Int. J. Plant Sci. 165(6):957–964. 2004.

1058-5893/2004/16506-0004$15.00

THE PERIDIAL DEVELOPMENT AND DEHISCENCE MECHANISM OF *CRYPTENDOXYLA HYPOPHLOIA*, A CLEISTOTHECIAL ASCOMYCETE ISOLATED FROM THE BODIES OF ARTHROPODS

M. D. Greif,[1] A. Tsuneda, and R. S. Currah

Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada

Cryptendoxyla hypophloia is a rarely reported cleistothecial ascomycete that has a cephalothecoid peridium comprising six to eight plates that split apart at maturity to expose the ascospores. Three new isolates from the bodies of live-trapped insects represent circumstantial evidence supporting an older hypothesis that this fungus is dispersed by arthropods and provided fresh material in which to examine the development of the ascocarp and its unusual active dehiscence mechanism. Vegetative hyphae envelop coiled gametangia to form the earliest stages of the development of the peridium and then accommodate the increasing volume of the expanding centrum tissues by branching and tip growth. Peridial plates are defined early in ascocarp ontogeny and at maturity consist of thick-walled, radially arranged hyphae that have transverse wall thickenings. Cells between plates and underlying dehiscence lines are thin walled. On drying, the radially arranged hyphae of the outer peridial layer contract, rupturing the thin-walled cells around the periphery of each plate along previously formed dehiscence lines. As plates flatten and evert, the ascocarp opens to expose the ascospores, ostensibly for pickup by arthropod carriers. Meristematic growth could not be confirmed in the developing ascocarps of *C. hypophloia* even though it has been implicated in the growth of the peridium in other cephalothecoid taxa.

Keywords: fungal dispersal, convergent evolution, ascocarp dehiscence, cephalothecoid, Sordariales, Pseudeurotiaceae.

Introduction

A variety of ascocarp forms have resulted from the evolutionary optimization of the mechanisms required to disperse the ascospores. Disk-shaped apothecia permit the unhindered, forcible ejection of ascospores from an exposed hymenial surface so they can be carried on air currents. Flask-shaped perithecia permit a similar ejection of ascospores or may extrude spores as a slimy mass that may adhere to an animal carrier. The ascocarps of many ascomycetes are cleistothecial and have a solid peridium that completely encloses their ascogenous cells and their products and often lack the ability to eject or extrude ascospores. Consequently, ascospore release and dispersal in cleistothecial species must rely on alternative mechanisms.

Recently, we obtained from the bodies of arthropods three different isolates of *Cryptendoxyla hypophloia* Malloch and Cain, a cleistothecial fungus originally placed in the Pseudeurotiaceae (Malloch and Cain 1970) but having molecular similarities to the Sordariales (Suh and Blackwell 1999). In *C. hypophloia*, ascospores are released from the ascus by dissolution of the ascus wall and accumulate within a peridium consisting of six to eight disarticulating plates. This distinctive peridial type, described first by von Höhnel (1917) in *Cephalotheca sulfurea* and later referred to as "cephalothecoid" by Malloch and Cain (1970), is particularly fragile at maturity, and shatter or breakage of the peridium by external agents is considered to be the primary means by which ascospores are released from these cleistothecial ascocarps (Chesters 1934; Parguey-Leduc 1970; Hawksworth and Booth 1974; Uecker 1977; Benny et al. 1980). The ascocarps of *C. hypophloia* may shatter in some instances, but usually they exhibit an active dehiscence mechanism that allows the ascocarp to open, flower-like, when ascospores are mature.

The cephalothecoid peridium presumably represents innovations adapted for specific environments because this peridial type has evolved in distantly related lineages including the Sordariales, Ophiostomatales, and Dothideales (Uecker 1977; Suh and Blackwell 1999). This widespread occurrence and reports of both active and passive (i.e., shattering) dehiscence in cephalothecoid ascomycetes indicate that a peridial wall composed of disarticulating plates represents a convergence of different ontogenetic sequences and anatomical features. Unfortunately, reports of the peridial development or ultrastructural mechanisms associated with dehiscence are rare and too few to make meaningful comparisons. Of three studies dealing with the development of cephalothecoid peridia (Chesters 1934; Uecker 1977; Benny et al. 1980), only one was done at an ultrastructural level, and it focused on a species (*Chaetomidium arxii*) in which dehiscence is passive (Benny et al. 1980). The structural components underlying active dehiscence have not been investigated, and, consequently, the development and structure of the cleistothecium and its retracting plates in *C. hypophloia* are unknown. This information is fundamental to teasing out the selective pressures that led to the evolution of the cephalothecoid peridium.

[1] Author for correspondence; e-mail mgreif@ualberta.ca.

Manuscript received February 2004; revised manuscript received April 2004.

Using light microscopy (LM) and scanning (SEM) and transmission electron microscopy (TEM) and *C. hypophloia* grown on agar-based medium, we describe the development and the structural basis of the active dehiscence mechanism of its cleistothecial ascocarp and describe how the cephalothecoid peridium in this species might function in the release and dissemination of ascospores in nature.

Material and Methods

Three isolates of *Cryptendoxyla hypophloia* were obtained from insects trapped in a southern boreal mixed wood forest 70 km east of Edmonton, Alberta, and deposited in the University of Alberta Microfungus Collection and Herbarium (UAMH). UAMH 10412, from *Mycetophagus californicus* (order Coleoptera, family Mycetophagidae), was studied in detail using a combination of LM, SEM, and TEM. UAMH 10413 and 10414, from a European ground beetle (order Coleoptera, family Carabidae) and a springtail (order Collembola), respectively, were examined by LM only. Isolates were cultured on cornmeal agar (CMA, Becton Dickinson Microbiology Systems) and on sterile moist filter paper.

For SEM, 5-mm disks of agar bearing immature and mature cleistothecia of *C. hypophloia* were fixed in unbuffered 2% glutaraldehyde for 4 h, placed in 2% tannic acid–2% guanidine hydrochloride overnight at 5°C, and postfixed in 2% OsO_4 for 3 h at room temperature. Fixed material was dehydrated in an ethanol series, taken to amyl acetate, critical-point dried in a Polaron E-3000 dryer using carbon dioxide, and then coated with gold and examined at 15 kV with a Hitachi S-510 electron microscope. For TEM, specimens were fixed in 2% glutaraldehyde and 2% OsO_4 in phosphate buffer at pH 7.3 and dehydrated in an ethanol series. Samples were then embedded in Spurr's resin. Ultrathin sections were stained with uranyl acetate and lead citrate. Photomicrographs of these samples were taken at 75 kV with a Hitachi H-7000 electron microscope.

Results

Development of the Cleistothecium

All stages in cleistothecium development could be located in cultures by 4 wk after inoculation. Cleistothecia were minute and white to pale brown when immature and then black, globose, and 70–115 μm in diameter (fig. 1*A*) when mature and ready to dehisce. At this stage, the upper portion of the peridium would split open when the lid of the petri dish was removed or when cultures were allowed to dry out (fig. 1*B*).

Cleistothecium development was initiated by the formation of short lateral branches (gametangia) that grew toward each other from adjacent vegetative hyphae (fig. 1C). On contact, one branch enlarged and encircled the other to form an ascogonium (fig. 1*D*, 1*E*). Six to eight branches arising from nearby vegetative hyphae then grew toward the ascogonium (fig. 1*F*). Each of these hyphae, once appressed to the ascogonium, branched repeatedly to form a cluster of loosely arranged cells. Hyphal branches originating within each cluster grew out at acute angles from parent hyphae and formed radiating parallel bands, giving rise to the peridial plate (fig. 1*G*). Paraphyses were absent, although within the centrum of the immature cleistothecium, larger sterile cells separated the more densely cytoplasmic and smaller ascogenous cells (fig. 2*A*). Sterile, thin-walled cells also formed a layer against the inner surface of the peridium (fig. 2*A*, 2*B*). At maturity, both the interascal cells and the cells lining the inner surface of the peridium had collapsed, leaving a cavity in the first instance and an amorphous residue in the second (fig. 2*C*). The outer peridium was composed of multiple layers of thick-walled hyphae (fig. 2*B*, 2*C*).

In squash mounts, immature cleistothecia contained clusters of young, club-shaped asci that had arisen from croziers and that were at different stages of development (fig. 2*D*, 2*E*). Under TEM, these clusters were irregularly disposed among the sterile cells of the centrum (fig. 2*A*). By TEM, immature ascospores were delimited by a double membrane system (fig. 2*F*) that later enclosed an inner electron-translucent and an outer electron-opaque layer of wall material (fig. 3*A*, 3*B*). At maturity, asci had deliquesced and ascospores adhered as a central mass within the cleistothecial cavity (fig. 2*C*).

Peridial plates were composed of parallel arrangements of thick-walled pigmented hyphae. Each plate developed radially from a central cluster of cells through the formation of acute lateral branches and tip growth (figs. 1*G*, 3*C*). Elongating hyphal tips from opposing fronts met and grew between each other so that the hyphae of adjacent plates were interdigitated (fig. 3*C*). By TEM, in long section, hyphae of the peridial plates were vacuolated at maturity and along the inner wall surface had pronounced thickenings that formed a banding pattern that was perpendicular to the direction of growth (fig. 3*C*, 3*D*). In immature cleistothecia, a mucilaginous material adhered to the outer surface of the peridium (figs. 1*G*, 3*E*).

Rows of thin-walled cells, equidistant from the points of origin of individual plates and perpendicular to the orientation of growth of peridial hyphae, were visible from very early stages of cleistothecium development (figs. 1*G*, 3*F*). By SEM each of these cells had a transverse groove in the cell wall (fig. 3*F*; fig. 4*A*, 4*B*) that aligned with those of neighboring cells to form the dehiscence lines that delineated incipient peridial plates (fig. 4*B*). By TEM, peridial cross sections showed that the hyphae underlying these lines lacked the pronounced wall thickenings described above (fig. 4C) and were present in only one or two layers (figs. 2*C*, 4*D*). The outer mucilaginous layer was not present when cleistothecia were mature (fig. 4*E*, 4*F*). Close to maturity, the peridium comprised six to eight well-defined polygonal plates (fig. 4*E*, 4*F*), each three to four cells thick (fig. 4*F*). The arrangement of plates and dehiscence lines gave the surface of the peidium an appearance resembling a soccer ball (fig. 4*B*, 4*E*, 4*F*).

Dehiscence of the Cleistothecium

At room temperature and under the lamp of the dissecting scope, the uppermost peridial plates of mature cleistothecia everted in 3–5 min, exposing the ascospores. Although eversion rates differed, a similar change in the shape of these plates occurred when dishes were left unsealed and placed in the dark at 4°C and 37°C. Regardless of temperature and illumination, placing a drop of sterile water on opened

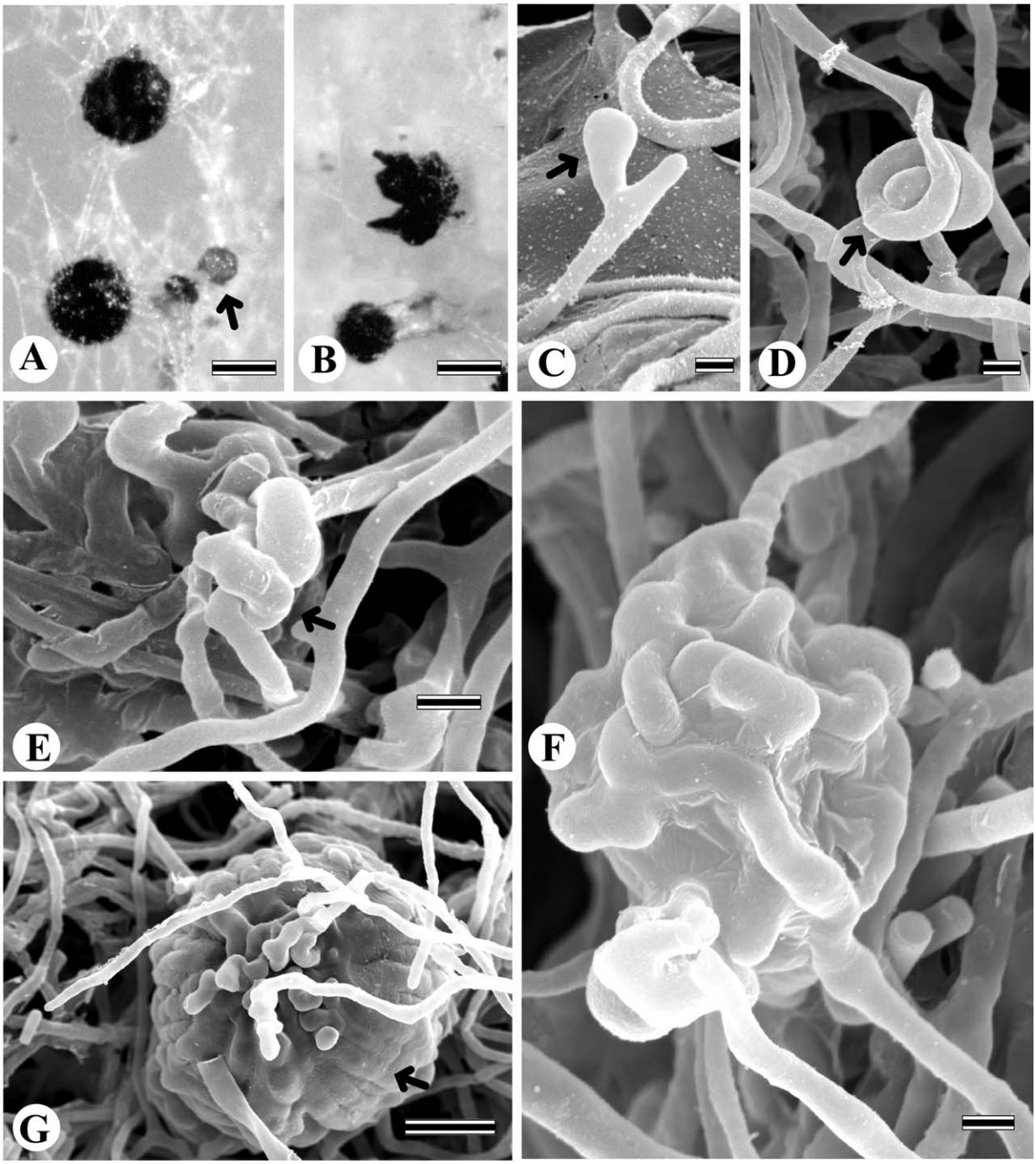

Fig. 1 Stages in the cleistothecial development of *Cryptendoxyla hypophloia*. *A*, Culture on filter paper showing mature black globose cleistothecia along with smaller pale immature cleistothecia (arrow). Bar = 77 μm. *B*, A cleistothecium that has opened. Bar = 77 μm. *C*, Gametangium initiated as a short side branch (arrow) from vegetative hypha. Bar = 2 μm. *D*, Gametangial branches (arrow) encircling each other upon contact. Bar = 2 μm. *E*, Ascogonial coil (arrow) formed from gametangia. Bar = 2 μm. *F*, Ascogonium enveloped by peridial initials that have originated from neighboring vegetative hyphae. Bar = 2 μm. *G*, Immature cleistothecium displaying cluster of loosely arranged cells. Hyphal branches originate from this cluster to form the peridium. Arrow indicates a dehiscence line. Bar = 10 μm.

cleistothecia caused the peridial plates to revert to their former shape, in 5–10 s in some cases. Subsequent cycles of drying and wetting caused a repeat of this eversion-inversion pattern. The first indication of plate eversion was a change in the shape of the cleistothecium from globose to polygonal as individual concave plates began to flatten. Soon afterward, fissures following the dehiscence lines opened across the thin-walled hyphal cells (fig. 1*B*; fig. 4*C*, 4*E*, 4*F*).

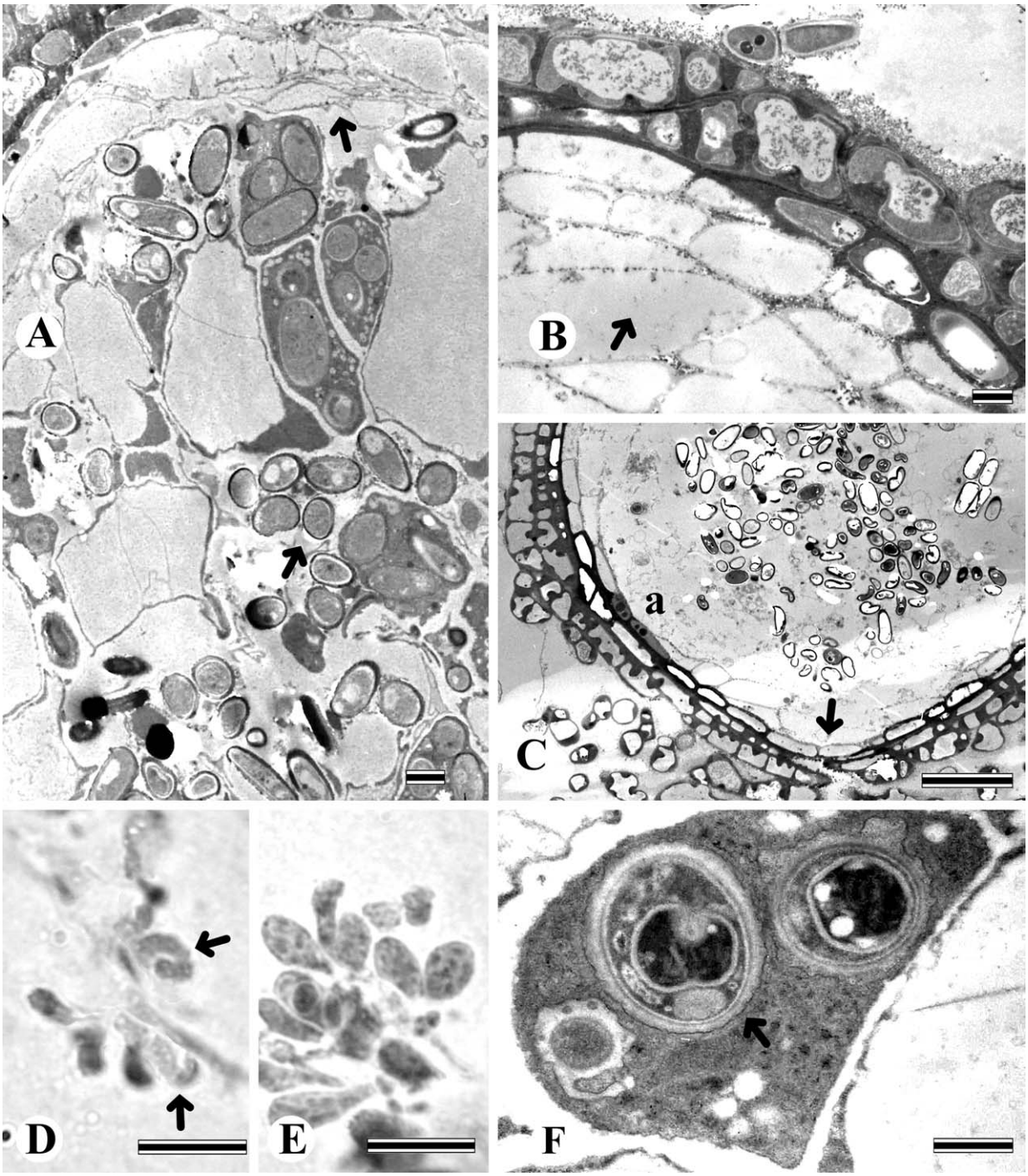

Fig. 2 Development of centrum and ascogenous tissue. *A*, Interior of cleistothecium displaying large sterile cells separating more densely cytoplasmic and smaller ascogenous cells. Sterile cells form a layer against the inner surface of the peridium (arrow). Ascospores are interspersed throughout the central cavity (arrow). Bar = 2 μm. *B*, Close-up view of peridial wall. Thin-walled cells (arrow) form a layer against thick-walled outer peridial layer. Bar = 2 μm. *C*, Cross section of mature cleistothecium. The large sterile cells and thin-walled inner peridial layer have collapsed, leaving behind an amorphous residue (*a*). Ascospores form a mass in the center of cleistothecial cavity. Arrow indicates position of dehiscence line in section. Bar = 10 μm. *D*, Formation of asci. Asci arise from croziers (arrows). Bar = 10 μm. *E*, Cluster of asci at different stages of development. Bar = 10 μm. *F*, Interior of ascus showing immature ascospores delineated by a double membrane (arrow). Bar = 1 μm.

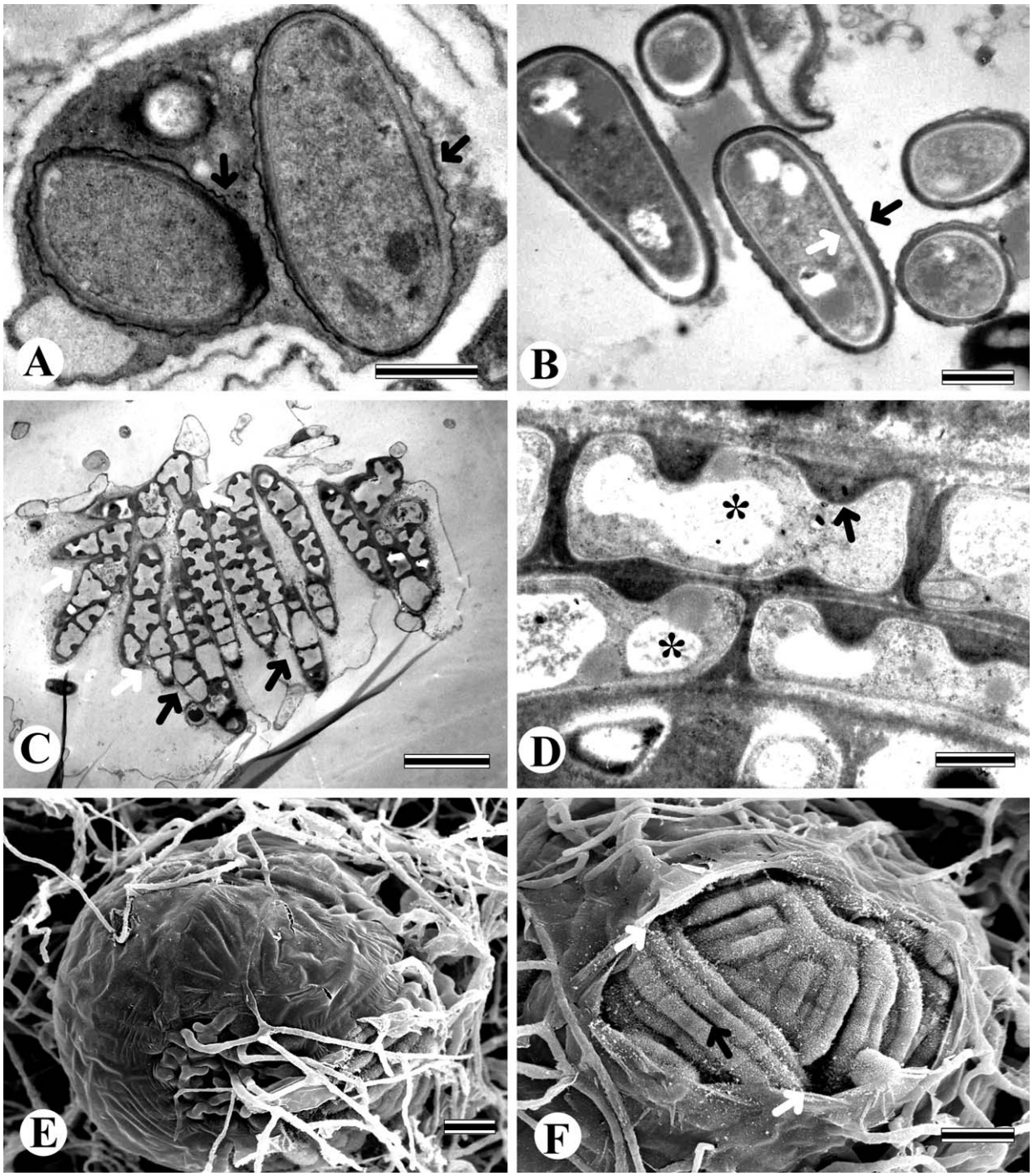

Fig. 3 Formation of ascospores and peridial plates. *A*, Ascospores delineated by an outer membrane (arrows). Bar = 1 μm. *B*, Liberated ascospores showing the mature ascospore wall composed of an electron-light inner layer (white arrow) and an electron-dense outer layer (black arrow). The outer layer is osmiophilic, indicating the presence of lipids. Bar = 1 μm. *C*, Long section through a developing peridial plate. The plate increases in size by tip growth and branching (white arrows). Interdigital growth (black arrows) is visible between opposing plates. Bar = 10 μm. *D*, Hyphae of the peridial plates are vacuolated at maturity (asterisks). Pronounced thickenings form a banding pattern visible along the inner wall surface (arrow). Bar = 1 μm. *E*, Immature cleistothecium bearing mucilaginous layer. Bar = 10 μm. *F*, Dehiscence line (black arrow) equidistant from the points of origin of individual plates (white arrows) in immature cleistothecium visible through ruptured mucilagenous layer. Bar = 10 μm.

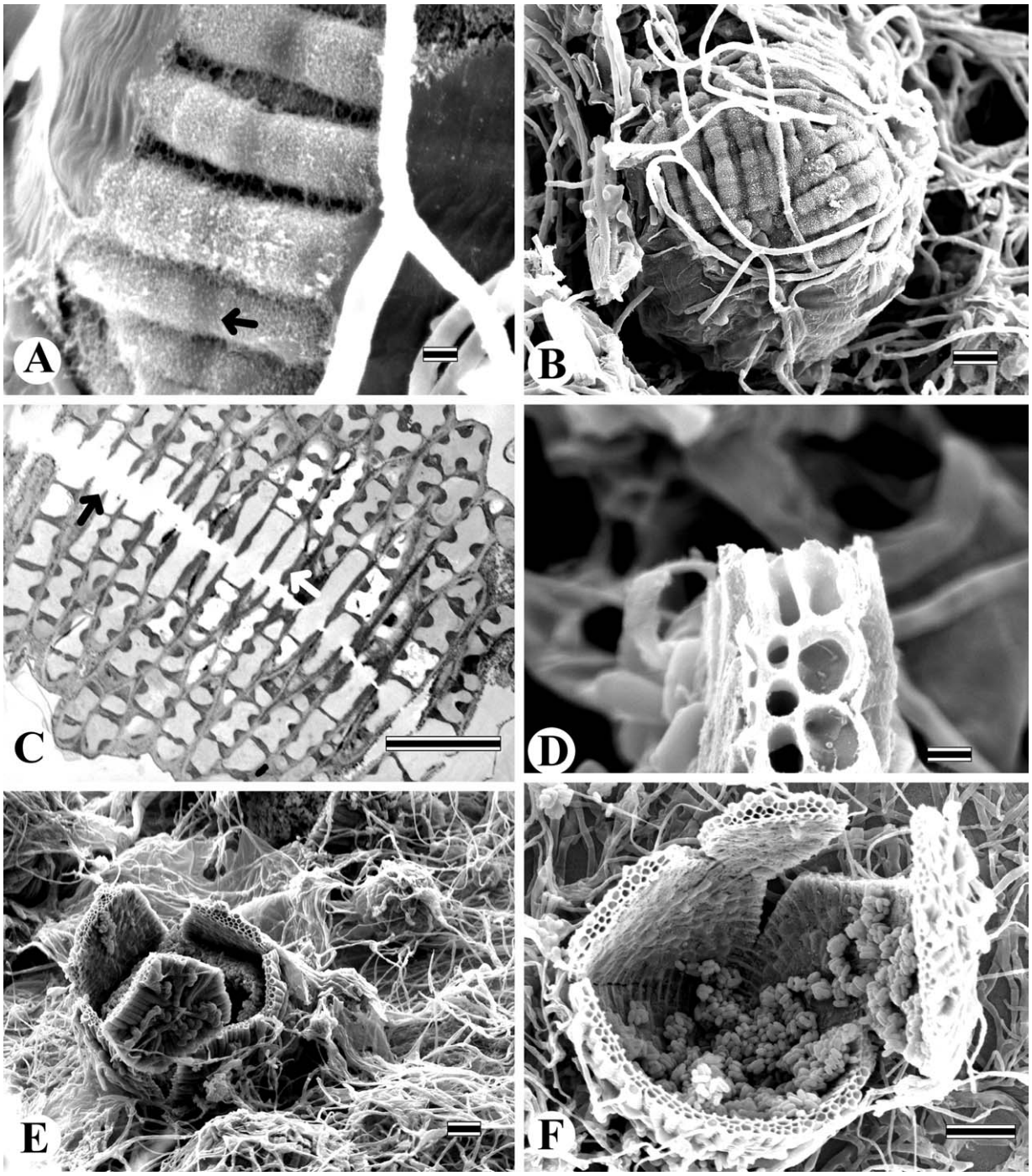

Fig. 4 Opening of cleistothecium. *A*, Close-up view of dehiscence line (arrow). Dehiscence line is visible as a transverse groove perpendicular to the direction of growth of the peridial cells. Bar = 2 μm. *B*, Dehiscence lines delineating peridial plates. Bar = 10 μm. *C*, TEM of dehiscence line. Cells are thin walled (white arrow), and rupturing occurs across midregion of dehiscence cells (black arrow). Bar = 10 μm. *D*, End view of peridial plate after dehiscence. Dehiscence lines are only one to two cell layers thick. Bar = 2 μm. *E*, Opened cleistothecium. The peridium is composed of six to eight well-defined plates, each three to four cells thick. Desiccation has caused the peridial plates to flatten and evert, rupturing the cleistothecium along the dehiscence lines. The outer mucilaginous layer is no longer present. Bar = 20 μm. *F*, Opened cleistothecium with a mass of ascospores at the bottom. Bar = 20 μm.

Eversion of the uppermost peridial plates exposed a conglobate and tightly adhering mass of darkly pigmented, unicellular ascospores in the lower portion of the peridium. In a few instances, plate eversion was extreme and caused the ascospore mass to roll out of the opened cleistothecium. Mature ascospores remained in a tight cluster when placed in a drop of water but dispersed readily in immersion oil.

Discussion

In the original taxonomic description of *Cryptendoxyla hypophloia*, Malloch and Cain (1970), using only LM and direct mounts, observed the development of the cleistothecia in cultured material from "simple coils," through the early stages of cleistothecium formation, and finally to the mature "subglobose to globose, black . . . nonostiolate ascocarps" that would "split open along the dehiscence lines and spread apart . . . under the drying effect of the microscope lamp" (p. 1818). Most of their preliminary observations concerning development are compatible with ours, although SEM and TEM provided us with an opportunity to make more detailed interpretations of the relationship between structure and function.

Features associated with centrum development agree closely with those reported by Malloch and Cain (1970), who also observed that asci arose from croziers. They did not report that the pattern of ascus maturation was asynchronous nor did they comment on the hydrophobic nature of the ascospore wall. During development, TEM shows that an osmiophilic substance is present in the outer layer of the ascospore wall, suggesting the presence of lipids, and is an observation compatible with the behavior of the ascospores when placed in water and in immersion oil. The hydrophobic nature of the ascospore wall may have a role in dispersal (see below).

Malloch and Cain (1970) were intrigued by the developmental sequence leading to the formation of the cephalothecoid peridium but were not able to confirm the origin of the "platelike complexes" comprising this structure. They did identify the bilayered nature of the peridial wall, noting the presence of a darkly pigmented outer layer of thick-walled, pigmented, "nearly square" cells and an inner layer of hyaline thin-walled cells. SEM observations indicate that the peridial tissues originate from vegetative hyphae that arise near conjoined gametangia and grow toward and become appressed to the ascogonial coil. The cells of the peridial hyphae along with the "nodular growths of wall material around the periphery of the septa and other points" (Malloch and Cain 1970, p. 1818) are, in part, a misinterpretation of the perpendicular banding pattern in the hyphae of the peridial plates. The transverse wall thickenings, in face view, do resemble septa and cause these hyphae to resemble files of square cells. The functional significance of the nodular growths and the squared cells in the outer layers of the peridial plates was not mentioned in the original description but is related to the peculiar active dehiscence mechanism in this fungus that is explained below.

On drying, peridial plates of the more or less globose cleistothecia lose their curvature, presumably because the radial arrangements of banded hyphal elements of the outer peridial layer contract as surface tension increases inside the cells. As the plates flatten, stress exerted on the thin-walled cells around the periphery of each plate causes these cells to tear, and a fissure develops along the dehiscence line. Similar dehiscence mechanisms occur in the sporangia of some bryophytes, leptosporangiate ferns (Raven et al. 1992), and the anthers of some angiosperms (Esau 1977). In these examples, localized bands of wall material in otherwise thin-walled cells allow columns of such cells to contract and, while doing so, pull apart the sporangial wall.

As the outer layers of thick-walled cells in the peridial plates continue to contract, the plates evert even further and expose the mass of ascospores within. Cells along the dehiscence lines that do not rupture act as hinges between plates, allowing the cleistothecium to open and close when moisture is removed or added and preventing the cleistothecium from shattering and collapsing in on itself. The mucilaginous sheath found on the immature cleistothecia and the hyaline, thin-walled layer of cells lining the interior of the peridium may serve to prevent premature drying and eversion of the peridial plates of *C. hypophloia*.

Among the cephalothecoid ascomycetes, some aspects of the origin and development of peridial plates in *C. hypophloia* may be unique. In this species, the number of peridial plates per cleistothecium is established early in development, and additional plates do not form as cleistothecia mature. Continued branching and tip growth increase the size of each plate in a more or less radial fashion and account for the characteristic pattern of interdigital growth of new hyphae along the expanding margins of peridial plates (figs. 3*C*, 4*C*). Tip growth permits the ascocarp to swell and ensures that the thin-walled cells destined to tear apart during dehiscence, and that develop early in ascocarp ontogeny, remain aligned as the peridium increases in surface area. In contrast, most previous observations of the development of cephalothecoid peridia implicate meristematic growth (subdivision and subsequent growth of cells) in the expansion (and proliferation) of plates. For example, in *Zopfia rhizophila* (Uecker 1977) and *Zopfia nicotiae* (Parguey-Leduc 1970), new peridial plates form by a meristematic process that is initiated between preexisting plates and after the dehiscence lines develop. This pattern also permits uniform expansion of the peridium (Uecker 1977). In *Chaetomidium arxii*, peridial plate initiation continues after the formation of the first set of peridial plates, and new dehiscence lines continue to form late in the development of the cleistothecium, although the specific mechanism of plate formation has not been reported (Benny et al. 1980). Chesters (1934) stated that growth and expansion of the cleistothecia of *Cephalotheca* species resulted from meristematic proliferation of new cells in cephalothecoid plates. In addition to meristematic growth, tip growth was exhibited in *Z. rhizophila* in the vicinity of dehiscence lines (Uecker 1977). Cells underlying these dehiscence lines first lost pigmentation and then elongated, growing in between the peridial cells on both sides of the dehiscence line, creating an expanding zone of cells between peridial plates.

A peridium of thick-walled cells is a character shared by all cephalothecoid fungi (Chesters 1934; Uecker 1977; Benny et al. 1980), but the transverse banding pattern in the peridial cells that seems to drive the plate eversion mechanism is, so far, unique to *C. hypophloia*. A similar dehiscence

mechanism may operate in *Cephalotheca reniformis* (Chesters 1934) and in *Batistia annulipes* (Samuels and Rodrigues 1989), but ultrastructural data are unavailable for these species. In other cephalothecoid genera, e.g., *Zopfia* (Parguey-Leduc 1970; Uecker 1977), *Chaetomidium* (Benny et al. 1980), *Apiosordaria* (Stchigel et al. 2000), and *Corynascella* (Guarro et al. 1997), eversion of peridial plates has not been reported, and the ascocarps of these representatives may simply shatter when mature.

Cryptendoxyla hypophloia was described as a monotypic genus with a *Chalara* anamorph by Malloch and Cain (1970), based on cultures derived from cleistothecia collected from under the bark of a dead maple tree in Ontario. Three additional specimens from similar habitats (beneath the bark of dead hardwoods) are mentioned in the original description and a fourth, from a standing dead aspen in Alberta, is reported in Lumley et al. (2000). Malloch and Cain (1970) observed numerous mites beneath the bark where their original collections were made and noticed that some mites had cleistothecia (whole and broken) and ascospores attached to their bodies or entrained among their hairs. Their suggestion, that *C. hypophloia* "is probably dispersed by arthropods" (p. 1818), is further supported by our isolates, which are the first reports of this fungus solely from the bodies of live-trapped insects. It was not possible to determine whether our isolates from insects were derived from ascospores, peridial tissue, conidia, or fragments of vegetative hyphae.

The functional relationship between this type of ascocarp dehiscence and ascospore dispersal by arthropod carriers remains speculative, but a potential scenario may be as follows. During periods of dry weather the peridia of cleistothecia, formed in cavities under bark or in logs, split open. At the same time, insects (or other arthropods), in seeking refuge from desiccating conditions (Daly et al. 1998), enter these cavities and come into contact with the exposed masses of the hydrophobic ascospores. These propagules adhere to the hydrophobic cement and wax layers on the carrier's exoskeleton and are eventually carried away to suitable new habitats. A relationship between cephalothecoid cleistothecia and arthropod dispersal agents has also been suggested for the brittle cleistothecia of *C. arxii* (Benny et al. 1980) and for *C. reniformis*, a species known from the galleries of woodboring beetles (Chesters 1934). The precise relationship between the behavior of carriers and ascocarp dehiscence mechanisms must remain speculative until direct observations of these phenomena can be made in the field or *in vitro*.

An *in vitro* approach was taken by Greif and Currah (2003) during an investigation of the functional basis of the reticuloperidium, another convergent type of peridial morphology that has developed in both the discomycetous Myxotrichaceae and the cleistothecial Onygenaceae. In these examples, when both fungi and insects were enclosed in a petri dish for observation, the stiff hairs investing the bodies of dipterans passed, lancelike, through the spaces in the peridial mesh, effectively attaching the ascocarp to the insect's body by ascocarp impalement. Similar direct observations of the interaction of insects and cephalothecoid ascocarps *in vitro* may lead to a clearer rationalization of the selective forces that have given rise to this peridial type. It would also be useful to know the potential role of conidia and hyphal fragments as dispersal agents. In addition, more isolates of these cephalothecoid fungi from their natural habitats and from arthropods would contribute substantially to the development of hypotheses concerning the evolution and function of the cephalothecoid ascocarp.

Acknowledgments

This research was supported by a Natural Sciences and Engineering Research Council Discovery Grant to R. S. Currah and a Challenge Grant in Biodiversity to M. D. Greif. We would like to thank Ichiko Tsuneda and M. Chen for technical assistance with the TEM images.

Literature Cited

Benny GL, DA Samuelson, JW Kimbrough 1980 Ascocarp development in *Chaetomidium arxii* (Chaetomiaceae) with special emphasis on the ontogeny of its cephalothecoid peridium. Bot Gaz 141: 347–352.

Chesters CGC 1934 Studies on British Pyrenomycetes. I. The life histories of three species of *Cephalotheca* Fuckel. Trans Br Mycol Soc 19:261–279.

Daly HV, JT Doyen, AH Purcell III 1998 Introduction to insect biology and diversity. 2d ed. Oxford University Press, Toronto. 680 pp.

Esau K 1977 Anatomy of seed plants. 2d ed. Wiley, Toronto. 550 pp.

Greif MD, RS Currah 2003 A functional interpretation of the role of the reticuloperidium in whole-ascoma dispersal by arthropods. Mycol Res 107:77–81.

Guarro J, AH Al-Saadoon, J Gené, SK Abdullah 1997 Two new cleistothecial ascomycetes from Iraq. Mycologia 89:955–961.

Hawksworth DL, C Booth 1974 A revision of the genus *Zopfia* Rabenh. Commonw Mycol Inst Pap 135:1–38.

Lumley TC, SP Abbott, RS Currah 2000 Microscopic ascomycetes isolated from rotting wood in the boreal forest. Mycotaxon 74:395–414.

Malloch D, RF Cain 1970 Five new genera in the new family Pseudeurotiaceae. Can J Bot 48:1815–1825.

Parguey-Leduc A 1970 Le développement des périthèces du *Lepidosphaeria nicotiae* A. Parguey. Bull Trimest Soc Mycol Fr 86: 715–724.

Raven PH, RF Evert, SE Eichhorn 1992 Biology of plants. 5th ed. Worth, New York. 791 pp.

Samuels GJ, KF Rodrigues 1989 *Batistia annulipes* and its anamorph, *Acrostroma annellosynnema*. Mycologia 8:52–56.

Stchigel AM, J Cano, J Guarro, HC Gugnani 2000 A new *Apiosordaria* from Nigeria, with a key to the soil-borne species. Mycologia 92:1206–1209.

Suh SO, M Blackwell 1999 Molecular phylogeny of the cleistothecial fungi placed in Cephalothecaceae and Pseudeurotiaceae. Mycologia 91:836–848.

Uecker FA 1977 Development and cytology of *Zopfia rhizophila*. Bot Gaz 138:420–427.

von Höhnel F 1917 Mycologische fragmente. Ann Mycol 15: 293–388.