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Endoconidiogenesis in *Endoconidioma populi* and *Phaeotheca fissurella*

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Abstract: Details of the development of endoconidia were basically the same in *Endoconidioma populi* and *Phaeotheca fissurella.* In both species, endoconidiogenesis involved (i) subdivision of conidiogenous mother cells by septation to form two to several daughter cells; (ii) accumulation of an electron-dense material between the daughter and mother cell walls; and (iii) separation of the daughter cells by septum schizolysis, accompanied by the dissolution of mother cell wall. Conidiomata of *E. populi* were unique in having a closed peridium and a locule filled with conidiogenous mother cells and, therefore, we proposed the new term, cleistopycnidium (pl. -a), for this structure. In the cleistopycnidium of *E. populi,* endoconidiation usually began in the core of the locule and spread outward. Release of endoconidia was by the degeneration of peridial cell walls.

Key words: black yeasts, cleistopycnidium, conidioma, endoconidium, meristematic black fungi, septum schizolysis, ultrastructure

INTRODUCTION

We recently found a dematiaceous fungus that formed endoconidia (i.e., conidia produced within a conidiogenous cell by cleavage of protoplast; Carmichael 1971) within darkly pigmented pycnidium-like conidiomata on trembling aspen, *Populus tremuloides* Michx. We proposed a new monotypic genus, *Endoconidioma,* to accommodate this taxon because it was distinct both morphologically and phylogenetically from the previously established endoconidial, hyphomycetous genera, *Phaeotheca* and *Hyphospora,* and from coelomycetous genera (Tsuneda et al 2004). Unlike *Endoconidioma,* both *Phaeotheca* and *Hyphospora* lack conidiomata with peridia (Sigler et al 1981, Ramaley 1996, Zalar et al 1999a) and, as far as the authors are aware, no coelomycetous taxa are known to form endoconidia. DNA sequence data for SSU (the small subunit of the nuclear rRNA gene) indicated that the *Hyphospora* state of *Comminutispora agavaciensis* Ramaley occurred in a large clade comprising Capnodiales, but *P. fissurella* Sigler, Tsuneda & Carmichael, the type species of the genus, was on its own branch in a basal position to the Capnodiales and Dothideales (Hambleton et al 2003). Whereas parsimony analyses of SSU and ITS (the internal transcribed spacer region of the nuclear rRNA gene) suggested that *E. populi* Tsuneda, Hambleton & Currah was phylogenetically close to Dothideales and allied anamorphs in *Kabatina* and *Hormonema* (Tsuneda et al 2004).

Endoconidiogenesis is not recognized in Ainsworth & Bisby's Dictionary of Fungi (Kirk et al 2001) as a pattern of conidiogenesis. This probably is because ultrastructural details on this process have been described only in species of *Phaeotheca.* In *P. fissurella* (Tsuneda and Murakami 1985), conidiogenous mother cells subdivided by septation to form two to several daughter cells that subsequently separated by septum schizolysis to become endoconidia. Dissolution or rupture of the mother cell walls brought about liberation of the endoconidia. De Hoog et al (1997) presented two TEM micrographs of meristematic cellular clumps in *P. triangularis* de Hoog & Beguin. Judging from these micrographs, this species closely resembles *P. fissurella* in endoconidiogenesis, although the authors did not describe the ultrastructural process. In *P. dimorphospora* DesRochers & Ouellette, cells consisting of chlamydospores converted to endoconidia that were liberated after exfoliation of the outer wall of the chlamydospore, as in mother cells of *P. fissurella* (DesRochers and Ouellette 1994). The scarcity of ultrastructural studies of endoconidiogenesis prompted us to carry out this comparative study to elucidate the ultrastructural details of endo conidial development and release in *E. populi* and *P. fissurella.* Because endoconidiogenesis of *P. fissurella* has been reported elsewhere (Tsuneda and Murakami 1985), the emphasis was on *E. populi.*

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MATERIALS AND METHODS

Fungal cultures used were *P. fissurella* (UAMH 4285) grown on potato-dextrose agar (PDA; Difco, Detroit, Michigan) at 18 C for 3 wk and *E. populi* (UAMH 10297) grown on 2% malt-extract agar (MEA; Difco) or cornmeal agar dextrose (CMAD; Difco) at 18 C for 1–3 mo. Thin sections (about 1 μm) for light microscopy (LM) were prepared only for *E. populi* using the method described in Tsuneda et al (2004). Methods for specimen preparation in scanning and transmission electron microscopy (SEM and TEM, respectively) were the same as described by Tsuneda et al (2001). All SEM samples were examined and photographed at 15 kV using a Hitachi S-510 microscope. For TEM, a Hitachi H-7000 electron microscope operating at 75 kV was used.

RESULTS

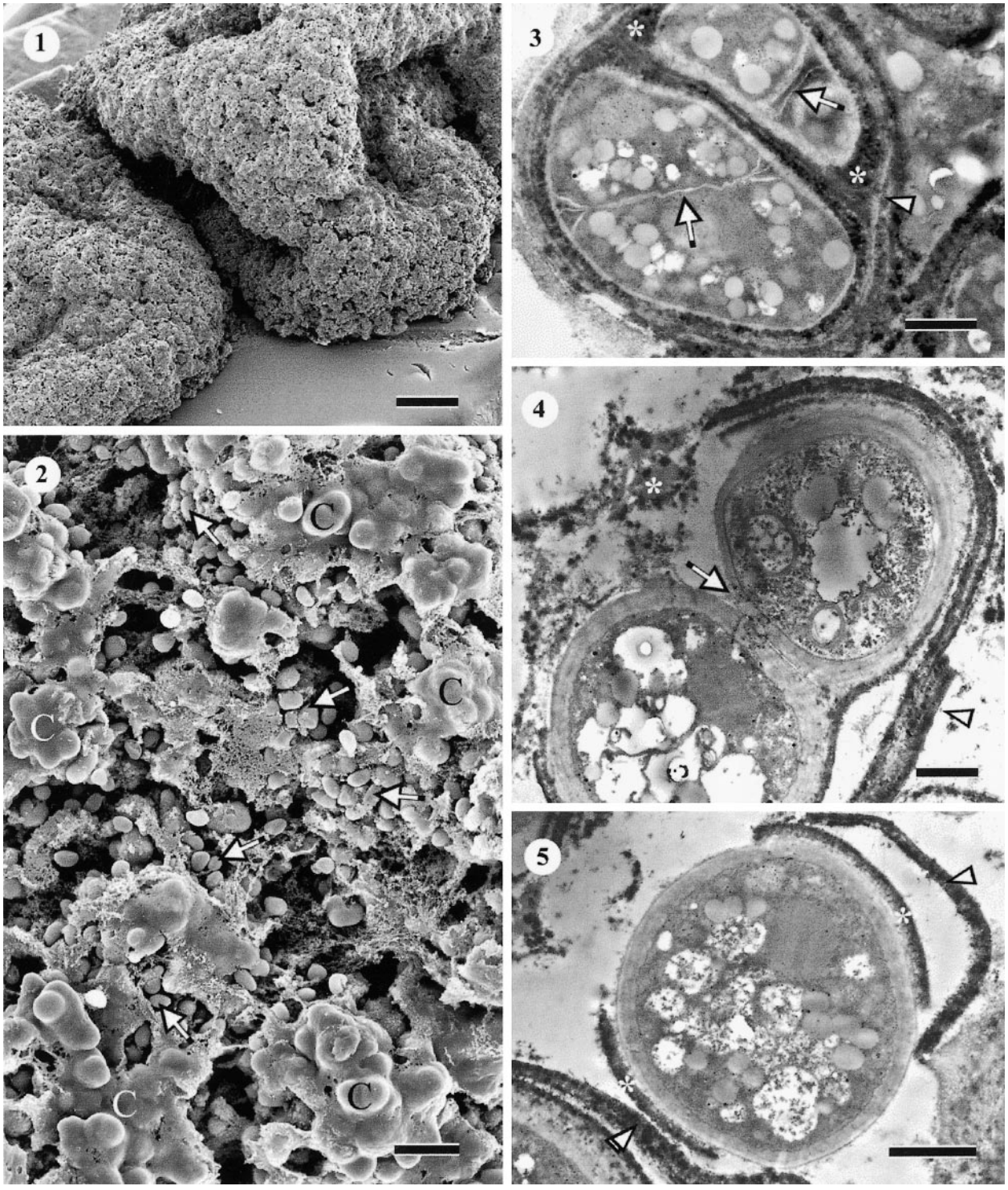
Endoconidiogenesis in Phaeotheca fissurella.—Colonies on PDA were black and cerebriform (FIG. 1), consisting of numerous dematiaceous, thick-walled cells. Upon maturation of the colony, all component cells were capable of becoming conidiogenous mother cells. Formation of endoconidia in the conidiogenous mother cells and their subsequent release occurred progressively from the surface to the inner region of the colony. Dissolution of the mother cell walls brought about the liberation of endoconidia (FIG. 2, arrows). TEM revealed the typical process of endoconidium development (FIGS. 3–5). First, conidiogenous mother cells subdivided by septation (FIG. 3, arrows) to form two to several daughter cells and subsequently mother and daughter cell walls were separated by an electron-dense, often granular material that accumulated between them (FIG. 3, asterisks). The daughter cells then were separated from each other by septum schizolysis (FIG. 4, arrow) that was accompanied by the dissolution of mother cell walls (FIG. 4, asterisk). Thus, the individual daughter cells (now endoconidia) were liberated (FIG. 2, arrows). Released endoconidia often bore remnants of the electron-dense material and mother cell wall (FIG. 5).

Endoconidiogenesis in Endoconidioma populi.—Unlike *P. fissurella, E. populi* produced subglobose to flask-shaped conidiomata on stromatic colonies consisting of numerous, darkly pigmented, thick-walled cells and hyphae (FIGS. 6, 7). Each conidioma was composed of a peridium and a locule filled with conidiogenous cells. All conidiomatal cells appeared markedly similar to vegetative stromatic cells, and there were no morphological distinctions between the incipient cells of peridium and locule (FIGS. 8, 9). Conidiogenesis usually began in the central area of the conidioma (FIG. 9, arrow) and gradually spread throughout the locule (FIGS. 10, 11). Endoconidia were liberated from openings that resulted from the dissolution of peridial cell walls (FIGS. 12, 13). Before wall dissolution, peridial cells formed endoconidia (FIG. 10, arrowheads), with one to several openings developing in a single conidioma (FIG. 12, arrows). TEM revealed that conidiogenous mother cells subdivided by septation (FIG. 14, arrows) and an electron-dense material accumulated between the mother and daughter cell walls (FIG. 14, white asterisk), as in *P. fissurella.* Dissolution of the mother cell walls progressed to the extent that they became amorphous and the daughter cells (endoconidia) appeared to be suspended in the amorphous matrix material (FIG. 15, asterisks). Endoconidia subsequently moved out of their mother cells, leaving remnants of septa in the newly vacated mother cells (FIG. 16, arrowheads); these soon further degenerated and became unidentifiable (FIG. 17d). The conidioma in FIG. 17 illustrates the stages in endoconidiogenesis, starting with the septation of mother cells and ending with the liberation of endoconidia.

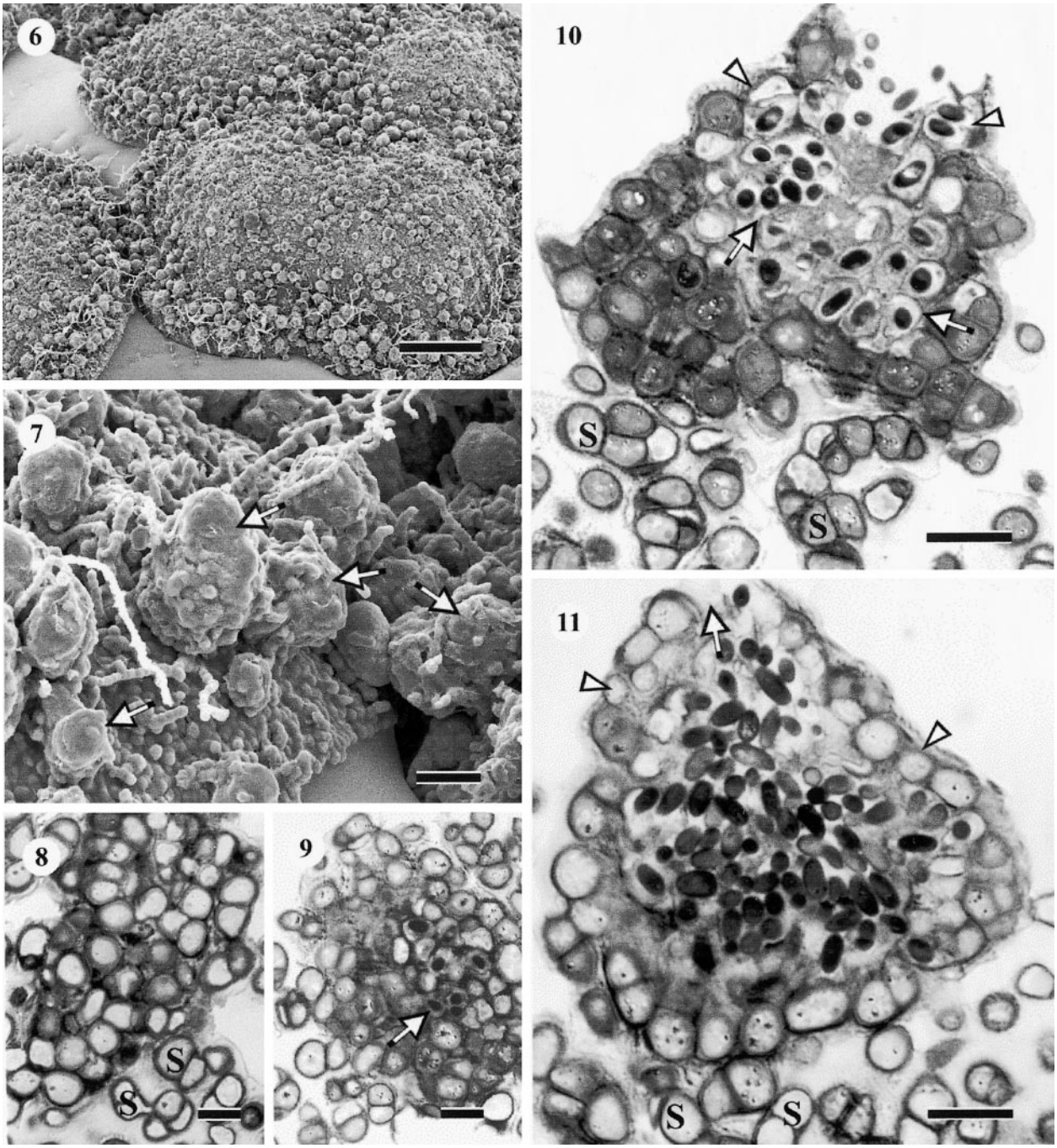
DISCUSSION

The developmental process for endoconidia in *E. populi* was basically the same as that in *P. fissurella* and included: (i) subdivision of conidiogenous mother cells by septation to form two to several daughter cells; (ii) accumulation of an electron-dense material between the daughter and mother cell walls; and (iii) separation of the daughter cells by septum schizolysis, accompanied by dissolution of the mother cell wall. The only recognizable difference was that dissolution of the mother cell wall was nearly complete in *E. populi* whereas it often was incomplete in *P. fissurella.* With regard to liberation of endoconidia, in *P. fissurella,* dissolution of mother cell walls alone allowed the release to the external environment, whereas in *E. populi,* the peridial cells also had to degenerate so that an opening was formed in the conidioma.

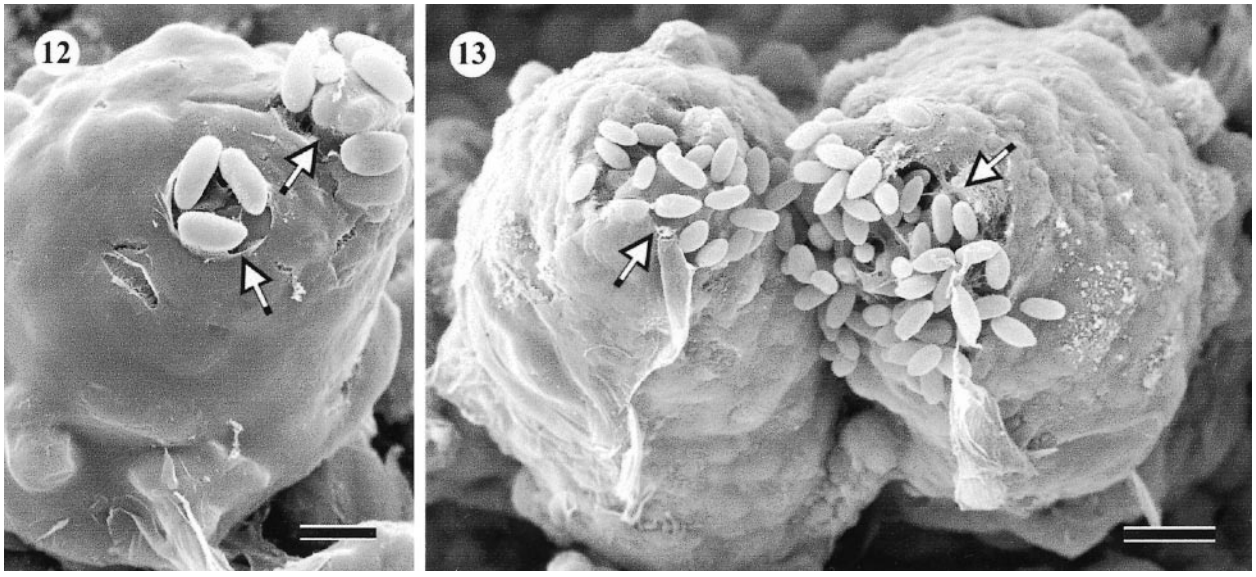
Ramaley (1996) reported that endoconidiogenesis in *H. agavaciensis* was initiated by repeated transverse and longitudinal divisions of hyphal cells. Some of the numerous cytoplasmic divisions were accompanied by wall formation, and the small conidiogenous cells thus formed produced endoconidia. Based on this description, *H. agavaciensis* is similar to *E. populi* and *P. fissurella* in endoconidiogenesis but ultrastructural studies of this process in *H. agavaciensis* are needed for confirmation. Likewise, a strain of *Capnobotryella renispora* Sugiyama developed propagules, analogous to endoconidia, within component cells of microsclerotia through a similar process, although only a single propagule occurred in each component cell (Hambleton et al 2003).



FIGS. 1–5. *Phaeotheca fissurella*. PDA, 3 wk, SEM (1, 2) and TEM (3–5). 1. Overall view of colonies. 2. Higher magnification of a colony surface. Dissolution of conidiogenous cell walls occurred at random sites to release endoconidia (arrows). Many conidiogenous cells are still intact (C). 3. A conidiogenous mother cell dividing by septation (arrows) to form daughter cells. Electron-dense material (asterisks) has accumulated between the daughter and mother cell walls (arrowhead). 4. Separation of daughter cells by septum schizolysis (arrow). The asterisk indicates the degenerated mother cell wall. The arrowhead indicates degenerating mother cell wall. 5. Liberated daughter cell (endoconidium) bearing remnants of the electron-dense material (asterisks) and mother cell wall (arrowheads). Scale bars: 1 = 500 μm; 2 = 20 μm; 3–5 = 2 μm.



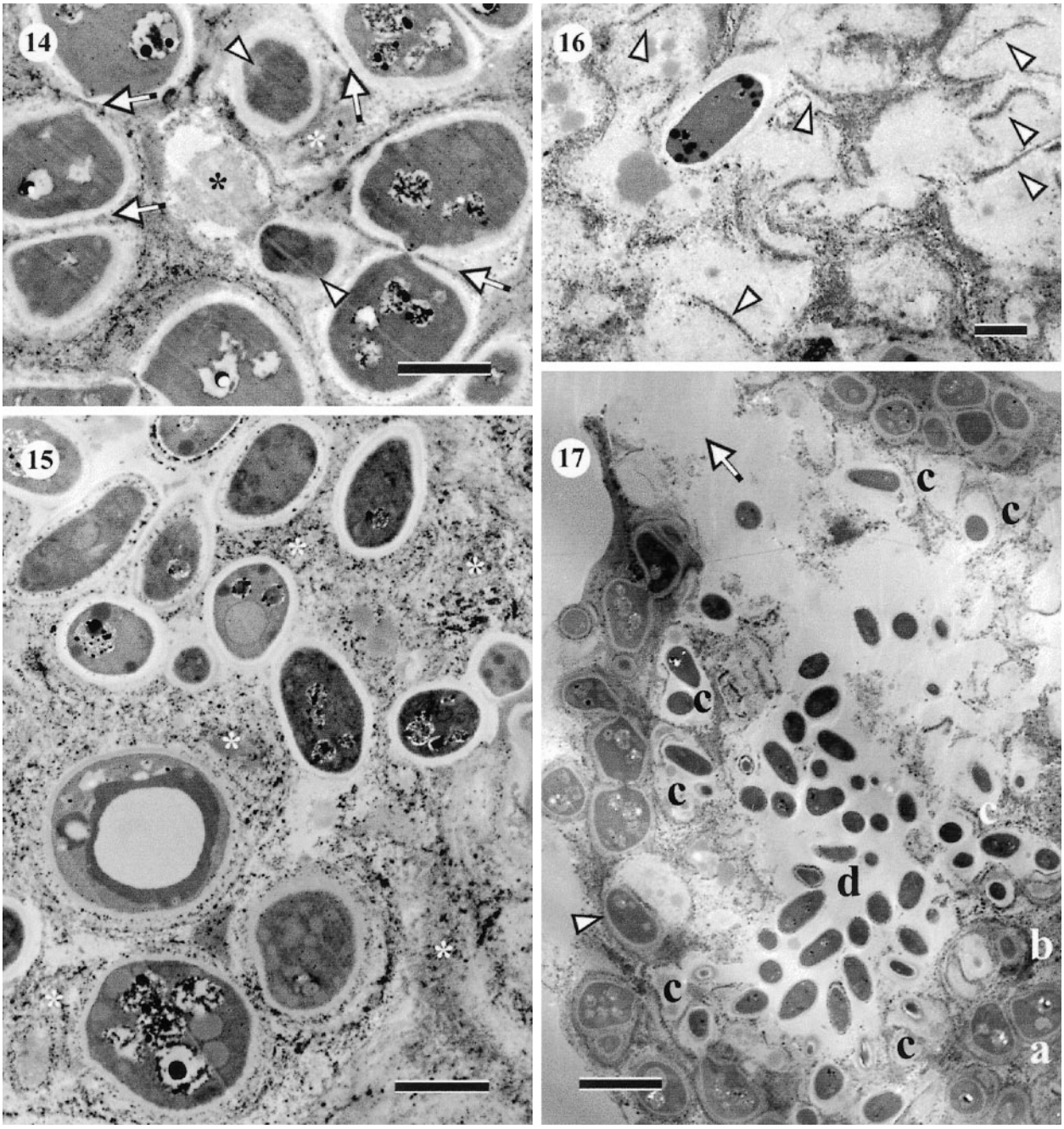
FIGS. 6–11. *Endoconidioma populi*. CMAD, 2 mo, SEM (6, 7). LM (oil immersion) of thin sections (8–11). 6. Overall view of colonies. 7. Enlarged view of a part of FIG. 6, showing developing conidiomata (arrows). 8. Immature conidioma arising from a stroma. Component cells of the conidioma appear markedly similar to those of the stroma (S). No sign of conidiation yet. 9. Initial stage of endoconidia formation (arrow) occurring at the central portion of a conidioma. 10. An actively sporulating conidioma. Outlines of conidiogenous (mother) cells are still discernible in the region indicated by the right arrow but not recognizable in the area indicated by the left arrow. Peridial cells also form endoconidia (arrowheads). S: cells of a stroma. 11. Conidioma packed with conidia. The arrow and arrowheads indicate an opening through which conidia are released and intact peridial cells, respectively. S: cells of a stroma. Scale bars: 6 = 400 μm; 7 = 20 μm; 8–11 = 10 μm.



FIGS. 12 and 13. SEM of conidiomata of *Endoconidioma populi* releasing conidia (arrows). MEA, 2 mo. Note that conidia were escaping from two different sites of the conidioma (arrows in FIG. 12). Scale bars: 12 = 5 μm; 13 = 10 μm.

Endoconidiogenesis has been reported in three medically important mitosporic fungi. Arthroconidia of *Coccidioides immitis* Stiles, the causal agent of coccidioidomycosis, invade the human body via the respiratory tract and convert to spherules that become multinucleate (coenocytic) and then segment to form numerous endospores within host tissue. As endospores emerge from the spherule, almost all are in packets bound by the smooth membranous outer layer (Cole and Sun 1985, Sun et al 1986). The term endoconidia, however, is not usually used for this fungus, probably because its endosporogenesis resembles, in some respects, sporangiosporogenesis. A previously undescribed synanamorph of *Wangiella dermatitidis* (Kano) McGinnis gave rise to cells that increased in size, became thick walled, and laid down septa to form multicellular, dematiaceous structures. The septa then split or schizolysed to separate endoconidia (Matsumoto et al 1990). In *Exophiala spinifera* (Nielsen & Conant) McGinnis, the cause of chromoblastomycosis, muriform cells formed within multinucleated giant cells in the dermis, and the muriform cells disarticulated from the outer wall of the parent cell and from each other to form endoconidia within the outer walls of the parent cells (Padhye et al 1996). Also, endoconidia are known to occur in *Aureobasidium, Hormonema, Sarcinomyces* and *Trimmatostroma* (De Leo et al 1999, Hermanides-Nijhof 1977, Wollenzien et al 1997, Zalar et al 1999b), but they develop only sporadically in undifferentiated hyphal cells and ultrastructural details of the developmental process have not been documented for these genera. It is interesting to note that the fungi mentioned above, except *C. immitis,* belong to a group of dematiaceous mitosporic fungi called ''black yeasts'' (Hermanides-Nijhof 1977) or ''meristematic black fungi'' (de Hoog et al 1999). We surmise that endoconidiogenesis is more prevalent in this group of fungi than presently known.

Conidiomata of *E. populi* are unique in that (i) the peridium is closed and the locule is filled with conidiogenous cells that are undifferentiated from peridial cells in morphology; (ii) conidia are produced endogenously in the locule cells; (iii) the peridial cells also are capable of forming endoconidia (FIG. 10, arrowheads); and (iv) more than two openings often occur per conidioma to ensure efficient release of endoconidia. What is the appropriate term for conidiomata with these characteristics? The pycnidium is defined as ''a frequently ± flask-shaped fungal tissue with a circular or longitudinal ostiole, the inner surface of which is lined entirely or partially by conidiogenous cells'' (Kirk et al 2001), and thus the term is inappropriate as are other existing terms for conidiomata, e.g. acervuli, sporodochia and synnemata. Therefore, we propose the new term, cleistopycnidium (pl. -a), for a conidioma with a closed peridium and a locule filled with conidiogenous cells. In the cleistopycnidia of *E. populi,* the peridial cells function not only as a protective layer but also as conidiogenous cells, as opposed to typical pycnidia whose conidiogenous cells clearly are differentiated from the peridial cells in both function and morphology.



FIGS. 14–17. TEM of different stages of endoconidiogenesis in *Endoconidioma populi*. CMAD, 2 mo. 14. Actively dividing conidiogenous cells that were forming daughter cells. Arrows and arrowheads indicate septa and developing endoconidia, respectively. White asterisk indicates electron-dense material while the black asterisk indicates a conidiogenous cell that appears to have been vacated. 15. Endoconidia surrounded by granular electron-dense material (asterisks). Walls of conidiogenous mother cells have been dissolved. 16. Remnants of septal walls (arrowheads) remained in a vacated conidiogenous area. One conidium is left behind. 17. Conidioma showing different stages of endoconidiogenesis. Letters (a, b, c, d) indicate progressive stages of cell wall dissolution of conidiogenous mother cells, leading to the release of endoconidia (d). The arrow indicates an opening created by the dissolution of peridial cell walls, through which endoconidia emerge. The arrowhead indicates a daughter cell (endoconidium) remained in a peridial cell. Scale bars: 14 = 4 μm; 15, 16 = 2 μm; 17 = 8 μm.

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