

# MYCOTAXON

Volume LXXIV(1), pp. 217-239

January-March 2000

## COMPUTER AIDED SYSTEMATIC EVALUATION OF MORPHOLOGICAL CHARACTERS OF THE OPHIOSTOMATOID FUNGI

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### ABSTRACT

*Ceratocystis sensu lato* includes over 100 species that are accommodated in three genera, *Ceratocystis sensu stricto*, *Ceratocystiopsis* and *Ophiostoma*. In the taxonomic history of these fungi, many different characters have been emphasised, including ascospore morphology, presence or absence of ascospore sheaths, anamorphs, conidium ontogeny, and others. These characters have often been applied inconsistently. For example, species with falcate ascospores were assigned to *Ceratocystiopsis*, while other groups of species having different ascospore forms were not given similar status. The advent of computer aided systematics has made it possible to analyse morphological characters objectively and to determine their influence on classification systems. Unfortunately, computer characters do not lend themselves to describe variation in morphological characters. Information is thus lost in the process of translating morphological characters into computer character states. This study describes a process of using binary code to describe morphological characters in terms of multi-state characters. This system allows for the inclusion of natural variation in computer aided parsimony. Furthermore, characters can be weighted equally, selectively or deleted to determine their effect on the taxonomic position of species being considered. Using this approach to code characters, species of *Ceratocystis sensu lato* were analysed using the parsimony analysis package PAUP. Morphologically, *Ceratocystis sensu stricto* forms a distinct monophyletic group to *Ophiostoma* and *Ceratocystiopsis*. Species of *Ceratocystiopsis* cluster with species of *Ophiostoma*. Results indicate that anamorph characters are important in distinguishing different groups in *Ceratocystis sensu lato*.

**KEYWORDS:** *Ophiostoma*, *Ceratocystis*, *Ceratocystiopsis*, morphology, character coding, phylogeny, computer systematics.

### INTRODUCTION

*Ceratocystis sensu lato* (s.l.) (Upadhyay, 1981) includes over 100 species that are presently accommodated in three genera, *Ceratocystis sensu stricto* (s.s.) Ellis & Halst., *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr. and *Ophiostoma* Syd. & P. Syd. (de Hoog and Scheffer, 1984; Wingfield *et al.*, 1993). Species in *Ceratocystis* have *Chalara* Corda anamorphs. *Ceratocystiopsis* spp. have several anamorph states

including *Sporothrix* Hektoen & C.F. Perkins, *Hyalorhinocladiella* H.P. Upadhyay & W.B. Kendr. and *Chalara* (Upadhyay & Kendrick, 1975). Anamorphs of species of *Ophiostoma* are found in the genera, *Sporothrix*, *Hyalorhinocladiella*, *Leptographium* Lagerb. & Melin and *Graphium* Corda (Mouton *et al.*, 1994).

The Ophiostomatoid fungi produce morphologically similar ascomata but have many different ascospore forms (Olchowecki and Reid, 1974). Griffin (1968) thus subdivided *Ceratocystis s.l.*, based on ascospore form and the presence or absence of sheaths around these spores. Upadhyay and Kendrick (1975) argued that species of *Ceratocystis s.l.* with sheathed, elongate to falcate ascospores were distinct and established the genus *Ceratocystiopsis* for these taxa. Ultrastructural studies have shown that sheaths represent secondary layers (Van Wyk *et al.*, 1993) which are the remains of the perithecial cavity during ascospore development (Van Wyk *et al.*, 1991). Therefore, the taxonomic distinction of genera in *Ceratocystis s.l.* based on sheathed and unsheathed ascospore morphology in *Ceratocystis s.l.* is of dubious value.

Upadhyay (1981) distributed species remaining in *Ceratocystis* into four sections based on ascospore morphology. Section **Ceratocystis** (Type *C. fimbriata* Ellis & Halst.) included species with hat, half-moon or cucullate-shaped sheaths; section **Ophiostoma** (Type *O. piliferum* (Fr.) Syd. & P. Syd.) to accommodate species without sheaths; section **Ips** (Type *C. ips* (Rumbold) Nannf.) was established to accommodate species with pillow-shaped sheaths; section **Endoconidiophora** (Type *C. coerulescens*, (Münch) B.K. Bakshi) accommodating species with elongate or inequilateral sheaths. The sections **Endoconidiophora** and **Ceratocystis** comprised the **Fimbriata** group, previously established by Olchowecki and Reid (1974). After having previously established *Ceratocystiopsis*, based on ascospore form, it is surprising that Upadhyay (1981) established these sections rather than assigning them generic status.

Despite the presence of different anamorphs in *Ceratocystis s.l.*, their application in the taxonomy of these fungi has been controversial. Upadhyay and Kendrick (1975) and Upadhyay (1981) recognised several anamorph genera in *Ceratocystis s.l.* These included, *Chalara* (Nag Raj and Kendrick, 1993); *Leptographium*-like (*Verticicladiella* Hughes, *Phialocephala* W.B. Kendr.) (Wingfield, 1985; Wingfield, 1993); *Graphium*-like (*Phialographium* H.P. Upadhyay & W.B. Kendr., *Graphilbum* H.P. Upadhyay & W.B. Kendr., *Hyalopesotum* H.P. Upadhyay & W.B. Kendr., *Pachnodium* H.P. Upadhyay & W.B. Kendr., *Pesotum* Crane & Schokn., *Graphiocladiella* H.P. Upadhyay) (Seifert and Okada, 1993); *Sporothrix*-like (*Hyalodendron* Diddens); and *Hyalorhinocladiella* H.P. Upadhyay & W.B. Kendr.) (de Hoog, 1993). *Graphium* is considered the synnematosus analogue of *Leptographium* (Upadhyay, 1981; Wingfield, 1993), while *Sporothrix* and *Hyalorhinocladiella* are similar to each other differing only in the presence or absence of conidiogenous denticles (de Hoog, 1993). Conidia develop in the *Chalara* anamorphs of *Ceratocystis* and *Ceratocystiopsis* enteroblastically by ring-wall building, while conidia in the anamorph genera of *Ophiostoma* are produced holoblastically by apical-wall building (with the exception of *Cp. falcata*) (Minter *et al.*, 1982; 1983). Based on conidium ontogeny, *Cp. falcata* should reside in *Ceratocystis*. This species is taxonomically placed in *Ceratocystiopsis* due to its

falcate sheathed ascospores. Recent studies using molecular data have shown that *Cp. falcata* is not phylogenetically related to species of *Ceratocystiopsis* (Hausner *et al.*, 1993).

Weijman and de Hoog (1975) argued for the separation of *Ceratocystis* and *Ophiostoma* due to the respective absence or presence of rhamnose and cellulose in their cell walls. Species of *Ceratocystis* lack rhamnose and cellulose in their cell walls. Furthermore, species of *Ceratocystis s.s.* are sensitive to low concentrations of the antibiotic cycloheximide. This is in contrast to the tolerance exhibited by species of *Ophiostoma* (Harrington, 1981; de Hoog and Scheffer, 1984).

Using molecular techniques such as ribosomal DNA sequencing, it has been possible to determine that *Ceratocystis* and *Ophiostoma* are phylogenetically distinct (Spatafora and Blackwell, 1993). Based on partial sequences of the small subunit ribosomal RNA gene it appears that *Ceratocystis* is phylogenetically best accommodated in the Microascales and *Ophiostoma* in Diaporthiales (Berbee and Taylor, 1992a, 1992b; Spatafora and Blackwell, 1993). Hausner *et al.* (1993) found that species of *Ophiostoma* and *Ceratocystiopsis* (with the exception of *Cp. proteae* M.J. Wingf. & P.S. van Wyk = *Gondwanamyces proteae* Marais & M.J. Wingf. and *Cp. falcata* (E.F. Wright & Cain) H.P. Upadhyay appear to form a monophyletic group, although a great degree of genetic diversity was found within this genus.

Three genera are currently accepted in the broader group of Ophiostomatoid fungi. These include: (1) *Ceratocystis*, with type species *C. fimbriata*; (2) *Ceratocystiopsis*, with type species *Cp. minuta* (Siem.) H.P. Upadhyay & W.B. Kendr.; and (3) *Ophiostoma*, and type species *O. piliferum*. There is a logical separation of species of *Ceratocystis* from species of *Ceratocystiopsis* and *Ophiostoma* based on conidium ontogeny. However, the distinction between *Ceratocystiopsis* and *Ophiostoma* is less clear, with species in these genera sharing various characteristics.

One of the problems in morphological systematics relates to how homology is recognised (Lutzoni and Vilgalys, 1995). The problem arises due to the difficulty of character interpretation (Tehler, 1990). The only way to test observed character homology is to expose all possible homology statements simultaneously to parsimony analysis (Tehler, 1990). With the advent of computer systematics, it has become possible to analyse large numbers of characters simultaneously. This has overcome the first step in treating morphological characters objectively.

One of the greatest challenges in morphological systematics relates to the interpretation of characters. A morphological character must be described in terms of quantitative grades (for example, ascospore size ranges) or qualitative characters (for example, light brown, brown to black or black pigmented ascospores) (Tehler, 1990). A further problem is encountered in unintentionally weighting characters selectively in data sets. Using different numbers of character states for different morphological characters introduces biased weighting. Examples of this can be found in every recent study analysing morphological characters (Lutzoni and Vilgalys, 1995; McLaughlin *et al.*, 1995; Petrini, 1993; Tehler, 1990). For example, Tehler (1990) assigned seven characters and 17 character states to ascospore morphology while five characters and 10 character states were used for pycnidia and conidia. Due to the difference in the

number of character states used to describe these characters, ascospore morphology will have greater weight in computer analysis. This may contribute to the incongruency encountered between morphological and molecular data sets.

"The objective for any systematist must be to present a phylogeny and classification with a minimum of "ad hoc" hypotheses. This is not accomplished by the partitioning of data." (Tehler, 1995). This sentiment reflects the ultimate aim of systematics, to simultaneously assess all data and derive holistic phylogenies. The term 'holistic phylogenies' is used to imply phylogenies that take all available characters (morphological and molecular) into consideration. However, this is not currently possible. Morphological data cannot currently be coded in such a way as to objectively reflect true morphology. What in reality is being compared is the taxonomist's perception of various characters. Using consensus trees for both morphological and molecular data is, we believe, the only way to realise a holistic approach to systematics (Lutzoni and Vilgalys, 1995; McLaughlin *et al.*, 1995; Nishida *et al.*, 1995; Tehler, 1995). This will only be truly possible when morphology and molecular characters can be treated at the same level without *a priori* weighting.

In this study, we make use of computer aided systematics to evaluate the various morphological characters used in the taxonomy of the Ophiostomatoid fungi. The aim of this study was to test the hypothetical taxonomy (of these fungi) proposed by Upadhyay (1981) and Seifert *et al.* (1993). Morphological characters were coded objectively and analysed using parsimony methods.

## MATERIALS AND METHODS

### SOURCE OF MORPHOLOGICAL DATA

The data used in this study were compiled from the literature and are based on published descriptions, measurements, photographs and sketches of taxa (Table 1). The greatest proportion of data were extracted from the monograph of Upadhyay (1981). In some cases descriptions were taken from original papers such as those for, *O. novo-ulmi* (Brasier, 1991); *C. virescens* (Davidson, 1944); *C. laricicola* (Redfern *et al.*, 1987); *O. cucullatum* and *O. flexuosum* (Solheim, 1986). Species with incomplete descriptions were excluded. These included *C. autographa* B.K. Bakshi, *C. denticulata* R.W. Davidson, *C. hyalothecium* R.W. Davidson, *C. pseudominor* Olchow. and J. Reid, *Cp. conicollis* (Olchow. and J. Reid) H.P. Upadhyay, *Cp. ochracea* (H.D. Griffin) H.P. Upadhyay, *Cp. retussi* (R.W. Davidson and T.E. Hinds) H.P. Upadhyay, *O. adjuncti* (R.W. Davidson) T.C. Harr., *O. coronata* Olchow. and J. Reid, *O. perparvispora* Hunt, *O. distortum* (R.W. Davidson) de Hoog and R.J. Scheff., *O. europhiodes* (E.F. Wright and Cain) H. Solheim, *O. grande* Samuels and Müller, *O. grandiocarpum* (Kowalski and Butin) Rulamort, *O. novae-zelandiae* (Hutchison and J. Reid) Rulamort, *O. penicillatum* (Grosn.) Siem., *O. polonicum* Siem., *O. polyporicola* Constant. and Ryman, *O. proliferum* (Kowalski and Butin) Rulamort, *O. serpens* (Goid.) Arx, *O. subanulatum* Livingston and R.W. Davidson, *O. valdivanum* (Butin) Rulamort, and *O. wagneri* (Goheen and Cobb) T.C. Harr. *Microascus longirostris* Zukal was included as outgroup to determine the homoplasy of morphological characters (Morton and Smith, 1963).

Table 1. List of taxa<sup>a</sup> used in analyses

1	<i>C. adiposa</i> (Butler) C. Moreau
2	<i>C. angusticollis</i> E.F. Wright & H.D. Griffin
3	<i>C. deltoideospora</i> Olchow. & J. Reid
4	<i>C. californica</i> DeVay, R.W. Davidson & Moller
5	<i>C. coerulescens</i> (Münch) B.K. Bakshi
6	<i>C. fagacearum</i> (Bretz) Hunt
7	<i>C. fimbriata</i> Ellis & Halst.
8	<i>C. laricicola</i> Redfern & Minter
9	<i>C. magnifica</i> H.D. Griffin
10	<i>C. moniliformis</i> (Hedgc.) C. Moreau
11	<i>C. paradoxa</i> (Dade) C. Moreau
12	<i>C. populicola</i> Olchow. & J. Reid
13	<i>C. radiculicola</i> (Bliss) C. Moreau
14	<i>C. stenospora</i> H.D. Griffin
15	<i>C. tenella</i> R.W. Davidson
16	<i>C. tubicollis</i> Olchow. & J. Reid
17	<i>C. virescens</i> (R.W. Davidson) C. Moreau
18	<i>Cp. alba</i> (DeVay, R.W. Davidson & Moller) H.P. Upadhyay
19	<i>Cp. collifera</i> Marm. & Butin
20	<i>Cp. concentrica</i> (Olchow. & J. Reid) H.P. Upadhyay
21	<i>Cp. crenulata</i> (Olchow. & J. Reid) H.P. Upadhyay
22	<i>Cp. falcata</i> (E.F. Wright & Cain) H.P. Upadhyay
23	<i>Cp. fasciata</i> (Olchow. & J. Reid) H.P. Upadhyay
24	<i>Cp. longispora</i> (Olchow. & J. Reid) H.P. Upadhyay
25	<i>Cp. minima</i> (Olchow. & J. Reid) H.P. Upadhyay
26	<i>Cp. minuta</i> (Siem.) H.P. Upadhyay & W.B. Kendr.
27	<i>Cp. minuta-bicolor</i> (R.W. Davidson) H.P. Upadhyay & W.B. Kendr.
28	<i>Cp. pallidobrunnea</i> (Olchow. & J. Reid) H.P. Upadhyay
29	<i>Cp. ranaculosus</i> T.J. Perry & Bridges
30	<i>Cp. spinulosa</i> (H.D. Griffin) H.P. Upadhyay
31	<i>O. abietinum</i> Marm. & Butin
32	<i>O. abiocarpum</i> (R.W. Davidson) T.C. Harr.
33	<i>O. aequivaginata</i> Olchow. & J. Reid
34	<i>O. ainoae</i> H. Solheim
35	<i>O. allantospora</i> H.D. Griffin
36	<i>O. araucariae</i> (Butin) de Hoog & R.J. Scheff.
37	<i>C. arborea</i> Olchow. & J. Reid
38	<i>O. aureum</i> (Rob.-Jeffr. & R.W. Davidson) T.C. Harr.
39	<i>O. bacillosporium</i> (Butin & Zimm.) de Hoog & R.J. Scheff.
40	<i>O. bicolor</i> R.W. Davidson & Wells
41	<i>O. brevicolla</i> (R.W. Davidson) de Hoog & R.J. Scheff.
42	<i>O. brunneo-ciliatum</i> Mathiesen-Käärik
43	<i>C. brunneocrinata</i> E.F. Wright & Cain
44	<i>O. cainii</i> (Olchow. & J. Reid) T.C. Harr.
45	<i>O. canum</i> (Münch) Syd. & P. Syd.
46	<i>O. clavigerum</i> (Rob.-Jeffr. & R.W. Davidson) T.C. Harr.

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- 47 *O. columnaris* Olchow. & J. Reid  
48 *O. conicolum* Marm. & Butin  
49 *O. crassivaginatium* (H.D. Griffin) T.C. Harr.  
50 *O. cucullatum* H. Solheim  
51 *O. davidsonii* (Olchow. & J. Reid) H. Solheim  
52 *O. dryocoetidis* (W.B. Kendr. & Molnar) de Hoog & R.J. Scheff.  
53 *O. epigloeum* (Guerrero) de Hoog & R.J. Scheff.  
54 *O. flexosum* H. Solheim  
55 *O. francke-grosmaniae* (R.W. Davidson) de Hoog & R.J. Scheff.  
56 *O. grandifoliae* (R.W. Davidson) T.C. Harr.  
57 *O. huntii* (Rob.-Jeffr.) de Hoog & R.J. Scheff.  
58 *O. introcitrina* Olchow. & J. Reid  
59 *O. ips* (Rumbold) Nannf.  
60 *O. leptographioides* (R.W. Davidson) Arx  
61 *O. leucocarpa* R.W. Davidson  
62 *O. megalobrunneum* (R.W. Davidson & Toole) de Hoog & R.J. Scheff.  
63 *O. minus* (Hedgc.) Syd. & P. Syd.  
64 *O. multiannulatum* (Hedgc. & R.W. Davidson) Fr.  
65 *O. narcissi* Limber  
66 *O. nigrocarpum* (R.W. Davidson) de Hoog  
67 *O. nigrum* (R.W. Davidson) de Hoog & R.J. Scheff.  
68 *O. nothofagi* (Butin) Rulamort  
69 *O. novo-ulmi* Brasier  
70 *O. obscura* (R.W. Davidson) Arx  
71 *O. olivaceapinii* R.W. Davidson  
72 *O. olivaceum* Mathiesen  
73 *O. piceae* (Münch) Syd. & P. Syd.  
74 *O. piceaperdum* (Rumbold) Arx  
75 *O. piliferum* (Fr.) Syd. & P. Syd.  
76 *O. pluriannulatum* (Hedgc.) Syd. & P. Syd.  
77 *O. populinum* (T.E. Hinds & R.W. Davidson) de Hoog & R.J. Scheff.  
78 *O. robustum* (Rob.-Jeffr. & R.W. Davidson) T.C. Harr.  
79 *O. rostrocoronatum* (R.W. Davidson & Eslyn) de Hoog & R.J. Scheff.  
80 *O. rostrocylindricum* (R.W. Davidson) Arx  
81 *O. sagmatospora* (E.F. Wright & Cain) H. Solheim  
82 *O. seticolle* (R.W. Davidson) de Hoog & R.J. Scheff.  
83 *O. sparsum* (R.W. Davidson) de Hoog & R.J. Scheff.  
84 *O. stenoceras* (Robak) Melin & Nannf.  
85 *O. tetropii* Mathiesen  
86 *O. torticiliata* Olchow. & J. Reid  
87 *O. tremulo-aureum* (R.W. Davidson & T.E. Hinds) de Hoog & R.J. Scheff.  
88 *O. triangulosporum* Butin  
89 *O. trinacriforme* (Parker) T.C. Harr.  
90 *O. ulmi* (Buisman) Nannf.
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<sup>a</sup> C. refers to *Ceratocystis*, Cp. to *Ceratocystiopsis* and O. to *Ophiostoma*.

## CHARACTER CODING

A total of eight morphological characters, four for the anamorph and four for the teleomorph, were used to describe taxa (Table 2a, 2b). Anamorph characters included type of conidiophore (mononematous and/or synnematous), conidiogenesis, type of anamorph (*Chalara*, *Graphium*, *Hyalorhinoclaadiella*, *Leptographium* and *Sporothrix*) and conidial morphology. Teleomorph characters included ascoma base, ascoma neck, ostiolar hyphae and ascospore morphology. Characters such as shape of peridial cells, ascomatal ornamentation, length of ostiolar hyphae and conidiophore dimension, were excluded because they were often not mentioned in descriptions.

Morphological characters were described in terms of multi-state characters (Table 2a, 2b). Qualitative characters were described using numerical ranges published by Grylls and Seifert (1993) in a synoptic key to the Ophiostomatoid fungi. Binary states (0 and 1) were used to indicate the presence or absence of character states for the various characters (Table 3).

Where more than one anamorph was present in a taxon, the characters of the synanamorph were also included in the analysis. In the event that only one anamorph was present for a taxon, the anamorph was used as synanamorph for comparative purposes. In taxa where the anamorph is unknown (*C. magnifica* and *C. stenospora*), anamorph characters were treated as missing data.

## PARSIMONY AND DISTANCE ANALYSIS

A data set (Table 3) established for each taxon using a wide range of character states (Table 2a, 2b), was analysed using parsimony methods. The most parsimonious tree was derived using heuristic search techniques of PAUP 3.1.1 (Phylogenetic Analysis Using Parsimony) (Swofford, 1993). Unweighted character states were analysed using PAUP and the most parsimonious tree produced. Characters were equally weighted using fractional weighting of character states (Table 4). This was achieved by weighting individual character states. The fractional weight of character states was determined by dividing the greatest number of character states used for each character by the number of character states pertaining to that particular character. Using this approach, a monophyletic outgroup was determined and used to root trees in subsequent analyses.

To simplify later analyses, a subset of 55 taxa was chosen based on the groupings of the tree resulting from equally weighted characters (Fig. 1). Bootstrapping (1000 replicates) was done to calculate the confidence intervals of the different branching points on the tree. To determine the contribution of different characters (Table 2a, 2b) to tree topology, perithecial base (1-3, 4-10), perithecial neck (11, 12-21, 22-26), ostiolar hyphae (27, 28-30), ascospore morphology (31-37, 38-43, 44-47), conidiophore (48-49), conidiogenesis (50-51), anamorph type (52-57) and conidial morphology (58, 59-64, 65-69, 70-73, 74-79, 80-84, 85-88), were weighted consecutively, twice as heavily as any other character. Characters were also deleted from analyses consecutively to determine the effect of the absence of characters. *Microascus longirostris* was included in the analysis to determine the effect of homoplasy of morphological characters used to determine tree topologies.

Table 2a. Teleomorph character states used in the morphology data set.

Characterstate	1	2	3	4	5	6	7	8	9	10
<b>1. Perithecial base</b>										
1-3 Base pigmentation	dark brown to black	light brown or olivaceous	white or hyaline	-	-	-	-	-	-	-
4-10 Diameter ( $\mu\text{m}$ )	<50	50-100	100-150	150-200	200-300	300-500	>500	-	-	-
<b>2. Perithecial neck</b>										
11 Neck	present / absent									
12-21 Length ( $\mu\text{m}$ )	<50	50-100	100-200	200-300	300-500	500-750	750-1000	1000-1500	1500-5000	>5000
22-26 Width at apex	<10	10-20	20-30	30-45	>45	-	-	-	-	-
<b>3. Ostiolar hyphae</b>										
27 Ostiolar hyphae present / absent	present / absent	-	-	-	-	-	-	-	-	-
28-30 Type	divergent	parallel	convergent	-	-	-	-	-	-	-
<b>4. Ascospore</b>										
31-37 Shape	oblong to ellipsoidal	curved, allantoid, orange section	fussiform x falcate	appearing sheathed	secondary wall hat shaped	secondary wall pillow shaped	secondary wall same shape as ascospore	-	-	-
38-43 Length ( $\mu\text{m}$ )	<3	3-5	5-7	7-10	10-20	>20	-	-	-	-
44-47 Width ( $\mu\text{m}$ )	<2	2-3	3-4	>4	-	-	-	-	-	-



Table 2b. Anamorph character states used in the morphology data set.

Characterstate	1	2	3	4	5	6
<b>5. Conidiophore</b>						
48-49 Type	mononematous	symnematos	-	-	-	-
<b>6. Conidiogenesis</b>						
50-51 Type	holoblastic	enteroblastic	-	-	-	-
<b>7. Anamorph</b>						
52-57 Type	<i>Chalara</i>	<i>Graphium</i>	<i>Hyalorhinocladiella</i>	<i>Leptographium</i>	<i>Sporothrix</i>	other
<b>8. Conidia / Anamorph</b>						
58 Septation	present / absent	-	-	-	-	-
59-64 Shape	globose	ellipsoidal to ovate	oblong to ellipsoidal	clavate	Y or T shaped	fussiform
65-69 Length (µm)	<5	5-7	7-10	10-20	>20	-
70-73 Width (µm)	<2	2-3	3-4	>4	-	-
<b>Conidia / Synanamorph</b>						
74-79 Shape	globose	ellipsoidal to ovate	oblong to ellipsoidal	clavate	Y or T shaped	fussiform
80-84 Length (µm)	<5	5-7	7-10	10-20	>20	-
85-88 Width (µm)	<2	2-3	3-4	>4	-	-

Table 3. Coded data matrix for taxa (Table 1)<sup>a</sup> coded according to character states in Table 2.

Character	1	2	3	4	5	6	7	8
1	100000111	-100000001	1001100-1100-1100-010010010001100001110-01-01-100000-11100000110001111100000110001111000001100011					
2	1000110000	-10000110000010000-1100-1000100111000011000110001-10-01-010000-001100111011100011001110011101110						
3	1001100000	-1001110000010000-1000-0100000010000100010-10-01-000010-011101110001000011101110001000						
4	1000110000	-1001110000001100-1100-10001010011000110010-10-01-001000-011000100001100011000100001100						
5	1000110000	-1000001110001000-1100-010000011000100010-10-01-000010-0110011110111001100111101110						
6	1000011000	-1000001100001000-1100-01001001000100010010-01-01-100000-0110000011100001011000011100001						
7	1000110000	-101100000011000-1110-1000101001000100010010-10-01-000010-011110110000011011110110000011						
8	1000000110	-1000110000000001-1110-11001001001110011010-01-01-100000-011000011100011100110011000111100110						
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<sup>a</sup> Taxa numbers correspond to those listed in Table 1.

Table 4. Fractional weighting of character states to equally weight characters.

Morphological character	Number of character states	Equal weighting of characters <sup>a</sup>
Teleomorph		
1. Perithecial	10	310
2. Perithecial	16	193
3. Ostiolar	4	775
4. Ascospore	20	182
Anamorph		
5. Conidiophore	2	1550
6. Conidiogenesis	2	1550
7. Anamorph	6	516
8. Conidia	31	100

<sup>a</sup> Equal weight of characters =  $(x \div y) \times 100$ , where,  
 x - largest number of character states per character (31),  
 y - number of character states of this character.

## RESULTS

Using the heuristic search option on the unweighted data set resulted in 20 equally most parsimonious trees. All 20 tree topologies were incongruent with the accepted classification of the Ophiostomatoid fungi (Seifert *et al.*, 1993; Upadhyay, 1981). Species of *Ceratocystis* were thus interspersed with species of *Ophiostoma* and *Ceratocystiopsis* without any obvious pattern consistent with our knowledge of this group.

In the analysis of equally weighted characters using fractional weighting, three trees rooted to midpoint were produced (Fig. 1). The tree topologies were similar, differing only within clusters. The tree topology was such that the species were divided into two groups i.e. species of *Ceratocystis* and species of *Ophiostoma* and *Ceratocystiopsis*. Notable exceptions were species presently residing in *Ceratocystis* (*C. angusticollis*, *C. californica*, *C. deltoideospora*, *C. populicola*, *C. tenella* and *C. tubicollis*) that grouped with species of *Ophiostoma*. *Ceratocystiopsis falcata*, grouped with species of *Ceratocystis*. The *Ceratocystis* group formed a distinct monophyly and was used as outgroup in further analyses. Species of *Ophiostoma* and *Ceratocystiopsis* were found in separate clusters. Species with *Graphium* anamorphs grouped together, including certain species with *Graphium* as well as *Sporothrix* synanamorphs, (*O. araucariae*, *O. ulmi*, *O. novo-ulmi* and *O. piceae*). Species with *Sporothrix* or *Hyalorhinocladia* anamorphs occurred in two clusters. The first group included only species with *Sporothrix* anamorphs while the second group incorporated species with *Hyalorhinocladia* anamorphs and some species with *Sporothrix* anamorphs. Species with *Leptographium* anamorphs also resided in a separate cluster. The tree homoplasy index of morphological characters was 0.848 and the consistency index was 0.152.

Based on the dendrogram produced by equally weighting characters (Fig. 1), a subset of 55 taxa, representing the different groups, were selected for further analysis. A single most parsimonious tree was produced using the heuristic search option (Fig. 2). The tree was rooted using species of *Ceratocystis*, including *Cp. falcata*, as outgroup. The *Ceratocystis* group was separate from species of *Ophiostoma* and *Ceratocystiopsis*, with a 98% confidence interval (Fig. 2). The *Graphium* and *Sporothrix* groups both had bootstrap values of 100%. The bootstrap value for the *Leptographium* group was 87% while the *Hyalorhinocladia* group had a bootstrap value of 95%. The homoplasy index for the tree was 0.775 and the consistency index 0.225. When *M. longirostris* was included in the analysis, it was found to group within the *Leptographium* cluster (Fig. 3).

Weighting various characters did not always produce tree topologies that were congruent with the equal weighting of characters. Weighting perithecial base resulted in nine trees. The grouping topology was similar to that in Figure 2, with differences in the internal rearrangements of the *Graphium* and *Ceratocystis* groups. Weighting of the perithecial neck character produced two trees, with loss of resolution in the *Leptographium* and *Hyalorhinocladia* groups. Weighting of ostiolar hyphae resulted in nine trees. The *Ceratocystis*, *Graphium* and *Leptographium* groups, with the exception of *O. leptographioides*, maintained resolution. Taxa in the *Sporothrix* and *Hyalorhinocladia* groups were intermixed with *O. leptographioides*.

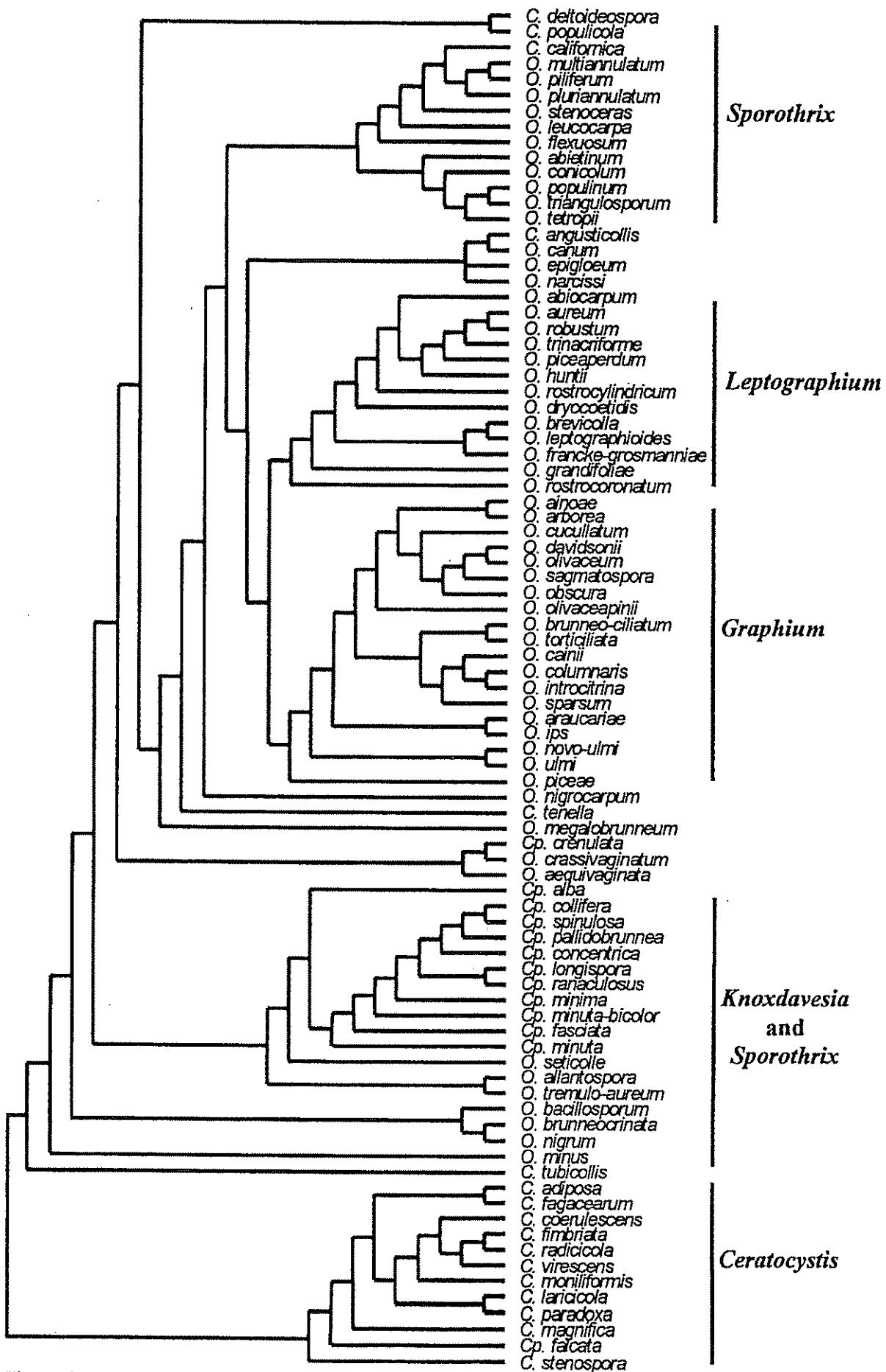


Fig. 1. Consensus of three most parsimonious trees.

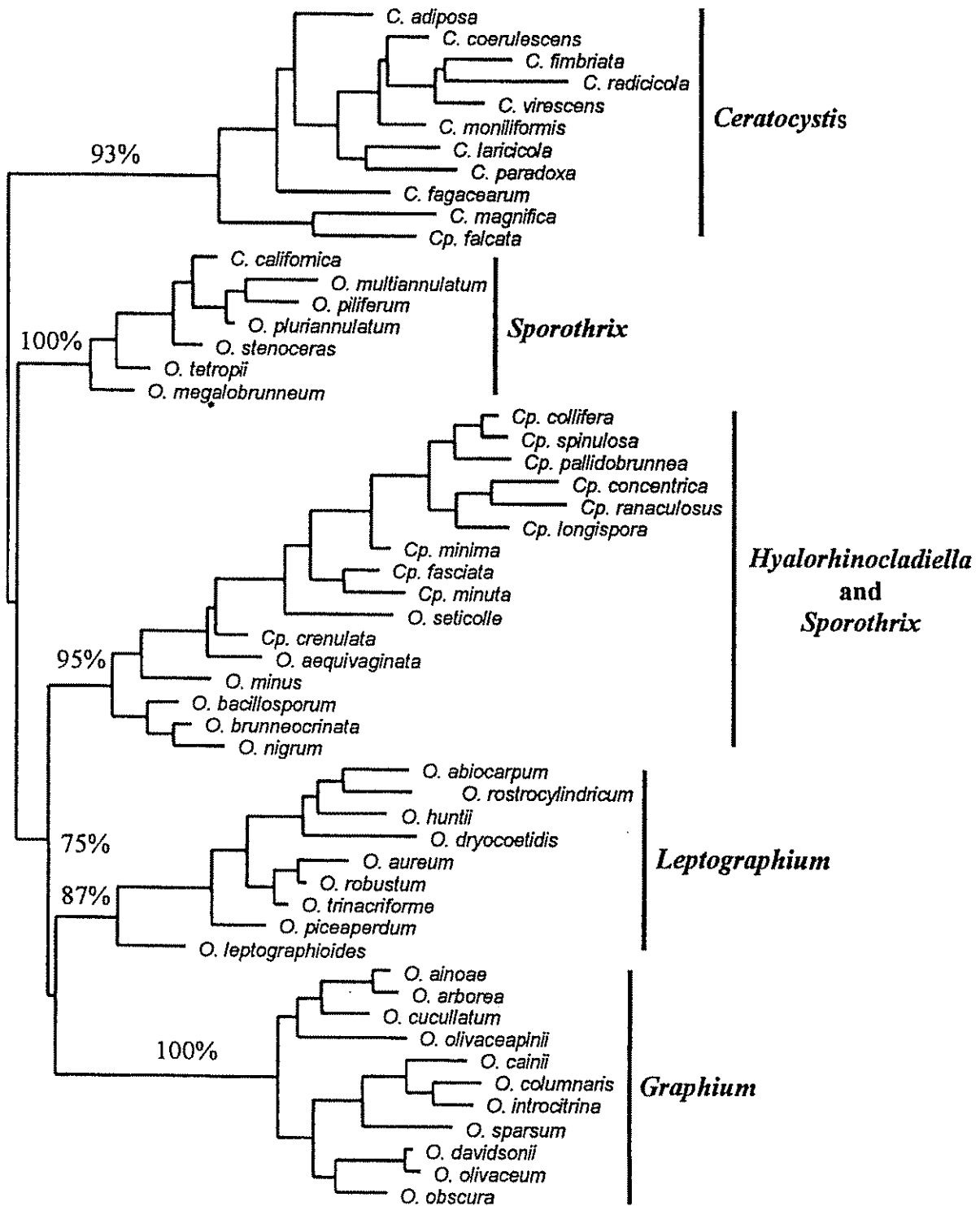


Fig. 2. Most parsimonious tree of selected taxa.



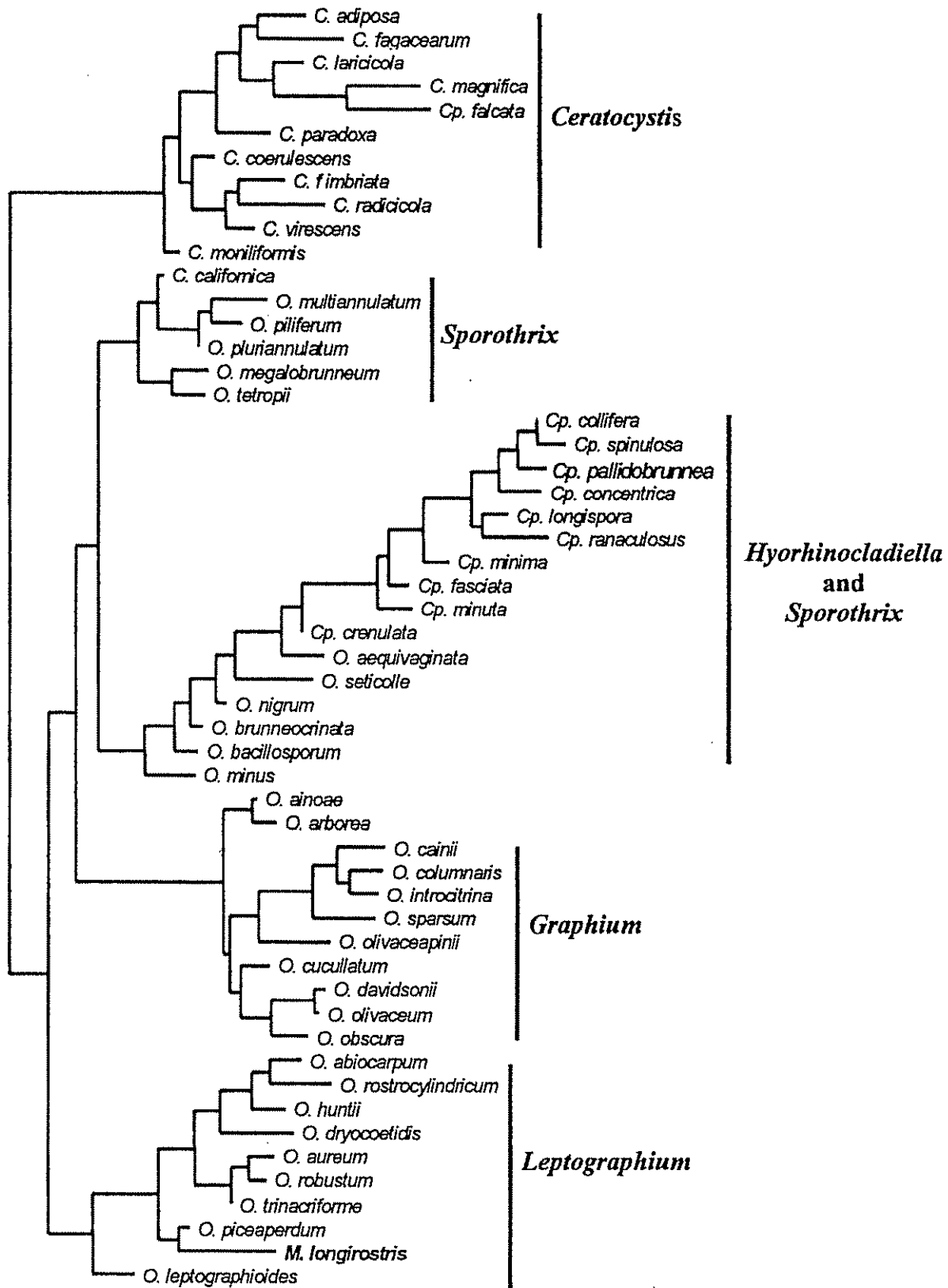


Fig. 3. Most parsimonious tree with *M. longirostris* included in the taxa selection.

Weighting ascospore morphology resulted in two trees with a loss of resolution in the *Hyalorhinocladiella* group.

Weighting anamorph characters had less of an effect on tree topology than it did with teleomorph characters. Weighting conidiophore and anamorph type, resulted in one tree with similar topology to that where characters were equally weighted. Weighting the type of conidiogenesis character produced three trees. The different groups were maintained with internal rearrangements occurring in the *Graphium* group in all the trees. Weighting of conidial morphology produced four trees of which the *Hyalorhinocladiella* group was unresolved and internal rearrangements occurred in the *Graphium* and *Sporothrix* groups.

Deleting characters from analyses had a greater effect on tree topology than did weighting. Deleting perithecial base resulted in 10 trees, all having the same group topology as that in Figure 2, but with internal rearrangements. Excluding perithecial neck resulted in 15 trees. *Ceratocystis magnifica* grouped within the *Leptographium* group and rearrangements occurred in the *Hyalorhinocladiella* and *Leptographium* groups. Deletion of ostiolar hyphae resulted in two trees. Here, species in the *Leptographium* group and *C. virescens* were unresolved. Deleting ascospore morphology resulted in nine trees, in which the *Graphium* and *Sporothrix* groups were maintained. The *Leptographium* group included *C. magnifica* while *Hyalorhinocladiella* was split into two separate clusters.

Deletion of anamorph characters had a similar effect on tree topology as did the deletion of teleomorph characters. Excluding type of conidiophore resulted in three trees. Here, *Ceratocystis* and *Sporothrix* groups were maintained while taxa in the *Graphium*, *Hyalorhinocladiella* and *Leptographium* groups were intermixed. Deleting type of conidiogenesis from the analysis produced five trees in which the *Graphium* and *Sporothrix* groups were maintained. The *Leptographium* group included *C. magnifica* while the *Hyalorhinocladiella* group included *Cp. falcata*. Removing anamorph type resulted in 12 trees in which the groups *Ceratocystis*, *Sporothrix* and *Graphium* were maintained. The *Hyalorhinocladiella* group included *O. leptographioides* with the grouping of other taxa in the *Leptographium* group, unresolved. Deleting conidial morphology from the analysis resulted in 89 trees. All the groups, *Ceratocystis*, *Graphium*, *Sporothrix*, *Leptographium* and *Hyalorhinocladiella* were maintained with rearrangements of taxa occurring within groups.

## DISCUSSION

In this study of published morphological characters, the grouping of species in *Ceratocystis s.l.* based on anamorph type suggests that this character is important in the delineation of phylogenetic groups. The exception is that species with *Hyalorhinocladiella* anamorphs form a group that includes species with *Sporothrix* anamorphs. Benade *et al.* (1996) found that the *Hyalorhinocladiella* anamorph is similar to *Sporothrix* and that there is apparently a continuum of developmental stages between these two genera. The clustering of species with similar anamorph type may ultimately form a sound basis of different genera within *Ophiostoma sensu lato*.

Weighting individual character states can be used to cancel the effect of assigning different numbers of character states to different characters. Analysis of equally weighted morphological characters of the Ophiostomatoid fungi produced tree topologies reasonably congruent with those from studies using molecular data (Berbee and Taylor, 1992a; 1992b; Hausner *et al.*, 1993; Spatafora and Blackwell, 1993). Results from parsimony analysis, of equally weighted morphological characters, support the view that *Ceratocystis s.s.* is a distinct genus from *Ophiostoma*. Furthermore, in analyses testing the homology of all morphological characters simultaneously, there does not appear to be any justification for separating species of *Ceratocystiopsis* from *Ophiostoma*. This finding based on morphological data is congruent with molecular data that suggests that *Ceratocystiopsis* and *Ophiostoma* form a monophyletic group (Hausner *et al.*, 1993).

Using parsimony analysis of equally weighted characters with unrooted trees (rooted to midpoint), species of *Ceratocystis* (with the exception of *C. angusticollis*, *C. californica*, *C. deltoideospora*, *C. populicola*, *C. tenella*, *C. tubicollis* and the inclusion of *Cp. falcata*) were identified as a monophyletic group. We can support the exclusion of *C. angusticollis*, *C. californica*, *C. deltoideospora*, *C. populicola*, *C. tenella*, *C. tubicollis* from *Ceratocystis* based on the fact that these species have anamorphs reminiscent of those in *Ophiostoma* and these species should be transferred to the latter genus (Seifert *et al.*, 1993).

The inclusion of *Cp. falcata* in *Ceratocystis* is justifiable as this fungus is more reminiscent of species in *Ceratocystis*. *Ceratocystiopsis falcata* has a *Chalara* anamorph, which is characteristic of species of *Ceratocystis s.s.* However, sequence, RFLP and hybridisation data (Hausner *et al.*, 1993; Viljoen *et al.*, 1999) suggest that *Cp. falcata* is phylogenetically distant from species of *Ceratocystis*. Ascospore morphology on its own is, therefore, not a good characteristic on which to base genera, as has previously been shown by Hausner *et al.* (1993). It would appear that the characters used in the taxonomy of the Ophiostomatoid fungi are highly convergent. This is supported by high homoplasy values for the trees resulting from PAUP analyses.

Consecutive weighting of morphological characters was useful in determining the effect of those characters on tree topology. Thus, weighting different morphological characters changed tree topology to a greater or lesser extent. Weighting perithecial base, conidiophore, conidiogenesis and anamorph type, did not affect tree topology. However, weighting perithecial neck, ostiolar hyphae, ascospore and conidial morphology resulted in a loss of resolution in one or more groups of taxa. Furthermore, no single morphological character, when weighted, resulted in the simultaneous loss of resolution in all groups of taxa. Therefore, the effect of perithecial neck, ostiolar hyphae, ascospore and conidial morphology on the taxonomy of these fungi is collective. This suggests that groups of characters, rather than single characters, should be used simultaneously in the taxonomy of these fungi. This is unfortunately not possible without the aid of computer based systematics. Results underline the importance of character description in computer systematics.

Removing morphological characters from analyses, placed the weighting of characters in perspective. Perithecial base was the only character that did not change tree

topology. Perithecial neck, ostiolar hyphae, ascospore morphology, conidiophore, conidiogenesis, anamorph type and conidial morphology contributed to a wide range of changes in tree topology. No single deleted character resulted in loss of resolution of all the different groups. For example, deleting anamorph type did not change the distinction between *Ceratocystis*, *Graphium* and *Sporothrix* groups. The *Leptographium* group was unresolved with *O. leptographioides* included in the *Hyalorhinocladiella* group. Therefore, the distinction between the groups of taxa, is not solely based on a single morphological characteristic. Furthermore, the low consistency index values and high homoplasy values confirm that the morphological characters, used in the taxonomy of the Ophiostomatoid fungi, are highly convergent. However, the high bootstrap values for the different nodes of the tree is an indication that the grouping of taxa within the trees is valid and is not a result of any single character.

Species of *Microascus* are morphologically similar to the Ophiostomatoid fungi. However, analysis of sequence data (Berbee and Taylor, 1992a; 1992b; Spatafora and Blackwell, 1993) has shown that *Microascus* is phylogenetically distinct from *Ophiostoma*. The inclusion of *M. longirostris* in the *Leptographium* group (Fig. 4) is, therefore, an indication that many of the morphological characters used in the taxonomy of these fungi are highly convergent. This is supported by the high homoplasy value (0.775) for the tree topology.

Computer analysis of morphological characters is error prone, because of the plastic nature of these characters and the way in which they are expressed in a data base. This bias is introduced by reducing a character to an exclusive state, regardless of the variation within that character. Under these conditions, the taxonomist must make the decision that one character state, above all others, best describes a taxon character. Using a binary system to indicate the presence or absence of multi-state characters does not restrict morphological characters from being expressed as selective character states in a data matrix. The advantage of this approach is that the data matrix will reflect shared character states and shared characters. Furthermore, using a combination of multi-state character states in conjunction with a binary system, allows the expression of natural variation within a coded data matrix. The binary coding thus makes it possible to assign each character equal weight or to weight certain characters preferentially. This is useful in determining the effect that different characters have on tree topologies, and can be used to identify divergent or convergent morphological characters.

This study provides an objective taxonomic hypothesis for the Ophiostomatoid fungi based on equally weighted morphological characters. In summary, the genus *Ceratocystis* s.s. is distinct from *Ophiostoma* while species of *Ceratocystiopsis* and *Ophiostoma* form a monophyletic group. Despite the high degree of homoplasy of characters, results strongly suggest that anamorph type may be an important character on which further subdivisions, in *Ceratocystiopsis* and *Ophiostoma*, may be based.

We have attempted to address the problems pertaining to character description and different numbers of character states, by using an inclusive approach and equal weighting of morphological characters. The effect is evident when comparing the result of unweighted versus equally weighted characters. Only the analyses of equally

weighted characters produced tree topologies congruent to hypothetical classifications. Furthermore, it appears that collections of morphological characters, rather than single characters, are important in the taxonomy of the Ophiostomatoid fungi. The taxonomic hypothesis arising from this morphology study should now be tested using less subjective data sets, such as those derived using molecular techniques.

### ACKNOWLEDGEMENTS

This research was supported by the South African Foundation for Research and Development. We would like to express our thanks to the reviewers who made a valuable contribution to the manuscript.

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