

Ascospore development in *Ceratocystis sensu lato* (Fungi): a review

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ABSTRACT

Ceratocystis, *Ceratocystiopsis* and *Ophiostoma* are important pathogens of trees and some agricultural crops and have recently been found on proteas and forest trees in South Africa. Taxonomic controversy exists regarding these genera and ultrastructural studies on the development of asci, uniquely shaped ascospores and centrum structure are inadequate. This review summarises current knowledge of ascospore shape and development of the centrum obtained from light and electron microscope studies of *Ceratocystis sensu lato*. Important questions requiring further investigations are outlined. It is furthermore proposed that additional ultrastructural studies are required to clarify the current taxonomic disagreement in this group. Such studies could also identify relationships between these fungi and other Ascomycetes.

UITTREKSEL

Ceratocystis, *Ceratocystiopsis* en *Ophiostoma* is belangrike patogene van bome en sommige landbougewasse en is ook onlangs in Suid-Afrika op proteas en in woudbome gevind. Daar bestaan meningsverskille oor die taksonomiese plasing van hierdie genera en ultrastrukturele ondersoeke van die ontwikkeling van askusse, uitsonderlike askospore en sentrumstruktuur van die askokarp is tans ontoereikend. Hierdie literatuurbespreking bied 'n opsomming van lig- en elektronmikroskopiese ondersoeke van askosporvorm en sentrumontwikkeling by *Ceratocystis sensu lato*. Belangrike vraagstukke wat verdere navorsing vereis, word uitgewys. Vervolgens word voorgestel dat bykomstige ultrastrukturele ondersoeke noodsaaklik is om die huidige taksonomiese meningsverskille op te klaar. Sodanige ondersoeke kan moontlik ook die verwantskappe tussen hierdie fungi en ander Ascomycetes aantoon.

INTRODUCTION

Ceratocystis Ell. & Halst. *sensu lato* includes the genera *Ophiostoma* H. & P. Sydow, *Ceratocystis sensu stricto* and *Ceratocystiopsis* Upadh. & Kendr. (DeHoog & Scheffer 1984; Upadhyay 1981; Upadhyay & Kendrick 1975; Weijman & DeHoog 1975). These organisms are important plant pathogens, especially of trees (Boyce 1961; Clark & Moyer 1988; Marion & French 1967; Smith 1967; Wismer 1961; Wood & French 1963) and have recently been recorded from South Africa associated with pine bark beetles (Wingfield & Marasas 1980) and insect-infested *Protea* L. inflorescences (Wingfield *et al.* 1988). *Ceratocystis s.l.* are characterized by ostiolate perithecia, evanescent asci and, in many cases, ascospores with galeate sheaths. The development of asci and ascospores in *Ophiostoma* and *Ceratocystis* has received little attention. As far as we are aware, no cytological study exists on ascospore development in *Ceratocystiopsis*, despite the importance of these organisms as plant pathogens and the significance that such studies could have on their taxonomy and determining evolutionary relationships.

The generic concept in *Ceratocystis s.l.* has been the basis of controversy and has changed regularly since the first cytological studies of these fungi in 1925. In a number of cases, the same fungi have been studied under different names which could lead to confusion. In this review we follow the taxonomic scheme of De Hoog & Scheffer (1984) where *Ceratocystis* and *Ophiostoma* are treated as distinct genera. Currently used names of fungi treated in

older literature under different taxonomic schemes are given in Table 1 with the appropriate synonymies.

The first ultrastructural examination of ascospore development of *Ceratocystis s.l.*, was that of Stiers (1976) on *C. fimbriata* (Ell. & Halst.) Elliot. Subsequent studies were on *O. stenoceras* (Robak) Melin & Nannf. (Garrison *et al.* 1979) and *O. ulmi* (Buism.) Nannf. (Jeng & Hubbes 1980a, 1980b). These studies, however, give little insight into centrum organization. Moreover, ascospores of *Ceratocystis s.l.* have unique shapes and previous studies have provided almost no information on the structure or development of these spores.

The aim of this review, is to compile data from past cytological and recent ultrastructural studies, to summarize current knowledge of centrum structure and organization as well as ascospore shape, in *Ceratocystis s.l.* The possible implications of these observations in the taxonomy of *Ceratocystis s.l.* and their relationships with other Ascomycetes, comparing certain ultrastructural features, are discussed.

CENTRUM DEVELOPMENT

Controversy exists as to whether species of *Ceratocystis s.l.* should be included in the Plectomycetes, characterized by closed ascocarps (cleistothecia) (Ainsworth *et al.* 1973; Malloch 1981), or Pyrenomycetes, with ostiolate ascocarps (perithecia) (Luttrell 1951). Luttrell (1951) includes members of *Ceratocystis s.l.*, with their distinctive ostiolate ascocarps, in the Pyrenomycetes. In contrast, Benny *et al.* (1980) considered the Ophiostomatales as an order of the Plectomycetes, with the Ophiostomataceae as the only family.

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TABLE 1.—Cytological studies of *Ceratocystis* and *Ophiostoma* from 1927 to 1981

Genus and species	Author
A <i>Ceratocystis adiposa</i> (Butl.) C. Moreau = <i>Ceratostomella adiposum</i> (Butl.) Sartoris = <i>Ophiostoma majus</i> (Van Beyma) Goid.	Sartoris (1927) Hutchinson (1950)
B <i>Ceratocystis autographa</i> Bakshi	Bakshi (1951)
C <i>Ceratocystis fimbriata</i> (Ell. & Ha!st.) Elliot = <i>Ceratostomella fimbriata</i> Ell. & Ha!st.	* Stiers (1976) Andrus & Harter (1933) Gwynne-Vaughan & Broadhead (1936)
D <i>Ceratocystis moniliformis</i> (Hedgc.) C. Moreau	Moreau & Moreau (1952)
E <i>Ceratocystis paradoxa</i> (Dade) C. Moreau = <i>Ceratostomella paradoxa</i> Dade	Dade (1928)
F <i>Ophiostoma ips</i> (Rumb.) Nannf. = <i>Ceratostomella montium</i> Rumb.	Taylor-Vinje (1940)
G <i>Ophiostoma multiannulatum</i> (Hedgc. & Davids.) Hendrix = <i>Ceratostomella multiannulata</i> Hedgc. & Davids.	Andrus (1936)
H <i>Ophiostoma piceae</i> (Munch) Bakshi = <i>Ceratocystis piceae</i> Munch	Bakshi (1951)
I <i>Ophiostoma stenoceras</i> (Robak) Melin & Nannf. = <i>Ceratocystis stenoceras</i> (Robak) C. Moreau	* Garrison <i>et al.</i> (1979)
J <i>Ophiostoma ulmi</i> (Buism.) Nannf. = <i>Ceratocystis ulmi</i> (Buism.) C. Moreau	Rosinsky (1961) * Jeng & Hubbes (1980a)

A–J Current names of genus and species.

* Ultrastructural studies.

An important feature of the Pyrenomycetes is the organization of the centrum. Luttrell (1951) proposed a developmental scheme, where centrum development is initiated by the formation of sterile and fertile cells. The fertile cells are initiated by curved hyphal branches and the sterile cells by additional branching hyphae. These cells, surrounded by other hyphae, give rise to the maturing perithecium. The central multinucleate fertile cell or ascogonium lies above the centre of the perithecium, rather than at its base, the asci therefore develop progressively and basipetally. The sterile cells are distributed between the fertile ascogonial cells, and they have the important function of providing space for the irregularly developing asci, by disintegrating during ascus development.

Asci in the Plectomycetes are evanescent and the ascospores fill the cleistothecial centrum at maturity with no special discharging mechanism. Luttrell (1951) emphasized that the irregular distribution of the asci within the centrum of *Ceratocystis s.l.*, is reminiscent of the Plectomycetes. Redhead & Malloch (1977) concurred with this opinion and included *Ophiostoma* and *Ceratocystis* in this order. Furthermore, based on the presence of ascospores with galeate sheaths, these authors included the genera with numerous yeasts in the Endomycetaceae. Typically, Plectomycetes have closed ascocarps (Ainsworth *et al.* 1973), and thus *Ophiostoma* and *Ceratocystis* with beaked, ostiolate perithecia could equally be excluded from this group.

Malloch (1981) suggested that the Plectomycete centrum has evolved towards progressive simplification. Sterile tissue, the ostiole, as well as forcible discharge of spores would therefore have been lost. This argument would justify inclusion of *Ceratocystis s.l.* in the Plectomycetes,

despite their having ostiolate perithecia. Our knowledge of ascocarp development in these fungi is based on few examples with almost no ultrastructural information. Ultrastructural studies on the development of the centrum of *Ceratocystis*, *Ophiostoma* and related fungi could provide important clues clarifying their relationships.

The development of the centrum of *Thermoascus aurantiacus* and *Ceratocystis thermophile* is typical of that of the Plectomycetes, with asci arising from croziers formed by ascogenous hyphae (Ellis 1981a, 1981b). Ultrastructural studies of these species, however, provide insufficient information to make logical comparisons with *Ceratocystis s.l.* The single-celled nature of yeasts apparently related to *Ceratocystis s.l.* precludes comparisons of centrum development.

CYTOLOGY

The earliest cytological study of *Ceratocystis* was that of Elliott (1925) on *C. fimbriata*, associated with black rot of sweet potato. Subsequent investigations on this species were by Andrus & Harter (1933) and Gwynne-Vaughan & Broadhead (1936). Cytological studies on related fungi dealt with *Ophiostoma multiannulatum* (Hedgc. & Davids.) Hendrix (Andrus 1936), *C. paradoxa* (Dade) C. Moreau (Dade 1928), *C. adiposa* (Butl.) C. Moreau (Sartoris 1927; Hutchinson 1950), and *O. ips* (Rumb.) Nannf. (Taylor-Vinje 1940). This was followed by studies of *C. autographa* Bakshi (Bakshi 1951), *C. moniliformis* (Moreau & Moreau 1952) and *O. piceae* (Munch) Bakshi (Bakshi 1951).

Cytological characteristics of each species of *Ceratocystis s.l.* previously studied are given in Table 2. It is generally accepted in the Pyrenomycetes, that perithecia

are derived from coiled hyphae without fertilization (Luttrell 1951). Coiling of hyphae, was observed by different authors in all genera that have been studied (Table 2). Crozier formation has been reported for most species, except *C. adiposa*, *C. paradoxa* and *O. stenoceras* (Table 2). In reference to studies of *C. fimbriata* (Andrus & Harter 1933; Gwynne-Vaughan & Broadhead 1936) and *C. adiposa* (Sartoris 1927), Hutchinson (1950), in a study of *C. adiposa*, however, stated that a simple curved hypha with four terminal nuclei, is unconvincing evidence of crozier formation. The inference here was that crozier formation does not occur in *Ceratocystis s.l.* In contrast, crozier formation was illustrated in ultrastructural studies of *O. ulmi* (Jeng & Hubbes 1980b). Ascogonia were observed in all species (Table 2), although some authors described the ascogonium as either a fertile cell in *O. multiannulatum* (Andrus & Harter 1933) or ascogenous hyphae in *O. piceae* (Bakshi 1951) and *O. ips* (Taylor-Vinje 1940).

In developmental studies of *Ceratocystis s.l.*, reference is commonly made to sterile cells, which include both cushion and pseudoparenchymatous cells (Table 2). However, their function has seldom been discussed. Pseudoparenchymatous cells are dispersed amongst the ascogenous hyphae and their arrangement appears to characterise centrum organization (Table 2). Although the ascogenous hyphae generally emerge towards the base of the perithecium, they may also extend to the base of the neck, with the asci developing towards the centre (Table 2). The ascogenous hyphae are usually separated from the perithecial wall by the cushion cells, with asci forming irregularly throughout the centrum.





















According to Luttrell (1951), the ascogonium is located above the centre of the perithecium (Figure 1A), developing basipetally (Figure 1B). From available cytological studies we interpret centrum development in *Ceratocystis s.l.* as follows: ascus development is preceded by the development of ascogenous hyphae (= fertile cells) from the ascogonium. The ascogenous hyphae developing from the ascogonium, appear to form towards the base of the perithecium, with cushion cells (= sterile cells) surrounding them (Figure 1A). The ascogonium therefore develops basipetally (Figure 1B) and asci are formed by acropetally developing ascogenous hyphae (Figure 1C). Ascospores then mature basipetally (Figure 1D).

Gwynne-Vaughan & Broadhead (1936) stated that there is inadequate reference in most literature to the characteristic shape of the ascospores in *Ceratocystis s.l.* The ascospore shape of each species previously studied cytologically, reproduced from descriptions or original illustrations, is compared in Table 2. Hutchinson (1950) emphasized that the number of perithecial wall layers and ascus shape are inadequate criteria for determination of natural relationships. More emphasis should therefore be placed on detailed cytological and ultrastructural studies of centrum organization, as well as ascospore shape and development, as potential criteria in the taxonomy of *Ceratocystis s.l.*

ULTRASTRUCTURAL COMPARISON OF *CERATOCYSTIS S.L.* WITH OTHER ASCOMYCETES

Ultrastructural studies on ascus and ascospore development in the Ascomycetes are numerous (Dyby &

TABLE 2.—Cytological characteristics of perithecium development in *Ceratocystis* and *Ophiostoma*

	Coil	Crozier	Ascogonium	Cushion	Pseudo-parenchym	Perithecial layers	Centrum	Ascospore
A <i>C. adiposa</i>	+	-	+	?	?	2		
B <i>C. autographa</i>	+	+	+	+	-	2		
C <i>C. fimbriata</i>	+	+	+	+	-	2-4		
D <i>C. moniliformis</i>	+	+	+	+	?	?		
E <i>C. paradoxa</i>	+	?	+	+	+	?		
F <i>O. ips</i>	+	+	+	+	+	2		
G <i>O. multiannulatum</i>	+	+	*	-	+	3-4		
H <i>O. piceae</i>	+	+	**	+	?	2		
I <i>O. stenoceras</i>	+	?	+	?	?	2		
J <i>O. ulmi</i>	+	+	+	?	+	3		

A-E, ascospores with sheaths; F-J, ascospores without sheaths, except *O. ips*; + = present; - = absent; ? = unknown; * = described as fertile cell; ** = described as ascogenous hyphae.

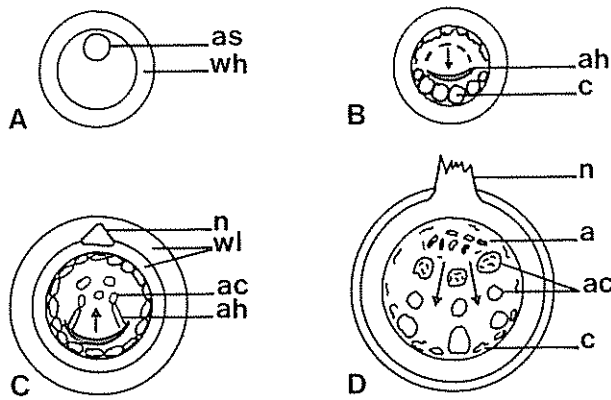


FIGURE 1.—Schematic diagrams of perithecium development in *Ceratocystis sensu lato*. A, ascogonium (as) formed above the centre of the perithecium surrounded by wall hyphae (wh); B, ascogonium developing basipetally forming ascogenous hyphae (ah) and cushion cells (c) forming surrounding layer; C, ascogenous hyphae developing acropetally forming asci (ac) and neck primordial cells (n) developing from perithecial wall layers (wl); D, evanescent asci maturing basipetally, releasing mature ascospores (a). Neck (n) elongating and cushion cells degenerating.

Kimbrough 1987; Furtado 1971; Honegger 1985; Mainwaring 1967; Merkus 1973; Van Brummelen 1987). In general, previous ultrastructural studies of ascospore development have investigated wall formation (Lynn & Magee 1972; Moens 1971), the morphology of nuclear and membrane structure (Hashimoto *et al.* 1960; Oso 1969) and the role of lomasomes and plasmalemmasomes (Marchant & Moore 1973). Ultrastructural studies of ascospore delimitation and development have also been conducted (Beckett 1981; Rosing 1985). A comprehensive review of this process has been provided elsewhere (Turian 1976). The latter review did not, however, cover *Ceratocystis s.l.*

Development of ascospores appears to be similar in all Ascomycetes. The spore walls are formed between two delimiting membranes (Ellis 1981a; Stiers 1976). In a preliminary investigation of *C. moniliformis* (Figure 2A)

and *O. minus* (Hedge.) H. & P. Syd. (Figure 2B) we have tentatively confirmed the presence of an electron transparent endospore and an electron dense episporic wall layer in ascospores of these species. Amongst fungi with evanescent asci, ultrastructural studies are available for *Thermoascus aurantiacus* Miede (Ellis 1981a) and *Chaetomium thermophile* La Touche (Ellis 1981b) as well as *Ceratocystis* spp. However, in the former fungi, ascospores are elliptically shaped with a characteristic germ pore and are therefore incomparable with *Ceratocystis s.l.*

Ascospore walls in *Ceratocystis sensu stricto* (Garrison *et al.* 1979; Jeng & Hubbes 1980a; Stiers 1976) are similar to those of certain yeasts (Bandoni *et al.* 1967; Beckett *et al.* 1973; Black & Gorman 1971; Hashimoto *et al.* 1960; Kreger-van Rij & Veenhuis 1975; Kurtzman & Ahearn 1976). Ultrastructural comparisons can therefore be made between ascospores with hat-shaped sheaths, in *Hansenula anomala* (Hansen) H. & P. Syd. (Bandoni *et al.* 1967), *Pichia spartinae* Ahearn, Yarrow et Meyers (Kurtzman & Ahearn 1976) and certain species of *Ceratocystis s.l.* To our knowledge these are the only fungi with hat-shaped galeate sheaths and they appear to be ultrastructurally indistinguishable.

CONCLUSION

With the exception of the hat-shaped ascospores in *Ceratocystis fimbriata*, the characteristic and unusual ascospores in other species of *Ceratocystis s.l.* have not been illustrated ultrastructurally. It is proposed that additional ultrastructural studies are required to clarify the current taxonomic disagreement in this group. Further ultrastructural studies on centrum organization, ascus development and ascospore shape may provide new keys to relationships between genera of these fungi. A better understanding of Plectomycetes/Pyrenomycetes relationships could result and might aid in interpretation of taxonomic and evolutionary relationships in the Ascomycetes as a whole

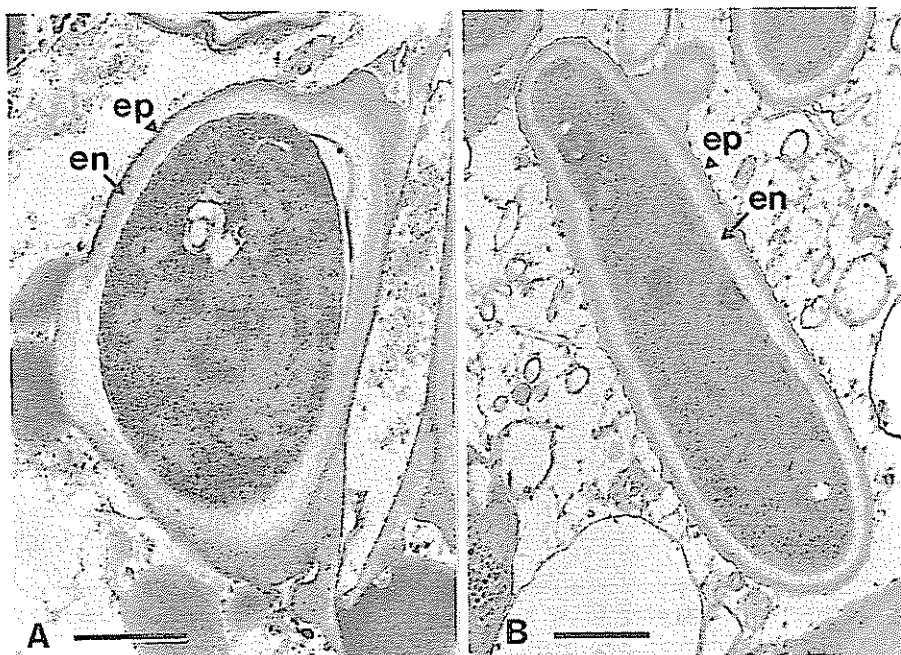


FIGURE 2.—Electron micrographs of mature ascospores. A, ascospore of *Ceratocystis moniliformis* with hat-shaped sheath (ep = episporic wall, en = endospore); B, elongated ascospore of *Ophiostoma minus*. Scale bar = 500 μ m.

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