Dung of deer	---	---	AY999150

^a Abbreviations: CBS: Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; IFO: Institute for Fermentation, Osaka, Japan;

* Sequences obtained from GenBank, isolate origin not given.

Table 2. Other reference sequences used in this study obtained from GenBank.

Species	28S rDNA	ITS rDNA
<i>Amphisphaeria umbrina</i>	AF452029	
<i>Apiosordaria nigeriensis</i>		AJ458148
<i>Apiosordaria verruculosa</i>	AY346258	
<i>Bombardia bombardia</i>	AY346263	
<i>Cercophora mirabilis</i>	AY346271	
<i>Chaetosphaeria innumera</i>	AY017375	
<i>Diaporthe pustulata</i>	AF408358	
<i>Immersiella immersa</i>	AY436408	
<i>Jugulospora rotula</i>	AY346287	
<i>Lasiosphaeria ovina</i>	AY436413	AY587931
<i>Lasiosphaeria sorbina</i>		AY587934
<i>Melanochaeta hemipsila</i>	AY346292	
<i>Pestalotiopsis versicolor</i>		AF409993
<i>Podospora anserina</i>		AY525771
<i>Podospora comata</i>		AF443849
<i>Podospora decidua</i>		AF443851
<i>Podospora ellisiana</i>		AY515360
<i>Podospora fimiseda</i>	AY346296	AY515361
<i>Strattonia carbonaria</i>	AY346302	
<i>Strattonia insignis</i>		AY277912
<i>Triangularia mangenotii</i>	AY346303	
<i>Valsella salicis</i>	AF408389	
<i>Xylaria hypoxylon</i>	U47841	
<i>Zygopleurage zygospora</i>	AY346306	

[TL], consistency index [CI], retention index [RI], rescaled consistency index [RC] and homoplasy index [HI] were calculated for trees generated under different optimality criteria. Clade stability was assessed in a bootstrap (BT) analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa. Kishino-Hasegawa tests (KH Test) (Kishino and Hasegawa, 1989) and Templeton test (Templeton, 1983) were performed in order to determine whether trees were significantly different. Trees were figured in Treeview (Page, 1996).

Model of evolution was estimated by using Modeltest 3.06 (Posada and Crandall, 1998). Posterior probabilities (PP) (Rannala and Yang, 1996; Zhaxybayeva and Gogarten, 2002) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes 3.0b4 (Huelsenbeck and Ronquist, 2001), using above estimated model of evolution. Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generations (resulting 10,000 total trees). The first 2,000 trees which represented the burn-in phase of the analyses, were discarded and the remaining

8,000 trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree.

Results

The 28S rDNA dataset comprised 852 sites, of which 4 ambiguous regions of 15 sites were excluded in the analysis. There were 167 parsimony informative characters (PIC) in this dataset. Both unweighted parsimony (UP) and weighted parsimony (WP) resulted in a single tree. KH and Templeton tests show that these trees are not significantly different ($P = 0.6500$, $P = 0.6547$). The single tree generated from the UP (TL = 651, CI = 0.528, RI = 0.649, RC = 0.343, HI = 0.472) is shown in Fig. 1.

ITS dataset with 36 strains have 802 characters, of which 12 ambiguous regions were excluded in the analysis. UP generated 118 trees, while weighted parsimony resulted in only one tree. These 119 trees are not significantly different based on KH and Templeton tests. The single tree generated from the WP (TL=358, CI=0.567, RI=0.729, RC=0.413, HI=0.433) is shown in Fig. 2.

The β -tubulin dataset comprised 606 characters after alignment, of which 4 ambiguous regions were excluded. Four trees and 2 trees, respectively, were generated from UP and WP. KH and Templeton tests reveal no significant difference among these trees. One of the two trees generated from the WP (TL=189, CI=0.545, RI=0.631, RC=0.344, HI=0.455) is shown in Fig. 3.

In all analyses, *Schizothecium* species constitute a monophyletic clade which received high statistical support (100% BT and 100% PP in the 28S rDNA tree, 90% BT and 100% PP in the ITS/5.8S rDNA tree, and 95% BT and 100% PP in the β -tubulin tree, Figs. 1-3). *Podospora* species were found to be polyphyletic and associated with species of *Apiosordaria*, *Bombardia*, *Cercophora*, *Lasiosphaeria*, *Triangularia*, *Zopfiella* and *Zygopleurage* (Figs. 1-3).

Discussion

Podospora was reviewed by Mirza and Cain (1969) and Lundqvist (1972), but the latter's definition of *Podospora* is narrower, as *Schizothecium* was reintroduced to accommodate some *Podospora* species. Lundqvist (1972) discussed the morphological differences between *Podospora* and *Schizothecium* and stated that the perithecia adorned with swollen agglutinated hairs or prominent protruding peridial cells, together with several other characters, are characteristics of *Schizothecium*. However, Furuya and Udagawa (1972) and Krug and Khan (1989) suggested that *Schizothecium* and *Podospora* should not

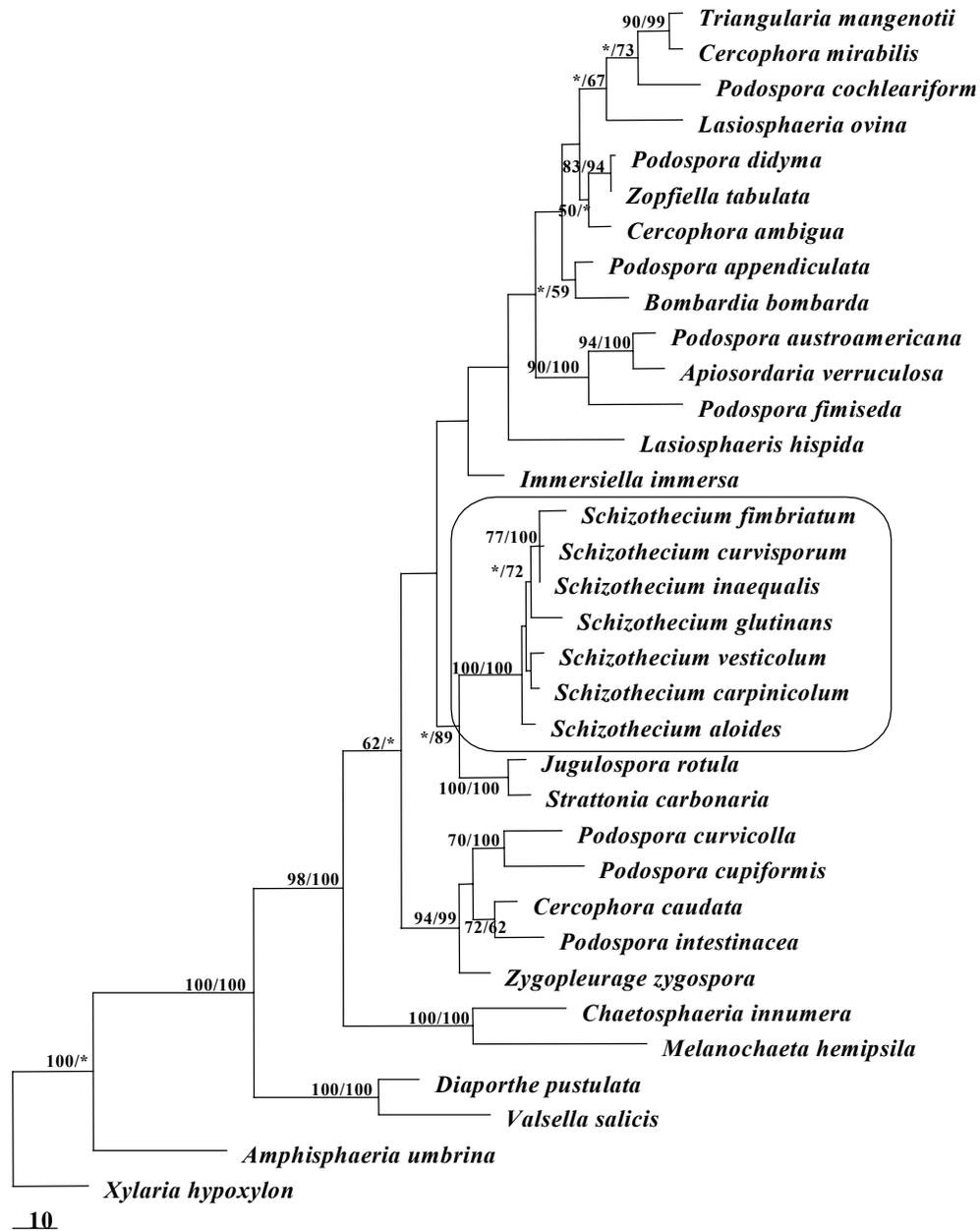


Fig. 1. The single tree generated from parsimony analysis based on 28S rDNA sequences (TL = 651, CI = 0.528, RI = 0.649, RC = 0.343, HI = 0.472). Data were analyzed with random addition sequence, unweighted parsimony and treating gaps as missing data. Values before the backslash are parsimony bootstrap (above 50%) while after are Bayesian posterior probabilities (above 50%). The tree is rooted with *Xylaria hypoxylon*. Asterisks (*) indicate clades which receive less than 50% support.

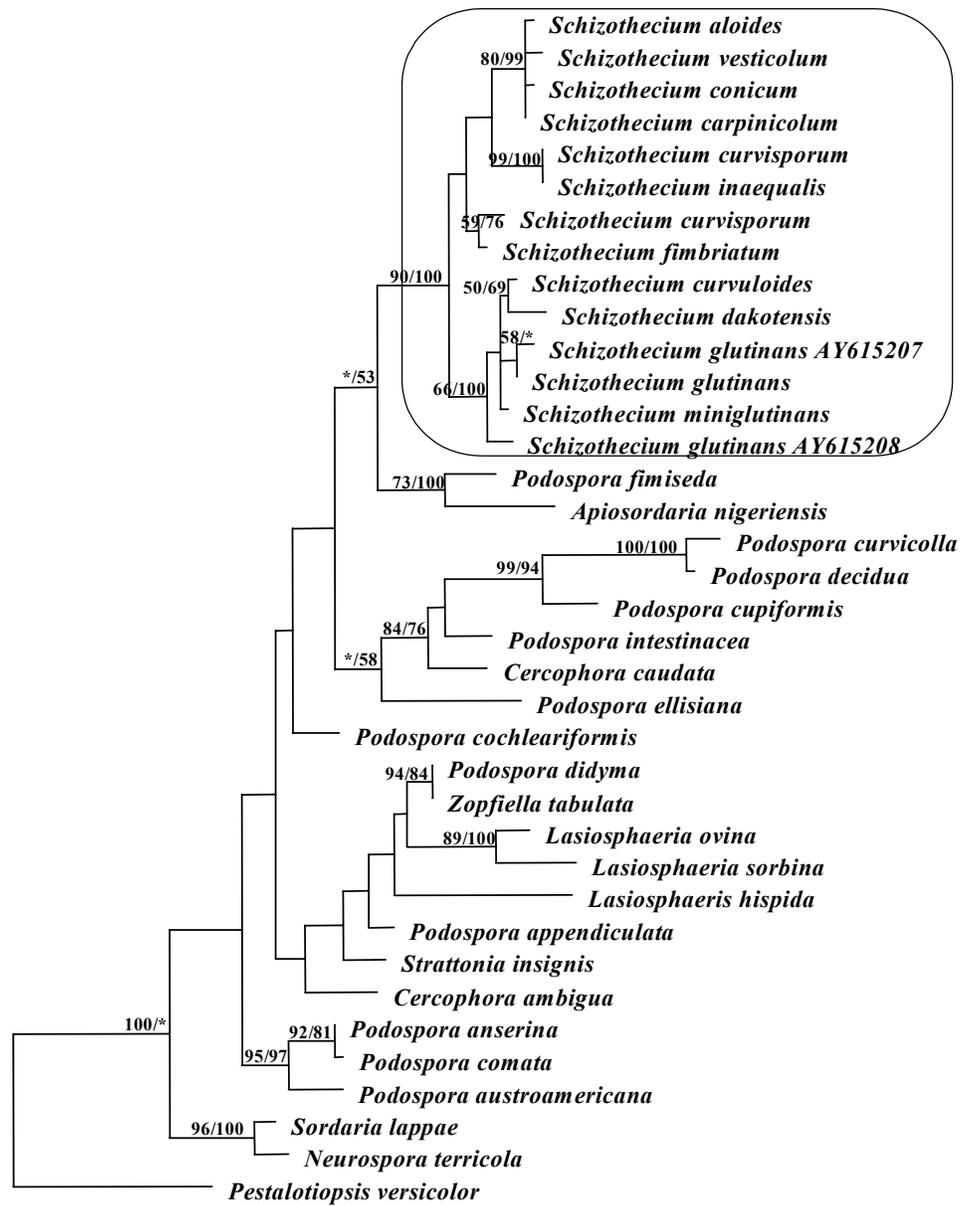


Fig. 2. The single tree generated from parsimony analysis based on ITS/5.8S rDNA sequences (TL=358, CI=0.567, RI=0.729, RC=0.413, HI=0.433). Data were analyzed with random addition sequence, weighted parsimony and treating gaps as missing data. Values before the backslash are parsimony bootstrap (above 50%) while after are Bayesian posterior probabilities (above 50%). The tree is rooted with *Pestalotiopsis versicolor*. Asterisks (*) indicate clades which receive less than 50% support.

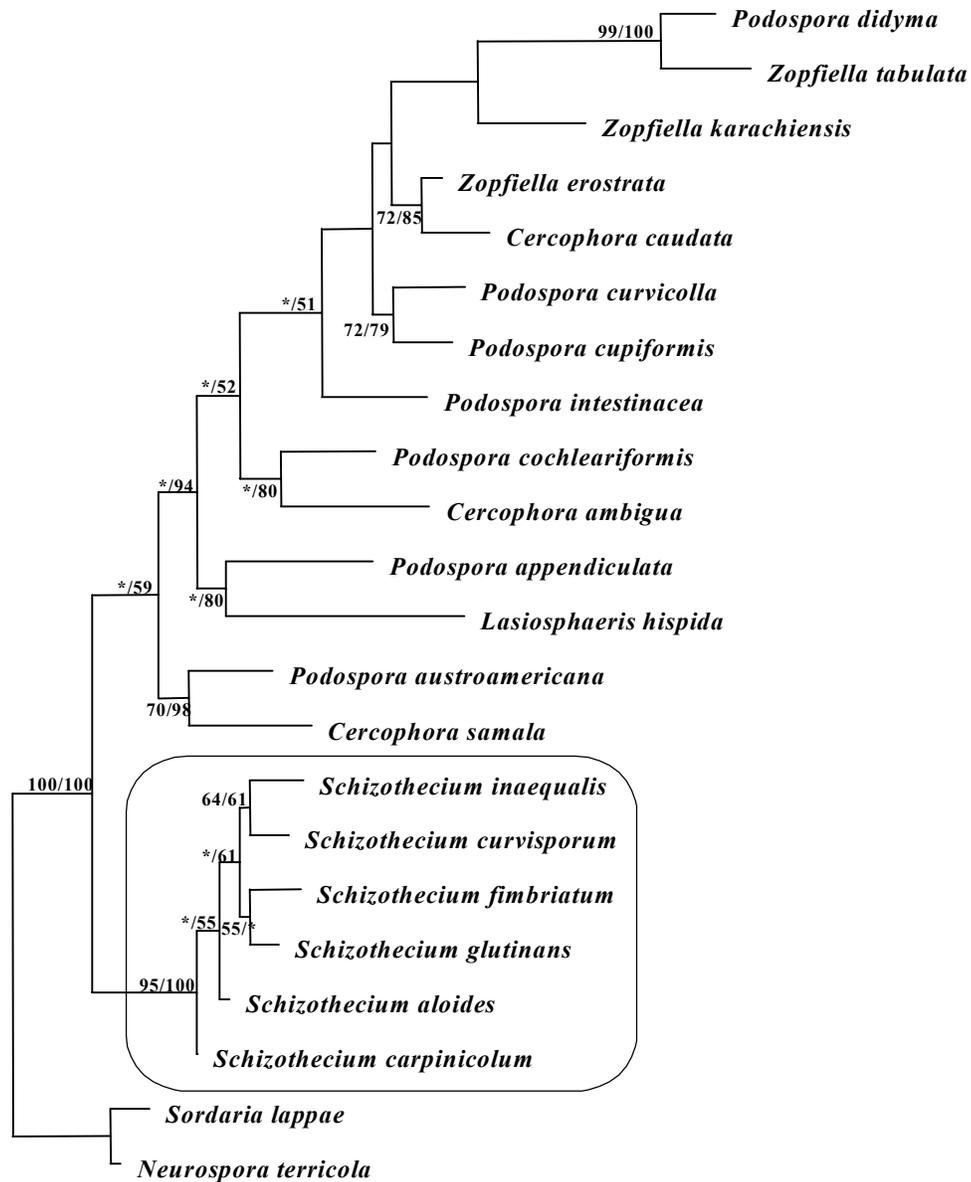


Fig. 3. One of the two trees generated from parsimony analysis based on β -tubulin sequences (TL=189, CI=0.545, RI=0.631, RC=0.344, HI=0.455). Data were analyzed with random addition sequence, weighted parsimony and treating gaps as missing data. Values before the backslash are parsimony bootstrap (above 50%) while after are Bayesian posterior probabilities (above 50%). The tree is rooted with *Sordaria lappae* and *Neurospora terricola*. Asterisks (*) indicate clades which receive less than 50% support.

be treated as different genera as a detailed justification to distinguish between them was not available. Similar views were shared by Bell and Mahoney (1995) and Stchigel *et al.* (2002). In the latest dictionary of fungi (Kirk *et al.*, 2001), *Schizothecium* was treated as a synonym of *Podospora*. To date, there has not been any comprehensive taxonomic review or phylogenetic assessment of these genera, although many named species under both genera are recognised.

Phylogenies inferred from rDNA and protein genes clearly indicate that *Schizothecium* species are monophyletic and belong to a unique and well supported group. Our results also demonstrate the importance of perithecial morphology (swollen agglutinated hairs, or with prominent protruding peridial cells) within this group of fungi. High parsimony bootstrap (BT) and Bayesian posterior probabilities (PP) support for this *Schizothecium* clade warrant its classification into a separate genus within *Lasiosphaeriaceae*. Results reported herein corroborate the taxonomic arrangement as postulated by Lundqvist (1972). *Podospora* species differ from *Schizothecium* species in having glabrous perithecia or perithecia with non-agglutinated hairs if present.

However, within *Podospora* and *Schizothecium*, there is still overlap of morphological characters, especially ascus and ascospore morphology. We agree with Bell and Mahoney (1995) that some other ascus and ascospore features of *Schizothecium* (e.g. lack of interascal filiform paraphyses, ascospores becoming septate at an early stage of development, pedicels being plasma-filled and persistent) mentioned by Lundqvist (1972) is somewhat obscure and relatively impractical in classification (Bell and Mahoney, 1995; Stchigel *et al.*, 2002). The perithecial morphologies of *Schizothecium* species, however are quite obvious and easily recognisable. In a recent phylogenetic study within *Sordariales*, Miller and Huhndorf (2005) have already demonstrated that ascomatal morphology is a better phylogenetic predictor than ascospore morphology. A similar phenomenon is reported here with respect to *Schizothecium*.

Morphologically speaking, *Schizothecium* species are characterised by ascomata adorned with swollen agglutinated hairs or prominent protruding peridial cells, cylindrical to clavate asci usually lacking an apical ring, and ascospore with a large brown cell and a small, hyaline pedicel, with or without gelatinous appendages. At the species level, the most useful characters to separate species within this genus are ascospore morphologies (Lundqvist, 1972). One point worth mentioning here is that ascospore shape appears to be phylogenetically informative at the species level. For example, *S. curvisporum*, *S. fimbriatum* and *S. inaequalis* are characterised by inequilateral ascospores, and in all phylogenetic trees, they are closely related (Figs. 1-3). Gelatinous appendages, another widely used ascospore character, however, seems to be

less informative in phylogeny and less practical in identification. For example, in the ITS tree (Fig. 2), *S. aloides*, *S. carpinicola*, *S. conicum*, *S. vesticola* cluster together with high support (80% BT and 99% PP). However, ascospores of *S. carpinicola* have no gelatinous appendages (Mouchacca, 1986); those of *S. aloides*, *S. conicum* and *S. vesticola* mostly have gelatinous appendages but ascospores without appendages were also observed in a same culture (Mirza and Cain, 1969; Bell and Mahoney, 1995). Even if molecular results do not provide a clear indicator about the utility of these characters, they are still very important in morpho-taxonomic studies.

In *Schizothecium*, the coprophilous species and the non-coprophilous species seem to be equally frequent. Several species have been reported from terricolous habitats such as *Schizothecium aloides* (from soil), *S. curvisporum* and *S. inaequalis* (from *Daucus carota*), *S. carpinicola* (from *Carpinus betulus*), *S. glutinans* (from *Arctostaphylos uva-ursi*) and even *S. vesticola* from an old gown (Mirza and Cain, 1969). Among them, *S. aloides*, *S. glutinans* and *S. vesticola* also occur in a coprophilous habitat (Lundqvist, 1972). The rest of *Schizothecium* species have been reported mainly from dung. *Schizothecium* therefore includes species from a wide range of hosts/habitats. Most *Schizothecium* species which are characterised by the absence of gelatinous appendages in their ascospores (e.g. *S. carpinicola*, *S. curvisporum*, *S. inaequalis*) appear to be restricted non-coprophilous habitats (Mirza and Cain, 1969; Mouchacca, 1986). Within the *Schizothecium* clade, there appears to be no clear cut phylogenetic relationship between coprophilous species and non-coprophilous species. On the other hand, most of the *Podospora* species are coprophilous in habitat (Mirza and Cain, 1969; Lundqvist, 1972). The presence of gelatinous appendages in many *Podospora* species have been generally assumed to be an adaptation to the coprophilous habitat as they may aid spore dispersal and attachment. *Podospora* species were found to be polyphyletic across the family *Lasiosphaeriaceae* and related to species of several genera with a variety of ascospore morphologies such as *Apiosordaria* (spinulose spores), *Zopfiella* (spores usually lacking gelatinous appendages) and *Zygopleurage* (spores with a long intercalary cell) (Figs. 1-3).

Mirza and Cain (1969) detailed 17 species which possess perithecia adorned with agglutinated perithecial hairs or prominent protruding cells. Lundqvist (1972) stated that there are 31 named species in *Schizothecium*, but only 17 species were listed and some of them are synonyms. Based on the present study and accessible literature, 24 species (with 7 new combinations) of *Schizothecium* are listed below as accepted species. *Schizothecium* was originally typified by *S. fimicola* (Corda, 1838). This species, as pointed out by Lundqvist (1972), is fully legitimate and a true *Schizothecium*. However, the

description of this species is incomplete and the type specimen is not available. The typification of *Schizothecium* is therefore, a necessity in future studies. The known anamorphic stages of *Schizothecium* species are all of the phialidic type (e.g. *S. aloides*, *S. conicum*, *S. fimbriatum*, *S. tetrasporum* and *S. vesticola*). The phialides are flask-shaped or elongate clavate, some with distinct collarettes. Spores are generally small, hyaline, and globose or ovoid.

Taxonomy

***Schizothecium* Corda, Icon. Fung. 2: 29. 1838.**

Ascomata perithecioid, immersed, semi-immersed or superficial, globose to subglobose, or pyriform-conical, light to dark brown, ostiolate, scattered or gregarious, upper part of the perithecia adorned with groups of swollen agglutinated hairs, or with prominent protruding peridial cells. Peridium pseudo-parenchymatous, membranaeous, consisting of light brown, angular to swollen-angular cells. Paraphyses usually lacking. Asci unitunicate, 4-spored, 8-spored or multi-spored, clavate to cylindrical, apically rounded, usually lacking an apical ring, pedicellate, dehiscing below the apex. Ascospores at first hyaline, becoming transversely uniseptate, with or without gelatinous appendages. Upper cells finally dark brown, fusiform-obovoid, smooth, with an upper germ pore. Pedicel hyaline, cylindrical, obclavate or obconical, usually long persistent. Anamorph phialidic.

Originally typified by *Schizothecium fimicola* Corda, Icon. Fung. 2: 29. 1838.

Accepted species

1. *Schizothecium aloides* (Fuckel) N. Lundq., Symb. Bot. Upsal. 20: 253. 1972.

- ≡ *Podospora aloides* Mirza & Cain, Can. J. Bot. 47: 2004. 1969.
- ≡ *Sordaria aloides* Fuckel, Jahrb. Nass. Ver. Nat. 27-28: 43. 1873.
- = *Ixodopsis fimicola* Karsten, Acta Soc. Fauna F1. Fenn. 2: 78. 1881.
- = *Podospora coronifera* (Grove) Cain, Can. J. Bot. 40: 459. 1962.
- = *Sordaria coronifera* Grove, Journ. Bot. 54: 85. 1916.

2. *Schizothecium alloechaetum* (J.H. Mirza & Cain) L. Cai, **comb. nov.**

- ≡ *Podospora alloechaeta* J.H. Mirza & Cain, Can. J. Bot. 47: 2003. 1969.

Notes: *Schizothecium alloechaetum* is morphologically similar to *S. glutinans*, *S. dakotense* and *S. dubium* (Mirza and Cain, 1969). This species produces two different types of perithecial hairs: short, swollen agglutinated hairs, and long, flexuous, non-agglutinated hairs (Mirza and Cain, 1969). The lack of molecular data on this species makes it relatively difficult, to

conclusively solve its taxonomic affinities. However, since multi-gene data have unambiguously place *S. glutinans* and *S. miniglutinans*, which also have two types of perithecial hairs, into *Schizothecium* (Figs. 1-3), *S. alloechaetum* should also be included in this genus.

3. *Schizothecium carpinicola* (Mouch.) L. Cai, **comb. nov.**

≡ *Podospora carpinicola* Mouch., Persoonia 13: 107. 1986.

Notes: This species is characterised by pyriform ascomata adorned with agglutinated hairs, cylindrical asci, and ascospores lacking a gelatinous appendages (Mouchacca, 1986). Multigene sequence data indicate that this is a species which should definitely be transferred to *Schizothecium* (Figs. 1-3). Morphologically, *S. carpinicola* is similar to *S. vesticola* in having cylindrical asci and a phialidic asexual stage. *Schizothecium carpinicola* is also similar to *S. curvisporum* and *S. inaequalis* in lacking a gelatinous appendages.

4. *Schizothecium cervinum* (Cain) N. Lundq., Symb. Bot. Upsal. 20: 253. 1972.

≡ *Podospora cervina* (Cain) Cain, Can. J. Bot. 40: 459. 1962.

≡ *Sordaria cervina* Cain, Univ. Toronto Studies, Biol. Ser. 38: 36. 1934.

5. *Schizothecium conicum* (Fuckel) N. Lundq., Symb. Bot. Upsal. 20: 253. 1972.

≡ *Podospora conica* (Fuckel) Bell & Mahoney, Mycol. 87: 379. 1995.

≡ *Cercophora conica* Fuckel, Jahrb. Nass. Ver. Naturk. 23-24: 245. 1870.

= *Podospora curvula* (de Bary ex Winter) Niessl, Hedwigia 22: 156. 1883.

= *Sordaria curvula* De Bary var. *coronata* Winter, Abh. Nat. Nat. Ges. Halle, 13: 102. 1873.

6. *Schizothecium curvisporum* (Cain) N. Lundq., Symb. Bot. Upsal. 20: 334. 1972.

≡ *Sordaria curvispora* Cain, Can. J. Res. C 26: 492. 1948.

≡ *Podospora curvispora* (Cain) Cain, Can. J. Bot. 40: 459. 1962.

7. *Schizothecium curvuloides* (Cain) L. Cai, **comb. nov.**

≡ *Podospora curvuloides* Cain, Can. J. Bot. 40: 453. 1962.

Notes: This species produces small clusters of short, agglutinated hairs around the perithecium neck. Ascospores are large, and provided with apical and basal gelatinous appendages. Morphologically, this species is similar to *S. dakotense* in the short, swollen agglutinated hairs. In the present study, *S. curvuloides* shows a close phylogenetic relationship with *S. dakotense* and *S. glutinans* (Fig. 2).

8. *Schizothecium dakotense* (Griff.) N. Lundq., Symb. Bot. Upsal. 20: 254. 1972.

≡ *Podospora dakotensis* (Griff) J.H. Mirza & Cain, Can. J. Bot. 47: 2016. 1969.

9. *Schizothecium dubium* (E.C. Hansen) N. Lundq., Symb. Bot. Upsal. 20: 254. 1972.

≡ *Podospora dubia* (E.C. Hansen) Niessl., Hedwigia. 22: 156. 1883.

≡ *Sordaria dubia* E.C. Hansen, Vidensk Meckbl. 59: 337. 1876.

10. *Schizothecium fimbriatum* (Bayer) Barrasa & Soláns, Revista Ibérica de Micología 6: 3. 1989.

≡ *Podospora fimbriata* (Bayer) Cain, Can. J. Bot. 40: 459. 1962.

≡ *Pleurage fimbriata* (Bayer) Page, Trans. Brit. Mycol. Soc. 40: 536. 1957.

≡ *Sordaria fimbriata* Bayer, Acta Soc. Sci. Nat. Moraviae 1: 111. 1924.

= *Bombardia lunata* Zickler, Planta, 22: 573. 1934.

11. *Schizothecium fimicola* Corda, Icon. Fung. 2: 29. 1838.

12. *Schizothecium formosanum* (Yei Z. Wang) L. Cai, **comb. nov.**

≡ *Podospora formosana* Yei Z. Wang, Mycotaxon 76: 383. 2000.

Notes: Although molecular data of this species is not available, the morphological character of this species (distinct, short, agglutinated hairs) is strongly suggestive of *Schizothecium* (Wang, 2000). This species produce two types of perithecial hairs, as those found in *S. glutinans* and *S. miniglutinans*. The ascospore morphology of this species is similar to that of *S. miniglutinans*. Two species are different on the number of ascospores in each ascus.

13. *Schizothecium glutinans* (Cain) N. Lundq., Symb. Bot. Upsal. 20: 254. 1972.

≡ *Podospora glutinans* (Cain) Cain, Can. J. Bot. 40: 460. 1962.

≡ *Sordaria glutinans* Cain, Univ. Toronto Studies, Bio. Ser. 38: 40. 1934.

14. *Schizothecium inaequalis* (Cain) N. Lundq., Symb. Bot. Upsal. 20: 334. 1972.

≡ *Podospora inaequalis* (Cain) Cain, Can. J. Bot. 40: 460. 1962.

≡ *Sordaria inaequalis* Cain, Can. J. Res. C 26: 489. 1948.

15. *Schizothecium linguiforme* (Cain) L. Cai, **comb. nov.**

≡ *Podospora linguiformis* (Cain) Cain, Can. J. bot. 40: 460. 1962.

≡ *Sordaria linguiformis* Cain, Univ. Toronto Studies, Biol. Ser. 38: 42. 1934.

Notes: This species is characterised by pyriform ascomata covered with short agglutinated hairs and long, flexuous, septate hairs intermixed with them. It resembles *S. glutinans*, *S. miniglutinans* and *S. alloechaetum* in having two

types of perithecial hairs (Cain, 1962; Mirza and Cain, 1969). Asci of this species are 16-spored and clavate-cylindrical, similar to *S. simile*. The ascospores of *S. linguiforme* are special in producing broad, hollow, linguiform apical gelatinous appendages. This species should be included in the *Schizothecium* based on its perithecial morphology.

16. *Schizothecium miniglutinans* (J.H. Mirza & Cain) N. Lundq., Symb. Bot. Upsal. 20: 254. 1972.

≡ *Podospora miniglutinans* J.H. Mirza & Cain, Can. J. Bot. 47: 2030. 1969.

17. *Schizothecium nannopodale* (Cain) L. Cai, **comb. nov.**

≡ *Podospora nannopodalis* Cain, Can. J. Bot. 40: 455. 1962.

Notes: This species should be transferred to *Schizothecium*. The perithecia are covered with obvious, clusters of agglutinated, brownish-black hairs. Cell of the hairs are swollen, measuring 8-25 × 6-12 μm (Cain, 1962). Ascospore lack a gelatinous sheath is also a character more typical to *Schizothecium* and similar to those found in *S. carpinicola*.

18. *Schizothecium nanum* N. Lundq., Symb. Bot. Upsal. 20: 255. 1972.

19. *Schizothecium oedotrichum* N. Lundq., Symb. Bot. Upsal. 20: 255. 1972.

20. *Schizothecium papillisorum* (Sharma) L. Cai, **comb. nov.**

≡ *Podospora papillispora* Sharma, Trans. Mycol. Soc. Jap. 17: 12. 1976.

Notes: *Schizothecium papillisorum* is morphologically similar to *S. aloides* in ascospore morphology (Sharma, 1976). The perithecial hairs of this species is similar to those of *S. glutinans* and *S. miniglutinans* in having two types of hairs. The presence of agglutinated hairs around the perithecium neck is indicative of its placement in *Schizothecium*.

21. *Schizothecium simile* (E.C. Hansen) N. Lundq., Symb. Bot. Upsal. 20: 256. 1972.

≡ *Podospora similis* (E.C. Hansen) Niessl, Hedwigia, 22: 156. 1883.

≡ *Sordaria similis* E.C. Hansen, Vidensk. Meddel. 1876: 59. 1877.

22. *Schizothecium squamulosum* (P. Crouan & H. Crouan) N. Lundq., Symb. Bot. Upsal. 20: 256. 1972.

≡ *Sordaria squamulosa* P. Crouan & H. Crouan, Florule Finistère. 22. 1867.

23. *Schizothecium tetrasporum* (G. Winter) N. Lundq., Symb. Bot. Upsal. 20: 256. 1972.

≡ *Podospora tetraspora* (G. Winter) Cain, Can. J. Bot. 40: 460. 1962.

≡ *Sordaria tetraspora* G. Winter, Hedwigia. 10: 161. 1871.

24. *Schizothecium vesticola* (Berk. & Broome) N. Lundq., Symb. Bot. Upsal. 20: 256. 1972.

≡ *Podospora vesticola* (Berk. & Broome) J.H. Mirza & Cain, Can. J. Bot. 47: 2044. 1969.

≡ *Sordaria vesticola* (Berk. & Broome) v. Hoehnel., Ann. Mycol. 16: 45. 1918.

≡ *Sphaeria vesticola* Berk. & Broome, Ann. and Mag. Nat. Hist. 3: 370. 1859.

= *Bombardia minuta* (Fuckel) Kirsch., Krypt. Fl. Brand. 7: 182. 1911.

= *Podospora minuta* (Fuckel) Niessl, Hedwigia, 22: 156. 1883.

= *Sordaria minuta* Fuckel, Jahrb. Nass. Ver. Nat. 27-28: 441. 1873.

Dubious or uncertain species

Schizothecium pilosum (Mouton) N. Lundq., Symb. Bot. Upsal. 20: 255. 1972.

This species (≡ *Podospora pilosa*, *Sordaria pilosa*) was listed as a species of *Schizothecium* by Lundqvist (1972) without description and justification. Mirza and Cain (1969) examined two specimens (Lundqvist 3379a and 3405a) but found only single, long hairs instead of agglutinated hairs. Cain (1934) also observed the non-agglutinated hairs only. This species is morphologically similar to *Podospora ellisiana* in ascomatal and ascospore morphologies. It is therefore, doubtful and not accepted here.

Schizothecium longicolle L. Ames, Sydowia, 5: 120. 1951.

The type specimen of this species is not available. Mirza and Cain (1969) have examined specimens deposited in TRTC (40528) and LPS (6842), and found the perithecia are covered with a few tufts of long, black, non-agglutinated hairs. This species is doubtful and not accepted here.

Schizothecium hispidulum (Speg.) N. Lundq., Symb. Bot. Upsal. 20: 254. 1972.

Lundqvist (1972) transferred *Sordaria hispidula* to *Schizothecium*. However, *Sordaria hispidula* is morphologically very similar to *Podospora conica* (= *Podospora curvula*), and based on a lots of specimens examined, they have been treated as synonyms (Mirza and Cain, 1969). We agree with Mirza and Cain (1969) that this species is same to *Schizothecium conicum* (≡ *Podospora conica*).

Key to *Schizothecium* species*

- | | | |
|----|---------------------------------------|-----------------------|
| 1. | Ascospores inequilateral..... | 2 |
| 1. | Ascospores not inequilateral | 4 |
| 2. | Ascospores concave on one side..... | <i>S. curvisporum</i> |
| 2. | Ascospores flattened on one side..... | 3 |

3.	Apical cells of the agglutinated hairs fimbriate, asci 8-spored, ascospores head 17-20 × 7.5-9.5 μm	<i>S. fimbriatum</i>	5
3.	Apical cells of the agglutinated hairs usually not fimbriate, asci 4-spored, ascospores head 17-24 × 10-13 μm	<i>S. inaequalis</i>	5
4.	Perithecia with agglutinated hairs or swollen protruding cells only		5
4.	Perithecia with agglutinated hairs or swollen protruding cells, as well as long, septate, flexuous hairs		15
5.	Asci 4-spored		6
5.	Asci more than 4-spored		7
6.	Ascospore head 19-22.5 × 11-13 μm	<i>S. tetrasporum</i>	6
6.	Ascospore head 12-14.5 × 7-9 μm	<i>S. nanum</i>	6
7.	Asci 8-spored		8
7.	Asci 16-spored, spore head 27-34 × 15-19 μm	<i>S. dubium</i>	8
7.	Asci 32-spored, spore head 18-24 × 13-15 μm	<i>S. dakotense</i>	8
8.	Pedicel small, more or less obconical		9
8.	Pedicel larger, cylindrical or clavate		10
9.	Ascospores uniseriate, spore head 11-14 × 7-8 μm, sometimes with gelatinous appendages	<i>S. cervinum</i>	9
9.	Ascospores biseriate, spore head 18-22 × 11-13 μm, without gelatinous appendages	<i>S. nannopodale</i>	9
10.	Ascospores uniseriate from the very beginning		11
10.	Ascospores biseriate from the very beginning		14
11.	Ascospore head longer than 22 μm		12
11.	Ascospore head shorter than 22 μm		13
12.	Ascospore head 22-27.5 × 14-19.5 μm, without gelatinous appendages	<i>S. carpinicola</i>	12
12.	Ascospore head 31-41 × 17-20 μm, upper gelatinous appendage grooved	<i>S. curvuloides</i>	12
13.	Perithecial neck with a conspicuous collar of swollen, agglutinated hair structure, up to 50 × 12 μm, cell of hair thick-walled, even claw-like, spore head 17-22 × 9-12 μm	<i>S. oedotrichum</i>	13
13.	Perithecial neck with short agglutinated hairs, cells of hair thin-walled, spore head 17-20 × 11-14 μm	<i>S. vesticola</i>	13
14.	Ascospore head 31-40 × 15-23 μm, agglutinated hairs very long forming a crown at the top	<i>S. aloides</i>	14
14.	Ascospore head 23-28 × 13-16 μm, agglutinated hairs much smaller and do not form a crown	<i>S. conicum</i>	14
15.	Asci 8-spored		16
15.	Asci more than 8-spored		18

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16. Ascospore head $46.5-60 \times 24-46.5 \mu\text{m}$ *S. papillisporum*
16. Ascospore head less than $40 \mu\text{m}$ long 17
17. Ascospore head $29-33 \times 19-22 \mu\text{m}$, spores uniseriate *S. glutinans*
17. Ascospore head $20-24 \times 12-17 \mu\text{m}$, spores uniseriate *S. miniglutinans*
17. Ascospore head $24-27 \times 15-19 \mu\text{m}$, spores biseriate *S. squamulosum*
18. Asci 16-spored 19
18. Asci more than 16-spored 20
19. Upper gelatinous appendage lash-like *S. simile*
19. Upper gelatinous appendage broad, hollow, linguiform *S. linguiforme*
20. Asci 32-spored, spore head $15.5-18.5 \times 11-13.5 \mu\text{m}$ *S. alloeochaetum*
20. Asci 64-spored, spore head $21-24.5 \times 13-15 \mu\text{m}$ *S. formosanum*

* *S. fimicola* is not included in this key because of its incomplete species description.

Acknowledgements

This study was funded by the Hong Kong Research Grants Council (HKU 7320/02M), National Natural Science Foundation of China (NSFC 3026002) and International Cooperation Research Foundation, Yunnan Province (2000C002). The University of Hong Kong is acknowledged for providing L.C. with a postgraduate scholarship and R.J. with a Hon. Assistant Professor. We are grateful to CBS and IFO for providing cultures. Helen Leung, Keith Cheung and Heidi Kong are thanked for laboratory assistance.

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(Received 15 December 2004; accepted 4 February 2005)