

## Ascus apical apparatus and ascospore characters in *Xylariaceae*

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**Abstract:** Members of *Xylariaceae* (*Ascomycota*) are recognized and classified mainly on the morphological features of their sexual state. In a number of genera high morphological variation of stromatal characters has made confident recognition of generic and specific boundaries difficult. There are, however, a range of microscopical characteristics which can in most cases make distinctions, especially at generic level, even in the absence of molecular data. These include details of the apical apparatus in the ascus (e.g. disc-shaped, inverted hat-shaped, rhomboid, composed of rings, amyloid, non-amyloid); position and length of the germ slit; and presence and type of ascospore wall ornamentation as seen by scanning electron microscopy (SEM). Unfortunately many of the classical studies on xylariaceous genera omitted these features and were undertaken long before the development of scanning electron microscopy. More recent studies have, however, demonstrated their value as diagnostic characters in the family. *Camillea* is for example, instantly recognizable by its rhomboid or diamond shaped apical apparatus, and the distinctive inverted hat or urniform type is usually prominent in *Xylaria*, *Rosellinia*, *Kretzschmaria*, and *Nemania*. At least six categories of apical apparatus based on shape and size can be recognized. Ascospore ornamentation as seen by SEM has been exceptionally useful and provided the basis for separating *Camillea* from *Biscogniauxia* and other xylariaceous genera.

**Key words:**

*Ascomycota*  
ascospores  
iodine reaction  
scanning electron  
microscopy  
systematics  
*Xylariales*

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### INTRODUCTION

*Xylariaceae* is one of the best-known and widely distributed families of *Ascomycota*. The majority of the species are wood inhabitants, and are particularly well represented in the tropics. Ju & Rogers (1996) recognized 38 genera, Whalley (1996) 40, and the number has grown to at least 76 (Lumbsch & Huhndorf 2010), although the total varies according to individual opinion and the status of several genera in the family awaits confirmation. The separation of genera and subsequent identification of taxa has been problematic mainly as a result of diversity of form and variation in many morphological characteristics (Whalley 1996, Rogers 2000). Genera within *Xylariaceae* were traditionally recognized on the basis of stromal form, stromal colour, and ascospore shape and dimensions (Fig. 1). As a consequence other important taxonomic features were neglected (Rogers 1979, Whalley 1996). Details of the ascus, including the apical apparatus, and ascospore topography were not considered. The subsequent advent of scanning electron microscopy (SEM) has demonstrated the value of spore ornamentation and details of stromatal surfaces (Læssøe *et al.* 1989, Whalley 1996). In this paper we assess the importance of these characteristics based on our experience and extrapolations from recent publications.

### METHODS

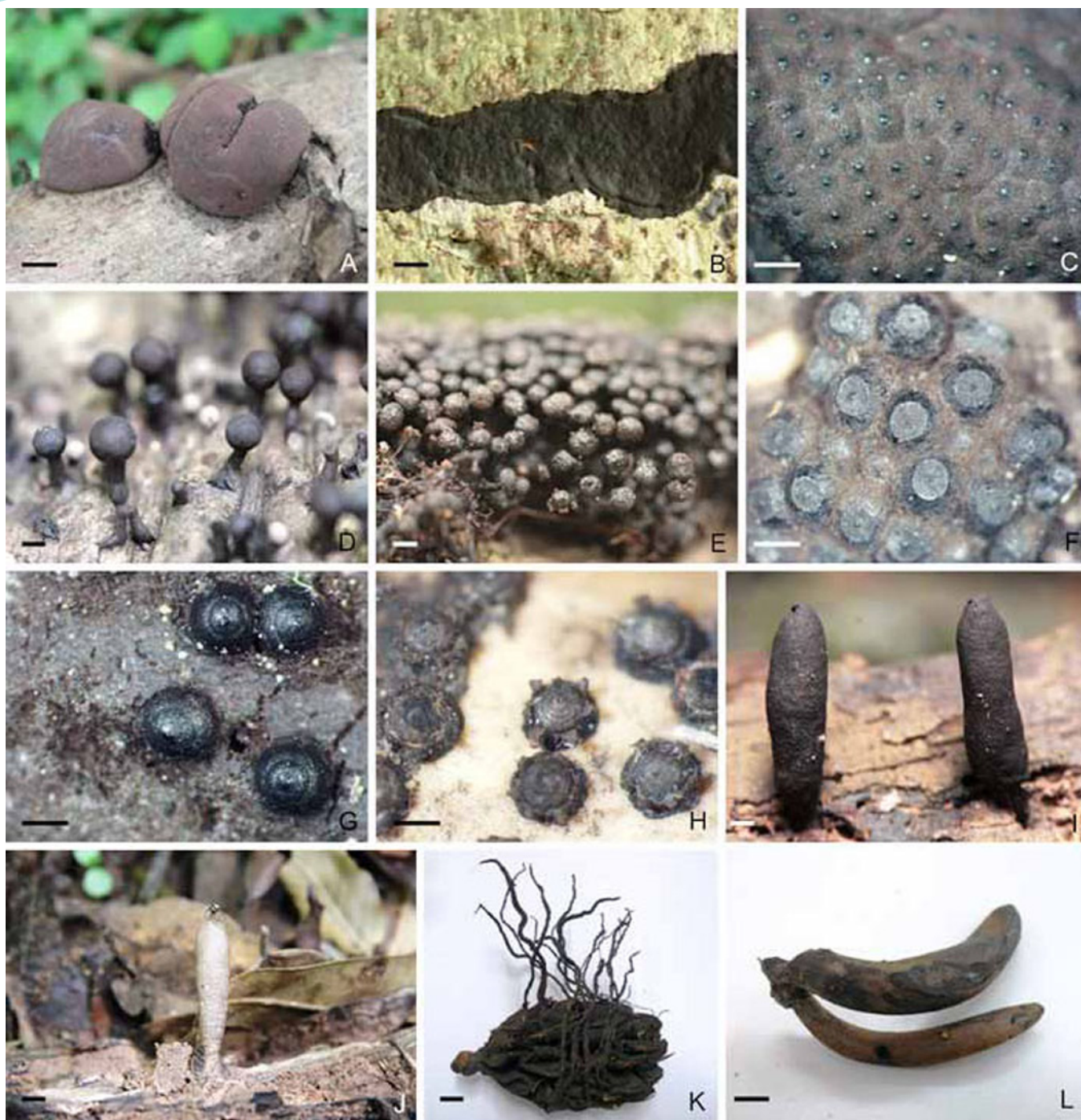
Squash preparations of asci and ascospores mounted in water, Melzer's iodine reagent, and lactophenol cotton blue were microscopically examined by bright field microscopy and differential interference contrast (DIC) light microscopy with an Olympus BH2 research microscope using x10, x40 and x60 dry objectives. Images were captured by Camera (INFINITY 1) and were analyzed by Infinity Analyze software provided with measurement functions and image enhancement options. For examination by SEM, small sections of dried stromata were mounted using Electrodag high conductivity paint (Acheson Colloids Company) on a 1cm diam aluminium stub. Additionally perithecial contents were spread on the surface of stubs. The specimens were sputter-coated with a film of gold approximately 500 Å thick in an Emitech K550X coating unit. The coated specimens were then loaded into a FEI (Quanta 200) ESEM (Environmental Scanning Electron Microscopy, 2008) and examined over a range of magnifications at an accelerating voltage of 5kV. Images for all methods were obtained using an image capture system (Oxford Instruments, INCA system, Oxford, UK).

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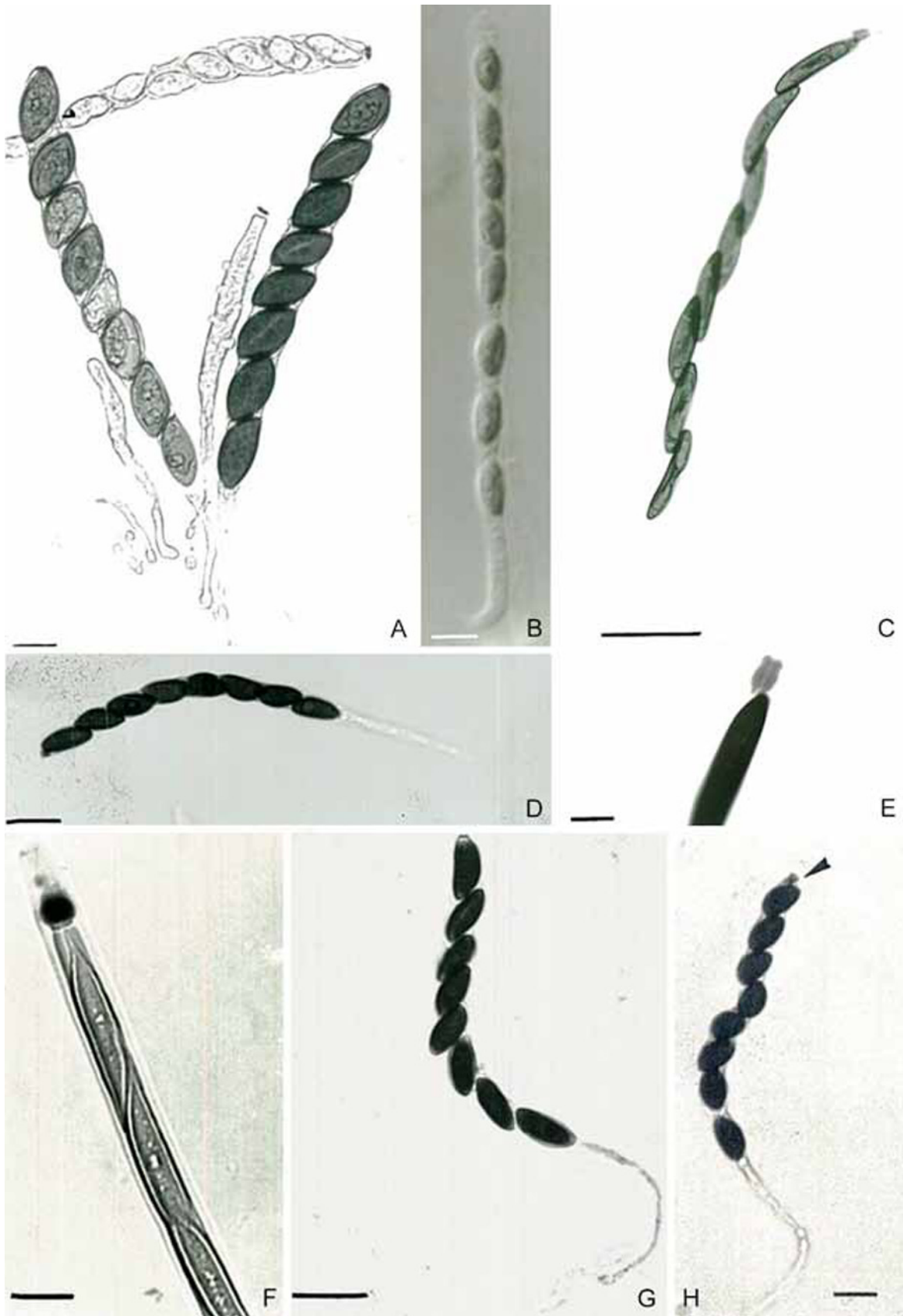
**Fig. 1.** Stromatal characteristics of some xylariaceous fungi. **A.** *Daldinia eschscholzii* (SUT 039) **B.** *Biscogniauxia capnodes* (SUT 212) **C.** *Hypoxylon monticulosum* (SUT 189). **D.** *Rhopalostroma lekae* (PK 148). *Kretzschmaria clavus* (PK 270). **F.** *Annulohypoxyylon bovei* var. *microspora* (SUT 025). **G.** *Rosellinia procera* (SUT113). **H.** *Astrocystis mirabilis* (SUT 051). **I.** *Xylaria* sp.(PK 017). **J.** *X. cubensis* (PK 108). **K.** *X. magnoliae* var. *microspora* (PH 072). **L.** *X. allantoidea* (PK 088). Bars A–B, I–L = 1 cm; C, F–H = 5 mm; D–E = 2 mm.

\* Collection abbreviations: AJSW = Liverpool John Moores University, UK; SUT = Suranaree University of Technology Nakhon Ratchasima, Thailand; ST Royal Forest Department, Bangkok, Thailand; SWU Srinakharinwirot University, Bangkok, Thailand – incorporating collections from national parks and forests of Thailand H (Khao Kra Yang Plantation, Phitsanulok Province), PK (Phu Kheo Wildlife Sanctuary, Chaiyaphum Province), and PH (Phu Hin Rong Kla National Park, Phitsanulok Province).

## RESULTS AND DISCUSSION

In most of the currently recognized genera of *Xylariaceae* the asci contain eight spores. Exceptions include *Wawelia*, with 4-spored asci (Minter & Webster 1983, Lundqvist 1992) and *Thuemenella* with 6-spored asci (Samuels & Rossman 1992). In general, the xylariaceous ascus is cylindrical and possesses a stipe. In *Biscogniauxia* the stipe is frequently short in relation to the spore-containing part of the ascus,





**Fig. 2.** Asci and different types of apical apparatus. **A.** *Hypoxylon fuscum* with disc-like apical apparatus stained in Melzer's reagent (AJSW 078\*). **B.** *Camillea selangorensis* ascus (IMI – isotype). **C.** *Kretzschmaria clavus* ascus with apical apparatus stained in Melzer's reagent (PK 270). **D.** *Nemania bipapillata* ascus with stipe (AJSW 693). **E.** *K. clavus* showing distinctive urniform apical apparatus stained dark blue in Melzer's reagent (PK 270). **F.** *C. fusiformis* with rhomboid apical apparatus stained in Melzer's reagent (MAW S21, IMI) **G.** *Hypoxylon lividicolor* ascus with long stipe (ST 1047 RFD). **H.** *Xylaria aristata* ascus with apical apparatus arrowed (ST 1411 RFD). Bars A–B, F–H = 10 µm; C–D = 25 µm; E = 5 µm.

whilst in *Xylaria* and *Kretzschmaria* the stipes are usually long. *Hypoxylon begae*, *H. haematostroma* and *H. polyporum* are notable within the genus for their very long stipes which appear to have diagnostic value (Ju & Rogers 1996). The apical tip of the ascus is usually rounded and encloses an apical apparatus which is mostly amyloid, staining blue in Melzer's iodine reagent. There are a number of taxa in which no apical apparatus can be seen by light microscopy although the possibility of some remnant structures cannot be excluded as such taxa have not yet been studied by transmission electron microscopy. The shape and size of the apical apparatus is one of the more important taxonomic features exhibited in *Xylariaceae* (Fig. 2). The general appearance of the apical apparatus has been successfully applied in taxonomic studies of the family (e.g. Munk 1957, Carroll 1963, 1964, Martin 1967, 1968a, b, 1969a, b, Krug & Cain 1974a, b, Francis 1975, Rogers 1979, Læssøe *et al.* 1989, van der Gucht 1995, Ju & Rogers 1996, Whalley 1996). Unfortunately, a number of important taxonomic studies in the family have not considered this feature. On the basis of shape and size, at least five types of amyloid apical apparatus can be recognized plus a category in which there is no visible apparatus:

- 1) Stacks of small rings, as in *Hypocopra* and *Poronia* (Krug & Cain 1974b, Jong & Rogers 1969).
- 2) Discoid or triangular, as in most species of *Hypoxylon s. str.* and *Daldinia* (Ju & Rogers 1996, Ju *et al.* 1997).
- 3) Broad band to discoid, as in *Biscogniauxia* (Ju *et al.* 1998).
- 4) Rhomboid to diamond-shaped in *Camillea* (Læssøe *et al.* 1989).
- 5) Inverted hat or urniform, as in *Xylaria*, *Rosellinia*, *Kretzschmaria* and *Nemania* (Petrini & Muller 1986, Whalley 1996, Rogers 2000).
- 6) No visible apical apparatus under the light microscope as in *Rhopalostroma* and most species of *Ascotricha* (Whalley & Thienhirun 1996, Hawksworth 1971)

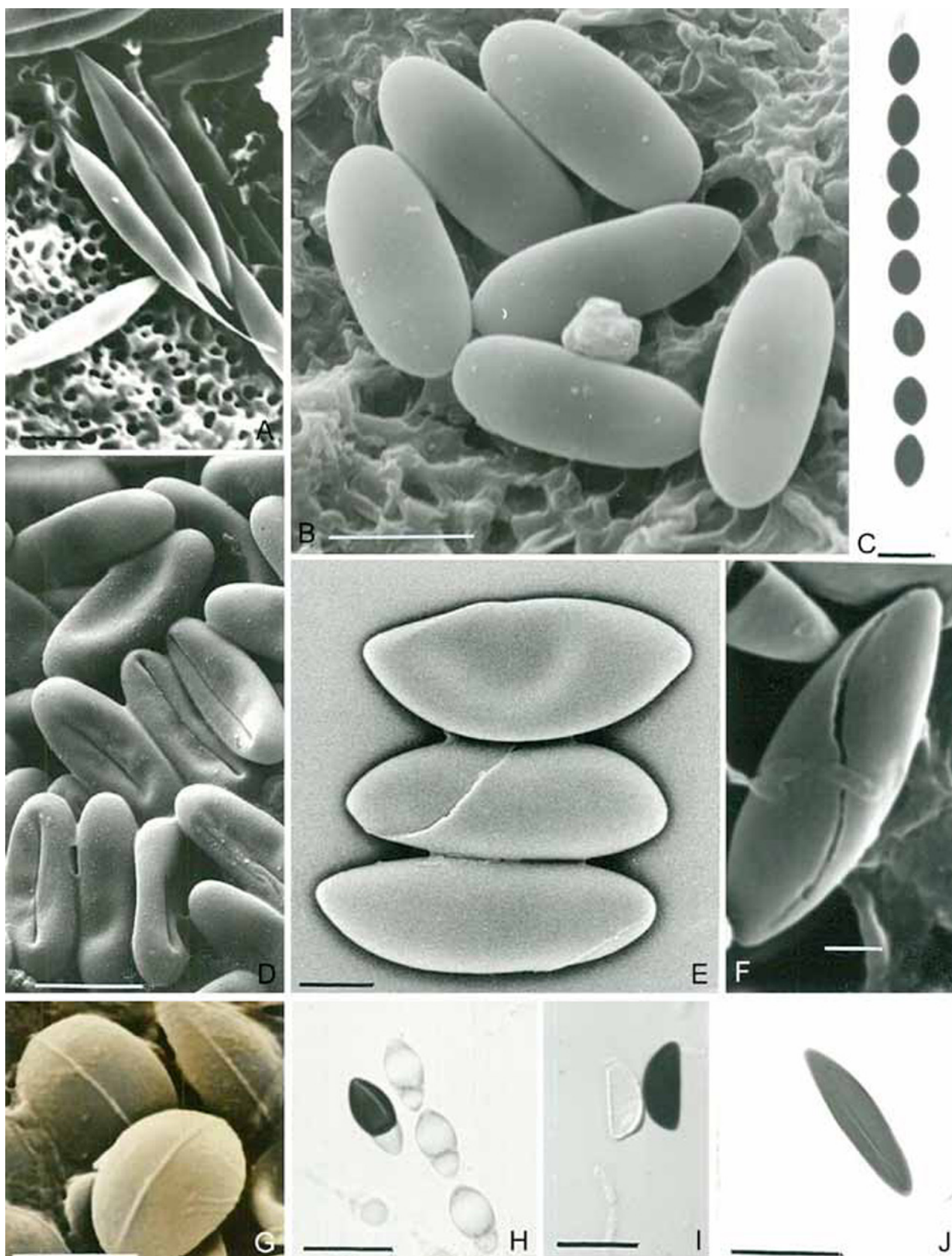
In most species the apical apparatus stains blue, usually dark blue, or occasionally reddish brown (dextrinoid) in Melzer's iodine reagent. The significance of the iodine reaction in the apical apparatus, including *Xylariaceae* has been discussed by Eriksson (1966), Kohn & Korf (1975), and Nannfeldt (1976). It has been shown that pre-treatment with potassium hydroxide (KOH) can induce a positive reaction in a previously iodine negative species (Nannfeldt 1976). Baral (1987) has questioned the effectiveness of Melzer's reagent demonstrating that Lugol's solution is superior in the detection of amyloidity in ascomycetes. Species of *Xylariaceae* can, however, be grouped according to the response of their apical apparatus to Melzer's reagent as:

- 7) Apical apparatus consistently iodine positive (blue).
- 8) Apical apparatus varying in its reaction to iodine, i.e. some collections give a positive amyloid reaction whilst other collections of the same species do not, as in *Hypoxylon cohaerens* and *Nemania serpens* (Pouzar 1985a, b, Petrini & Rogers 1986).
- 9) Apical apparatus consistently iodine-negative, as in *Hypoxylon intermedium* and *H. cercidicola* (Pouzar 1972, Ju & Rogers 1996).

The iodine positive nature of the apical apparatus is considered, however, to be a cardinal character of the *Xylariaceae* in spite of the presence of certain iodine negative taxa in what are undoubtedly taxa of the *Xylariaceae* (Rogers 1979, 1994, 2000).

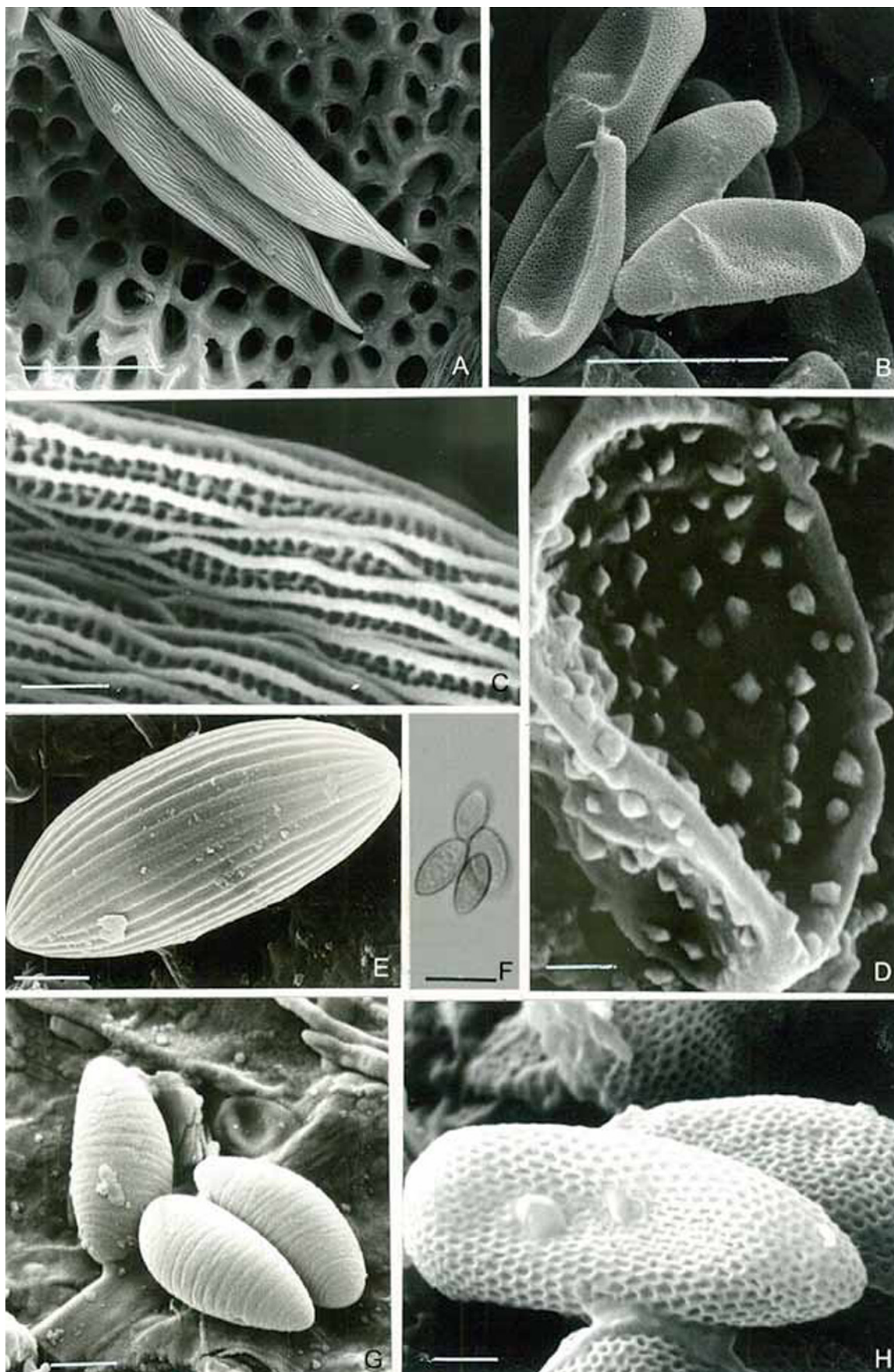
The structure of the apical apparatus appears to be relatively simple when studied by transmission electron microscopy (Greenhalgh & Evans 1967, Beckett & Crawford 1973, Griffiths 1973). Chadeaud (1942, 1973) proposed a much more complex structure on the basis of light microscopy, but many of his studies were carried out on old material with degenerating asci which might also be the case here. Regardless of structure or reaction to iodine, the function of the apical apparatus is not clear. Greenhalgh & Evans (1967) and Beckett & Crawford (1973) considered the apical apparatus to act as a sphincter through which the ascospores pass. Martin (1967a), however, was of the opinion that the ascospores bypass the apical apparatus during discharge and that the function of the apical apparatus was therefore unclear. Rogers (1979) suggested that the apical apparatus served as a strengthening device in the ascus and that it becomes everted, pushed to one side, or blown off by the ascospores once sufficient pressure has developed in the ascus. Certainly, the dimensions and shapes of many ascospores are not suited for passage through the central channel in the apical apparatus and the suggestion of Rogers (1979) is currently the most plausible. In a study of Barron's strain of *Nemania serpens* which unusually produces mature stromata in culture, Kenerley & Rogers (1976) demonstrated that the ascospores were passively discharged under wet conditions, but forcibly discharged under dry conditions.

The ascospores of most xylariaceous fungi are described as more or less bean-shaped (phaseoliform), single-celled, smooth walled, light to dark brown, and with a conspicuous germ slit usually running the full length of the spore (Rogers 1979). In reality, there is considerable variation on this basic theme (Fig. 2). In most species the ascospores are uniseriate in their arrangement in the ascus, but variation occurs in relation to their shape. The basic shape is ellipsoid, but this can become subglobose, oblong, fusiform, inequilaterally ellipsoid, navicular or broadly crescent-shaped. The ends can be narrowly or broadly rounded, attenuated, or apiculate. In *Biscogniauxia* species, which possess appendages, the loss of an appendage results in a truncate end (Whalley *et al.* 1990). In *Hypoxylon s. str.* and *Daldinia* the spores are usually inequilaterally ellipsoid, in *Biscogniauxia* they are more frequently subglobose, in *Xylaria* they are often broadly crescent-shaped, and in *Rosellinia* many are characterized by long attenuated ends (Petrini 1992). Most xylariaceous spores are brown, but range from light to medium or dark brown, sometimes appearing almost black. In *Camillea*, however, the spores are pale yellow or almost colourless, and almost all of them lack germ slits or pores, except for *C. labiatrix* which have a distinct slit (Rogers *et al.* 2002), and are ornamented. Their very pale colour, lack of a germ slit and presence of spore wall ornamentation, as observed by scanning electron microscopy, drew attention to the incorrect placement of many applanate species in *Hypoxylon*, which were subsequently transferred to *Camillea* (Rogers 1977, Læssøe *et al.* 1989). Thus, the genus *Camillea* is partially



**Fig. 3.** Variation in ascospore shape and germ slits. **A.** *Rosellinia bunodes* with elongated ascospore ends (AJSW 937). **B.** *Entoleuca mammata* with broadly rounded ascospores (AJSW 803). **C.** *Biscogniauxia nummularia* with broadly rounded ascospores. (AJSW 236). **D.** *Hypoxylon comedens* with straight germ slits 2/3 length of the spore (ST 1142 RFD). **E.** *Xylaria longipes* with spiral germ slit. (AJSW 576). **F.** *H. monticulosum* with spiral germ slits (SUT 189). **G.** *Rhopalostroma kanyae* with germ slit on the dorsal side of the ascospore (IMI 368200 – isotype). **H.** *Biscogniauxia anceps* showing bicelled spores and germ slit (AJSW 1009) **I.** *H. fuscum* showing dehiscent perispore following treatment with 10 % KOH (AJSW 078). **J.** *Kretzschmaria clavus* with straight germ slit almost full length of the ascospore (PK 270). A–B, D–G scanning electron micrographs. C, H–J bright field light microscopy. Bars A–B, D, G–H = 10 µm; C, E = 2 µm; F = 1 µm; I–J = 15 µm.





**Fig. 4.** Ascospore ornamentation. **A.** *Camillea fusiformis* longitudinal reticulate. (MAW S21, IMI). **B.** *C. tinctor* poroid (SUT 260). **C.** *C. fusiformis* details of reticulate ornamentation (MAW S21, IMI). **D.** *C. selangorensis* verrucose (IMI). **E.** *Nemaniam chestersii* longitudinal ribbed (AJSW433). **F.** *C. selangorensis* faint ornamentation by light microscopy (IMI). **G.** *Daldinia eschscholzii* transverse (SUT 039). **H.** *C. cyclops* poroid (MAW S18) A–E, G–H scanning electron micrographs. F, bright field light microscopy. Bars A–B, F = 10  $\mu$ m; C–D, G–H = 1  $\mu$ m; E = 2  $\mu$ m.

circumscribed on the basis of ascospore wall ornamentation which may be poroid, reticulate, or ribbed (*Camillea* subgen. *Camillea*), or echinulate to verrucose (*Camillea* subgen. *Jongiella*) (Læssøe *et al.* 1989, Rogers *et al.* 1991, Whalley 1995, 1996, Whalley *et al.* 1996, 1999).

Most xylariaceous ascospores are smooth walled, but ornamentation occurs spasmodically throughout the family (Figs 3–4). Thus, *Stromatoneurospora* possesses striate ascospores (Jong & Davis 1973), and some species of *Hypoxylon* s. str. have ascospores with faint transverse striations perpendicular to the long axis of the spore (Rogers & Candoussau 1982, Rogers 1985, van der Gucht & van der Veken 1992, Ju & Rogers 1996). Van der Gucht (1993) and Stadler *et al.* (2002) emphasized the significance of transverse striations of the ascospores in certain species of *Daldinia*. A single species of *Biscogniauxia*, *B. reticulospora*, exhibits reticulately ornamented ascospores (Ju *et al.* 1998), and the genera *Helicogermis* and *Spirodecospora* were erected mainly on the presence of a spiral ornamentation on the ascospores (Hawksworth & Lodha 1983, Lu *et al.* 1998). In their revision of *Hypoxylon*, Ju & Rogers (1996) placed considerable importance on ascospore ornamentation, noting that it can be found on the perispore, epispore, and/or beneath the epispore. Perispore ornamentation is evident in those taxa where perispores dehisce in 10 % potassium hydroxide. The ornamentation falls into two major patterns, which Ju & Rogers (1996) used as one of the three major characters to delimit the two sections of *Hypoxylon*. Transversely orientated, coil-like ornamentation can be found in sect. *Hypoxylon*, whereas a thickening of the perispore situated towards one end is almost universal in sect. *Annulata* (Ju & Roger 1996). It was also recognized that the conspicuousness of the coil-like ornamentation in sect. *Hypoxylon* is an important character at species level. This feature is useful in the separation of closely related taxa such as *H. anthochroum*, *H. duranii*, *H. fendleri*, and *H. retpela* (Ju & Rogers 1996). Epispore ornamentation appears to be rare in *Hypoxylon*, but shallow pits can be found in *H. rubellum* (Rogers *et al.* 1987), striations in *H. californicum* (Ju & Rogers 1996), and pleated folds in *H. rectangulosporum* (Rogers *et al.* 1992) and *H. thouarsianum* (Miller 1961). Transverse striations are also apparent in some *Daldinia* species (van der Gucht 1993, Stadler *et al.* 2002). Stadler *et al.* (2002) examined representative specimens of *Daldinia* species with the SEM and found that ornamentation of their outer spore layers were species-consistent. They reported them as having either smooth or transversely striated ascospores, with the striated spores always ellipsoid-equilateral to ellipsoid-inequilateral with narrowly rounded ends. Smooth ascospores were broadly ellipsoid to cylindrical. *Daldinia concentrica* was found to have very faint ornamentation, but this was only visible at  $\times 1000$  in an SEM. Ju *et al.* (1997) had previously found that ascospores of some species of *Daldinia* undergo perispore dehiscence in 10 % potassium hydroxide and have ornamentation similar to that exhibited by members of *Hypoxylon* sect. *Hypoxylon*. In *H. fragiforme* a shedding or eclosion, likened to the hatching of insect pupae, of the perispore in response to specific chemical stimuli has been interpreted as part of an intricate fungus-host recognition system (Chapela *et al.* 1990, 1991). Whether

this phenomenon occurs in other *Hypoxylon* species or indeed in other xylariaceous taxa has not been tested. In the coprophilous genera *Poronia*, *Podosordaria*, and *Hypocopra*, the ascospores are usually surrounded by thick gelatinous sheaths which are assumed to facilitate the spores adhering to plant materials, mainly leaf lamina (Rogers 1979).

Details of the asci and ascospores, in conjunction with features of any asexual stages (Ju & Rogers 1996), have proved to be valuable in making identifications, and they also provide insights into species groups and generic separations. However, knowledge on the distribution and patterns of extrolite chemicals in *Xylariaceae* and application of DNA technology has been pivotal in resolving boundary issues (Whalley & Edwards 1995, Stadler & Hellwig 2005, Triebel *et al.* 2005).

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