THE ULTRASTRUCTURE OF ASCOSPORE DELIMITATION IN *SACCOBOLUS KERVERNI*

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The mechanics of spore delimitation within the ascus have long been a topic of controversy among ascomycete cytologists. Lateral fusion of recurving astral rays around each nucleus following the mitotic, third division in the ascus (6), progressive differentiation of spore membranes in a granular layer of cytoplasm around each nucleus (5), fusion of epiplasmic vacuoles (8), and invagination of a peripheral membrane system in the ascus (3) all have been cited in attempts to describe this process under the light microscope. Carroll (2) and Reeves (12) provide more complete discussion of the process. Early observations of the ultrastructure of developing ascospores revealed only that following meiosis each nucleus somehow is surrounded by at least one membrane, outside of which the spore wall develops (7). Later studies have demonstrated that each spore is delimited by two membranes, an inner plasma membrane and an outer investing membrane, between which the spore wall is deposited (1, 4, 9–11, 12, 13). Recently, Reeves (12) has shown that in *Pyronema domestica* the spore membranes originate in an open-ended peripheral cylinder of double membrane which initially surrounds all eight nuclei in the ascus. As the membrane cylinder constricts between the seriate nuclei, it pinches out a row of ascospores. The present study was carried out to investigate the origin of the spore membranes in the discomycete, *Saccobolus*.

MATERIALS AND METHODS

Petri plates containing a Difco corn-meal agar culture medium (Difco Laboratories, Detroit, Mich.) supplemented with yeast and malt extracts were inoculated with the fungus and incubated at 25°C with a 12 hr, light-dark cycle. When a mycelial mat had covered about half of each plate, the cultures were subjected to cold shock at 4°C for 3–5 days and then were returned to room temperature. At the periphery of the old mycelium, where the new growth began, a dense ring of apothecia formed within which the asci developed in near synchrony. Small blocks of agar with developing apothecia on their upper surfaces were cut from the plates, trimmed of excess agar, and placed in 2% $KMnO_4$ for 2 hr (15 min *in vacuo*) at 4°C; they were then washed 6–8 times in distilled water and soaked for 4–6 hr in a 0.5% solution of uranyl acetate. The tissue was dehydrated in a graded alcohol series and embedded in an epoxy resin. Sections were cut with a diamond knife on an

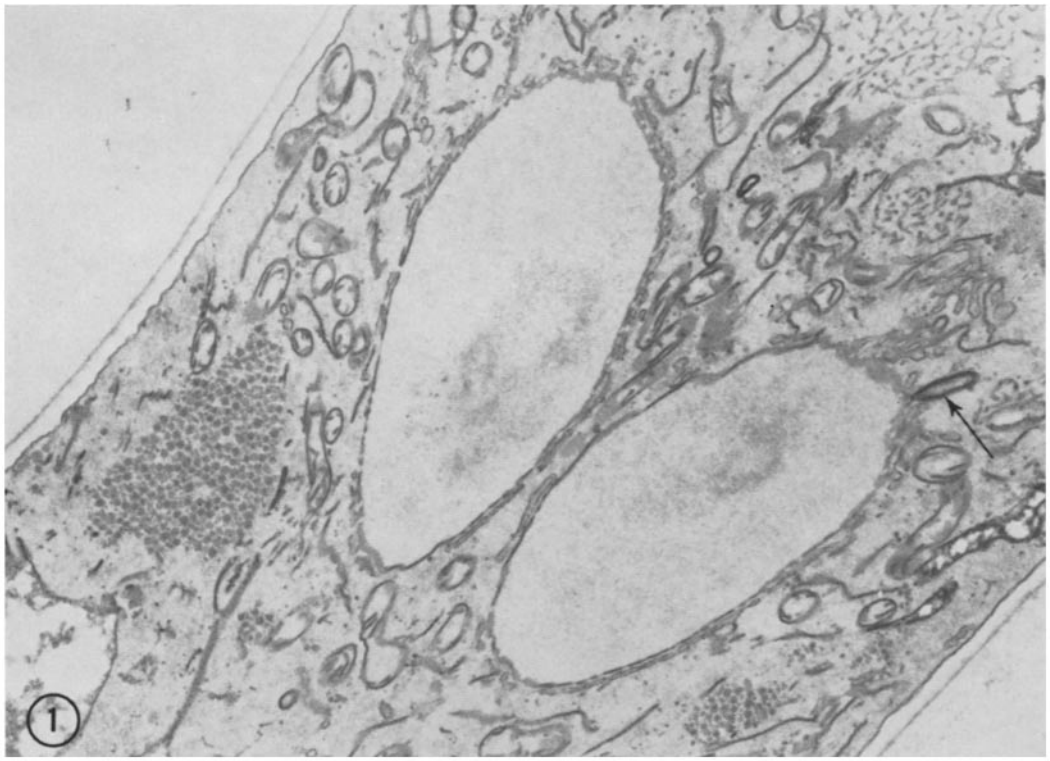

FIGURE 1 Central portion of the ascus after meiosis has begun. Note endoplasmic reticulum associated with the nuclei and bleb arising from the envelope of one of the nuclei (arrow). × 11,500.

ultramicrotome, stained on the grids for 15 min with Reynolds' lead citrate, and examined with an RCA 3-F electron microscope.

RESULTS

As meiosis commences within the young ascus, extensive associations of the endoplasmic reticulum (ER) with the nuclear envelope arise (Fig. 1). At certain points close to the nucleus, one or two ER cisternae condense and appress themselves against the nuclear envelope (Fig. 2). The nuclear envelope evaginates at these points, producing blebs 0.2–0.7 μ in diameter; single nuclear and ER elements of the blebs fuse, giving rise to membrane-bounded packets with a collapsed spherelike configuration (Figs. 3, 4). A cross-section through such a packet gives the impression of a closed vesicle bounded by two concentric double membranes (Fig. 5). The membrane in such "false" vesicles usually stains more densely than that in either the nuclear envelope or the endoplasmic reticulum (Fig. 1, 5); the interiors of the blebs, however, frequently stain less densely than the surrounding cytoplasm (Fig. 5). These membrane-bounded packets appear to migrate to the periphery of the ascus and there to unwind and fuse to produce a continuous system of double membrane. Various complicated profiles are seen during the early stages of fusion, and the topology of the process remains obscure (Figs. 6–8).

The forming membrane system first appears as an open-ended cylinder appressed to the ascus wall (Fig. 8). As blebbing from the nucleus continues, the new membrane adds on across the top and bottom of this cylinder, forming a more nearly complete sac (Fig. 9). At the same time, a final, mitotic, nuclear division takes place within, giving rise to eight nuclei. The peripheral membrane sac then invaginates, ultimately delimiting eight ascospores (Figs. 10, 11). Each spore finally is bounded by two membranes, a condition derived from the doubleness of the initial membrane sac (Fig. 11).

The blebbing of small, discrete, membrane-

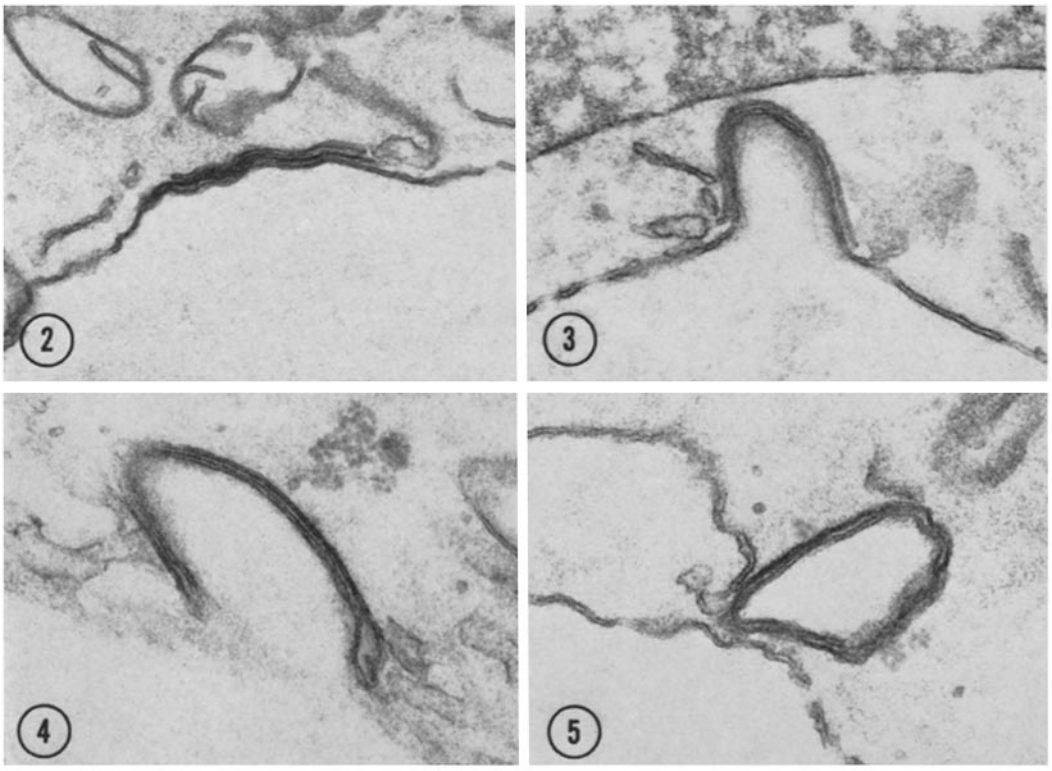

FIGURE 2 Area of presumptive blebbing on the surface of the fusion nucleus. ER cisternae are appressed to the nuclear envelope. × 45,000.

FIGURE 3 Nuclear envelope has evaginated. ER cisterna remains appressed, but still separate. × 45,000.

FIGURE 4 Stage in fusion of elements of the ER and nuclear envelope. × 45,000.

FIGURE 5 Cross section of nuclear bleb. × 45,000.

bounded packets from nuclear envelope during ascospore delimitation has been seen by the author in one other discomycete, *Ascodesmis sphaerospora* (2). However, these bodies have not been seen with the electron microscope during ascosporogenesis in either *Podospora anserina* (1) or *Pyronema domestica* (12). This discrepancy may be only apparent, a consequence of the fact that blebbing takes place rapidly and would be hard to find in nonsynchronized cultures. Alternatively, there may be real differences in the membrane sac ontogeny among different species of Ascomycetes. Reeves (12) suggests that long processes of the nuclear envelope which arise prior to ascospore delimitation in *Pyronema* are associated with the elaboration of membrane for the peripheral cylinder. If this is in fact the case, then here also the accretion of membrane for the peripheral system involves membrane synthesis at the surface of the nucleus. The differential staining properties of the membrane packets indicate that transfer of material from the nucleus to the spore-delimiting membrane system may be taking place. The eventual deposition of sculptured spore walls between the two membranes of this system following spore delimitation further suggests that information transfer also may be taking place.

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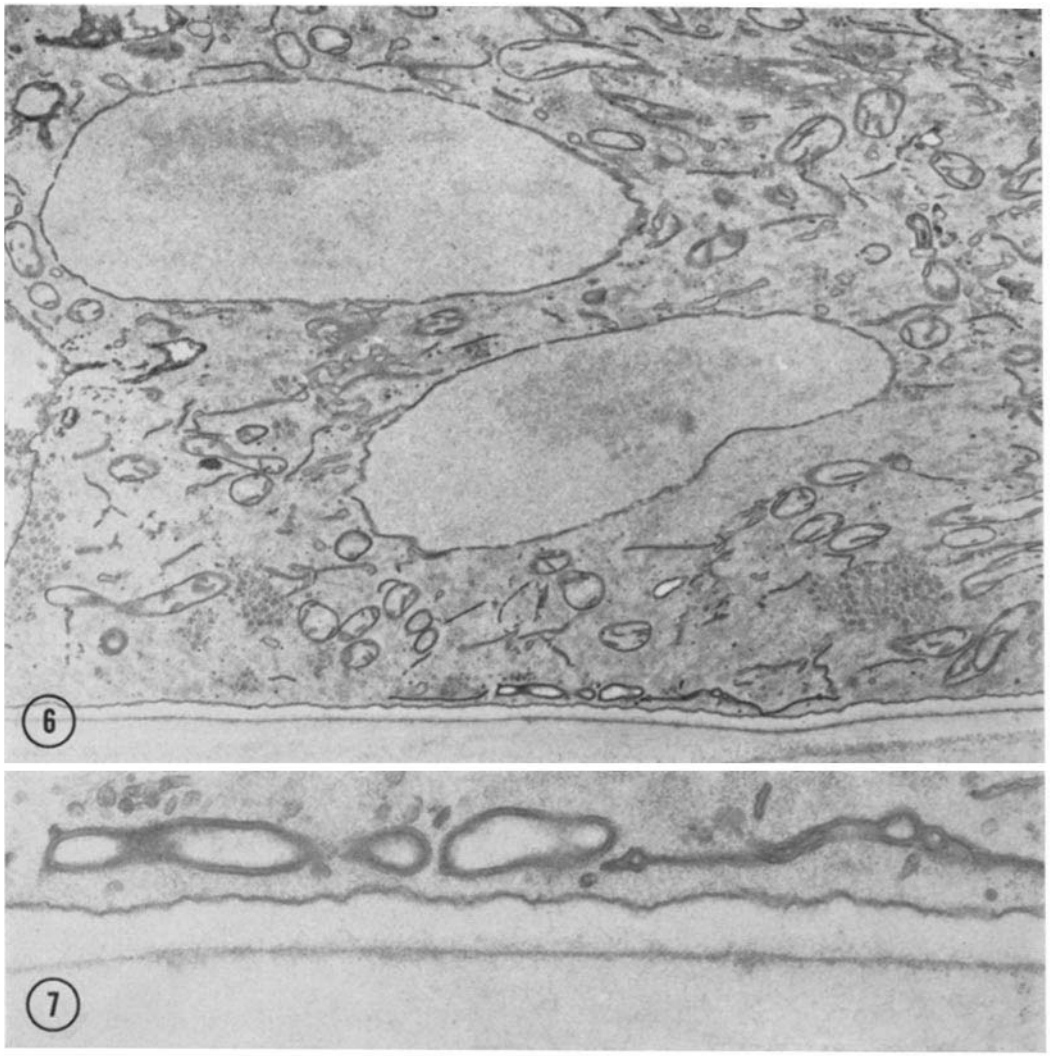

FIGURE 6 Membrane sac formation in the periphery of the ascus. × 10,500.

FIGURE 7 Detail of fusing membrane sacs shown in Fig. 6. × 41,000.

to the author. The work is based on a portion of a dissertation presented to the Faculty of the Graduate School of the University of Texas in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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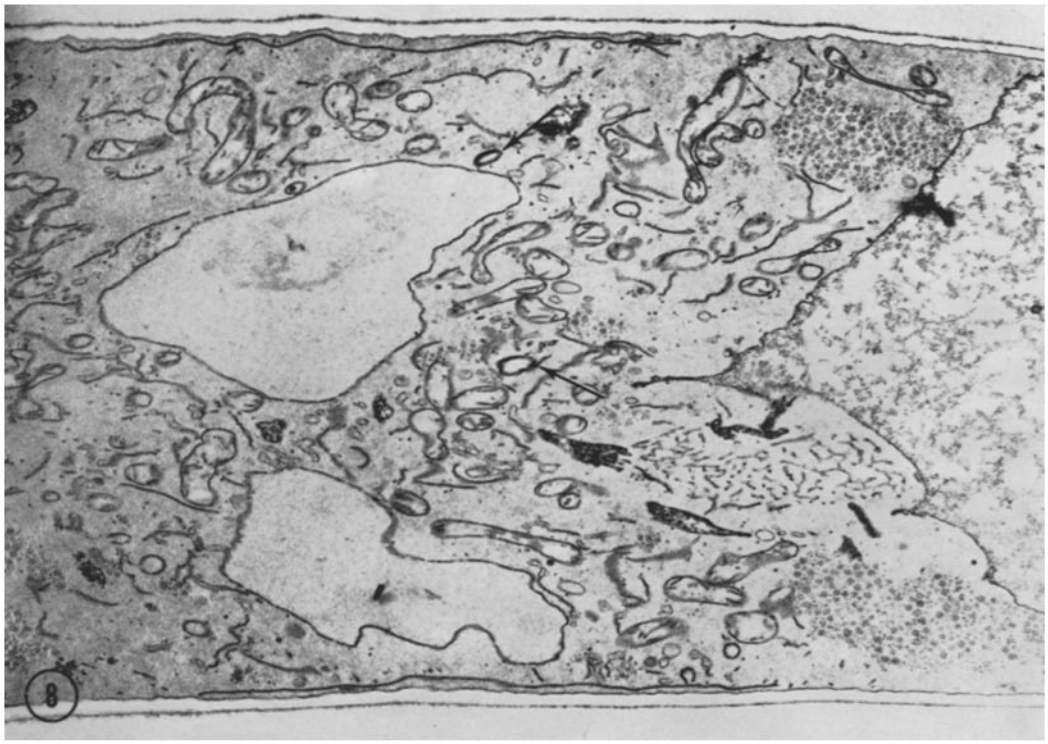

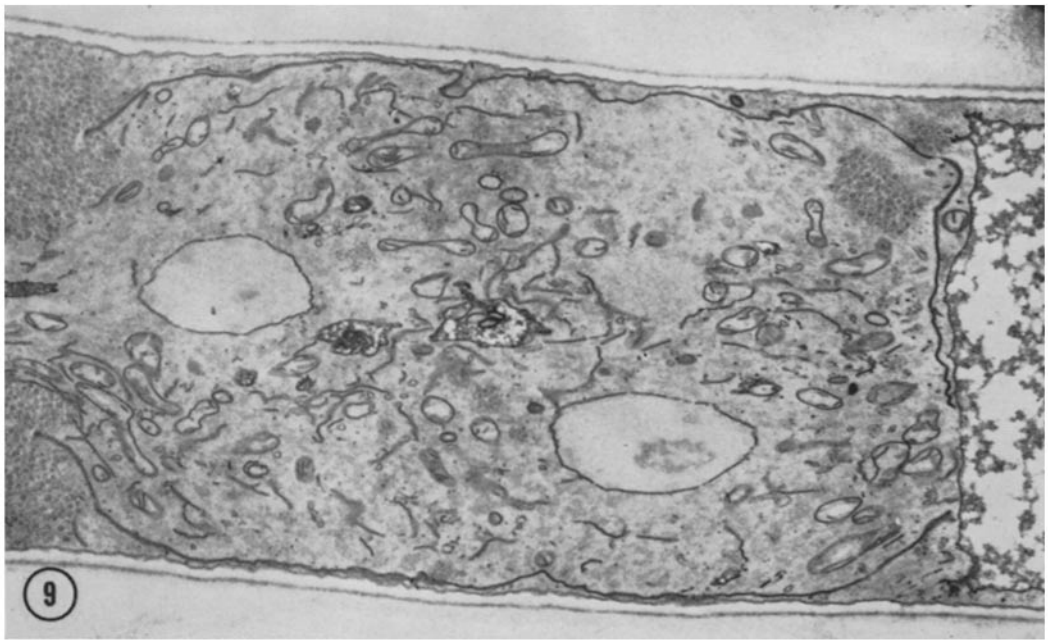

FIGURE 8 Longitudinal section of forming membrane sac, open cylinder stage. Note membrane packets in ascus cytoplasm (arrows). × 10,500.

FIGURE 9 Longitudinal section of forming membrane sac, ends of cylinder closing over. × 8,500.

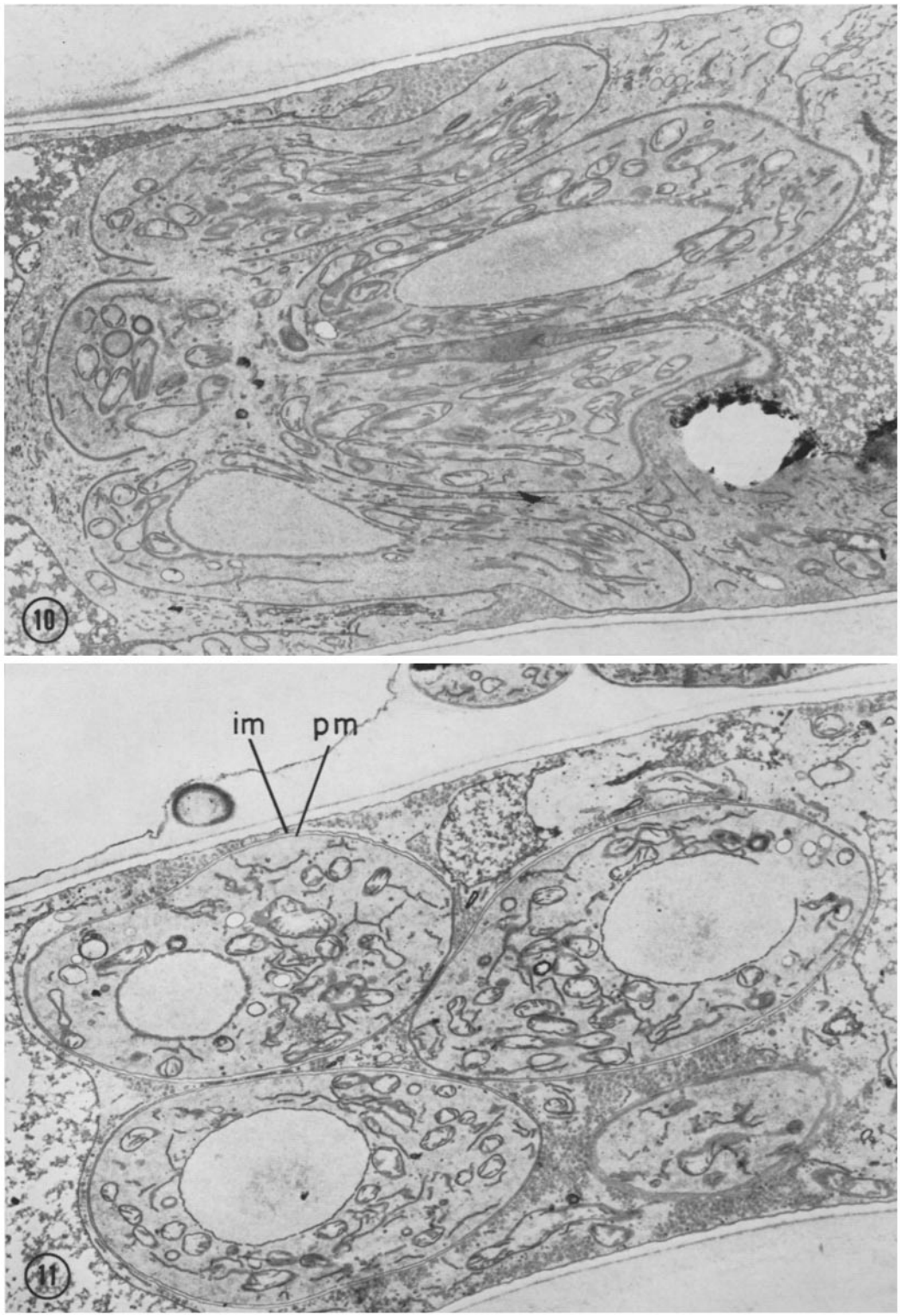

FIGURE 10 Longitudinal section of membrane sac at an advanced stage of invagination. Spore delimitation is almost complete. × 8,500.

FIGURE 11 Longitudinal section of ascus just after the completion of spore delimitation. Spores are surrounded by two membranes, the plasma membrane (*pm*) and the investing membrane (*im*). × 8,500.

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