# SPORE WALL ULTRASTRUCTURE OF PROTOSALVINIA[1]

## Wilson A. Taylor and Thomas N. Taylor

Department of Botany, The Ohio State University, 1735 Neil Avenue,
Columbus, Ohio 43210-1293

## ABSTRACT

*Protosalvinia* is an enigmatic fossil which has been historically assigned to several major taxonomic groups. Stratigraphically, the fossil occurs in a narrow range of Upper Devonian sediments. Tetrads of spores are associated with shallow depressions on the surface of approximately 5% of the specimens collected from the Ohio Shale in Columbus, OH. Spores are approximately 250 $\mu$m in diameter and have a spore wall which is composed of at least two distinct layers. The outer layer is coarsely laminated in regions where adjacent spores are in contact. Individual laminar units are thinnest toward the inside and gradually thicker toward the surface of the spore. In non-contact regions, the outer layer is composed of globular units. The inner layer of the wall has little discernable structure except for the presence of a distinct suture beneath the proximal trilete mark. This firmly establishes the meiotic nature of these structures. Comparison with eggs and tetraspores of several extant phaeophycean algae shows little similarity.

*PROTOSALVINIA* (Dawson, 1884) is an enigmatic Devonian fossil which has a long and confusing taxonomic history. In his original description of the fossil, Dawson allied it with the free floating water ferns ("rhizocarps of the Erian"). Since that time, other workers have suggested affinities with the brown algae (e.g., Schopf and Schwietering, 1970), or that the organism is an alga possessing features convergent with land plants (e.g., Niklas and Phillips, 1976). Most recently, the genus has figured prominently in discussions concerning the acquisition of terrestrial adaptations by early aquatic plants. Gray and Boucot (1979) cite biochemical and morphological data in support of a non-algal affinity for *Protosalvinia*, while Schopf (1978) argues in favor of assignment to the Phaeophycophyta based on morphology and occurrence in a strictly marine depositional setting. The key morphologic features which have prompted taxonomic assignment within the Phaeophycophyta are its thalloid organization, and the presence of tetrads of spores situated in depressions on what is believed to be the upper thallus surface. The present study was initiated to compare the ultrastructure of the spore wall of *Protosalvinia*, to that found in the single-celled reproductive stages of several representative extant thallose algae, in an effort to understand more accurately the taxonomic position and reproductive function of the spores of this interesting fossil.

MATERIALS AND METHODS—The specimens of *Protosalvinia* occur in a dark grey shale within the Upper Devonian Ohio Shale (Winslow, 1962; Russell, 1985). The locality is situated on the cutbank of Glen Echo Creek, four miles north of the center of Columbus, Ohio in Glen Echo Park. The fossils at this locality are assignable to *Protosalvinia arnoldii* (Schopf and Schwietering, 1970); in addition, the site also contains specimens of *P. ravenna*. They are highly resistant to physical and chemical weathering, and were easily removed intact from the rock surface with a pair of forceps. Thalli possessing packets of spores, which make up about 5% of the 1,000 or so specimens, were sonicated to remove excess sediment, and soaked overnight in concentrated hydrofluoric acid (46%). Spore tetrads were then mechanically removed from the dark rimmed depressions, often with a piece of the thallus still attached. Several specimens were cemented directly to standard SEM stubs with colloidal silver paint, coated with 100 A of gold in a Technics Hummer III sputter coater, and viewed with an Hitachi S-500 SEM operating at 20 KV.

The method which produced the most satisfactory results with regard to staining and penetration involved acetolysis of the tetrads (acetolysis enhanced staining affinity but did not alter the structural characteristics), dehydration in absolute ethanol, transfer to 100% acetone, and infiltration by Spurr low viscosity
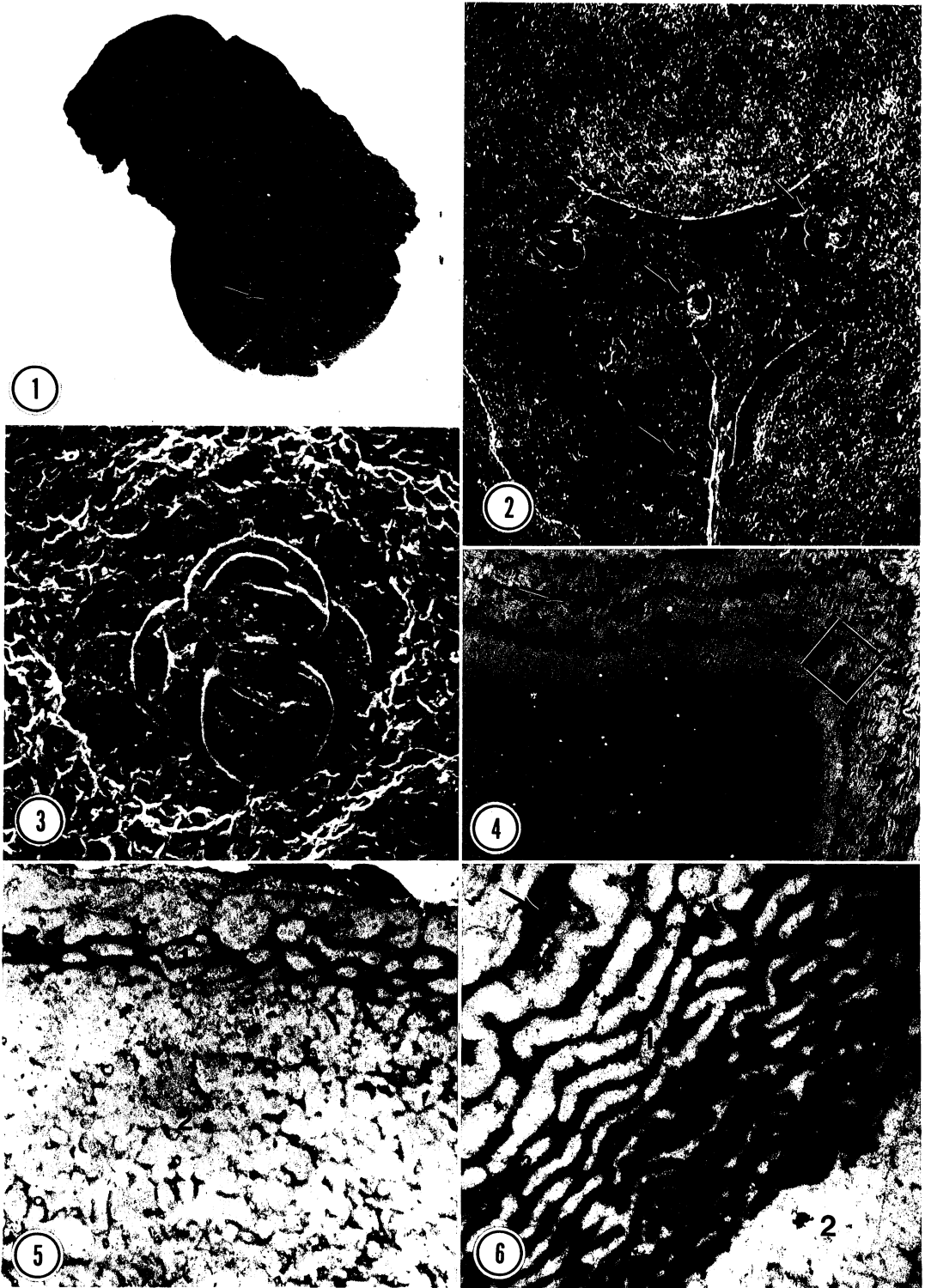
Fig. 1–6.   *Protosalvinia* sp. Fig. 1. *Protosalvinia ravenna.* **1.** Compressed specimen showing tetrads (arrow) located between the two flattened apical flaps. OSU Coll. 17,106. ×9. **2.** Tetrads of spores (arrows). ×23. **3.** Tetrad of spores in shallow depression on thallus surface. Parenchymatous or pseudoparenchymatous pattern on the thallus surrounds the depression. ×150. **4.** Point of contact (arrow) between two adjacent spores of a tetrad showing the bi-layered wall. Box identifies the protrusion of inner layer (2) into the outer layer (1) and indicates the position of the suture that

epoxy embedding medium (firm recipe), with increasing ratios of Spurr to acetone (1:3, 1:1, 3:1, 2 changes in pure plastic, 8–12 hr between each transfer). The resin was then polymerized in a 60 C oven, under vacuum, for 24 hr. Gold or silver sections were cut on a Sorval Ultracut using a diamond knife, and collected on Formvar coated copper slot grids (1 mm × 2 mm slot). Thin sections were stained with potassium permanganate (2% aqueous for 10 min), followed by uranyl acetate (2% aqueous for 10 min), and lead citrate (2% aqueous for 2 min). Specimens were then viewed in an Hitachi H-300 transmission electron microscope operating at 75 KV.

Extant algae used for comparison were either fresh (*Fucus* L. and *Ascophyllum nodosum* [L.] LeJol) or removed from herbarium sheets (*Dictyota dichotoma* [Hudson] Lamouroux and *Polysiphonia* Gerville). Eggs from the fresh material were extracted by placing receptacles in the sun. Upon drying, the thallus between the conceptacle pores shrinks away from the eggs, which are supported by sterile paraphyses. All of the extant material was fixed in 2% potassium permanganate (aqueous) for 30 min, and dehydrated in a graded ethanol series (40%, 60%, 70%, 85%, 95%, 100%, 10 min between changes). All other preparation procedures are identical to those listed above.

DESCRIPTION—*Protosalvinia*—The thallus of *Protosalvinia* is a discoid to irregularly shaped non-coalified compression (Fig. 1). Structurally, the thallus appears to represent a cuticle-like covering which reflects a parenchymatous or pseudoparenchymatous organization of the original plant body beneath. On the surface of approximately 5% of the specimens are 2–10 oval shaped depressions (Fig. 2), each containing a tetrad of spores (Fig. 3). The spores are smooth to irregularly pitted, and are often folded. Individual spores measure up to 200 μm in diam. Empty depressions are rarely seen, and partial tetrads were not observed. Removal of the tetrad from the thallus was usually not possible, and often resulted in the fragmentation of the spores. There is no apparent structural reason to explain this adherence.

The spore wall of *Protosalvinia* ranges from 4–10 μm in thickness, and consists of two distinct zones (Fig. 4, 13). In most instances, the material of which the spore wall is composed has no affinity for conventional heavy metal stains, and adequate contrast is obtained by the activity of the stain on the areas between the subunits—a sort of negative staining. Discrete structural entities are usually surrounded by darkly stained margins which apparently represent the reaction of the stains with the spaces in the wall. For this reason, the relative lack of spaces in the tightly packed inner portion of the spore wall renders interpretation of structural features difficult. The nature of the wall-bound substances which cause this staining reaction is not known. The inner zone represents from 60–85% of the total thickness of the wall.

The ultrastructure of the outer zone differs between regions where adjacent spores are in contact, and where they are not in contact. In non-contact regions, the outer zone appears globular (Fig. 5). In contact regions (Fig. 6), this zone is composed of 8–12 lamellae which consistently decrease in thickness toward the interior of the spore. The innermost lamellae may be as thin as 70 nm, with those toward the outer surface approximately 450 nm in thickness. These lamellae become convoluted especially near the spore equator and within approximately 20 μm of the proximal pole (Fig. 4). In other areas, the lamellae are smooth and compact, and the zone as a whole is relatively thin (Fig. 7). The maximum degree of convolution is seen at the proximal pole, and the resulting increase in thickness is primarily responsible for the elevated nature of the laesurae. Lamellae are sometimes fused in superposed layers (Fig. 7). The outer wall layer ranges from 1.3 to 4.0 μm, and represents from 15 to 40% of the total wall thickness. A transition zone exists within the outer spore wall region between the two laterally continuous zones of differing morphology previously discussed (Fig. 8, 13). This transition zone is thickened, and represents the curvaturae perfectae seen in transmitted light. Thickening usually involves both the inner and outer spore layers.

Beneath each of the laesurae is a faint suture which appears as a slightly darker staining line which bisects the inner spore wall perpendicular to the surface, and a protrusion of this layer into the lamellar units of the outer zone (Fig. 9). There is often an additional layer on the exposed surfaces of the spores (Fig. 8), and

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passes through the inner layer. A more detailed view of this region is illustrated in Fig. 9. ×4,000. **5.** Spore wall showing the globular units of the outer layer (1) in a non-contact region. ×10,000. **6.** Spore wall in a contact region. The surface of contact with the adjacent spore (arrow) is toward the upper left. Note the decreasing thickness of the lamellae toward the lumen of the cell (to the lower right). ×15,000.
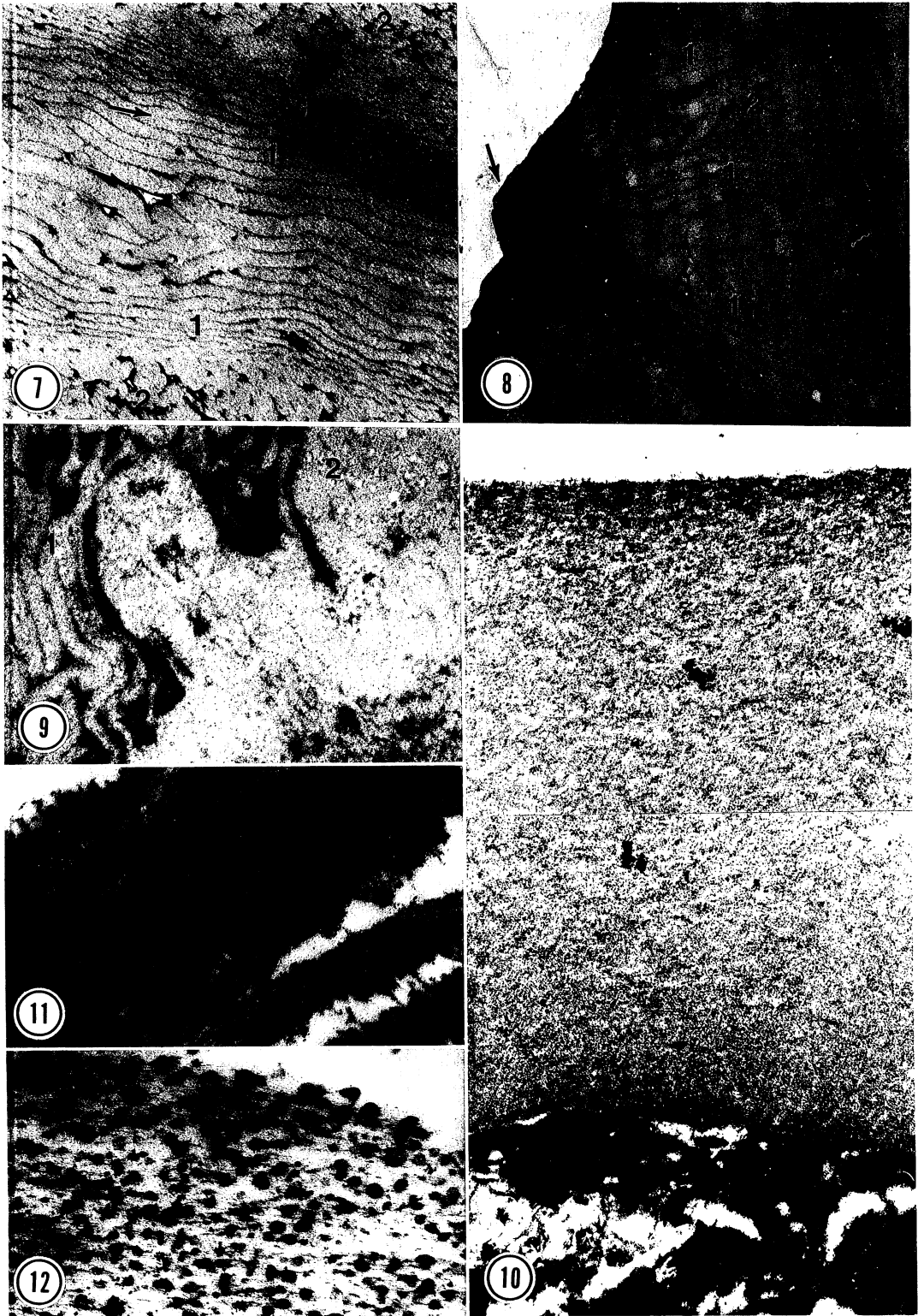
Fig. 7–12. Fig. 7–9. *Protosalvinia* sp. Fig. 10. *Dictyota dichotoma.* Fig. †1. *Ascophyllum nodosum.* Fig. 12. *Fucus* sp. 7. Outer wall regions of two adjacent spores in a contact area showing decreasing lamellae thickness in the outer layers (1) away from the appressed spore surfaces (lower arrow). Note the continuity of lamellae in superposed layers

occasionally between the thallus and spore, which may occupy spaces between adjacent globular subunits. This layer is variable in occurrence and thickness.

*Extant marine algae*—Single-celled reproductive phases of several extant marine algae were chosen as a basis for comparison to *Protosalvinia.* Representatives were chosen based on the following points of similarity. Morphologically, *Protosalvinia* is most reminiscent of a much reduced *Fucus.* A basic distinction between the two, however, is the fact that *Fucus* produces eggs in packets of eight. A related genus in the Fucales, *Ascophyllum,* produces eggs tetrahedrally in packets of four, a feature which is characteristic of the genus (Bold and Wynne, 1985). The tetraspore is another form of meiotic product produced by several groups of algae. One of these within the Phaeophycophyta that produces tetraspores is the Dictyotales. *Dictyota dichotoma* exibits classic dichotomous branching like that seen in some species of *Protosalvinia.* However, in the samples examined for the present investigation, the geometry of the tetrads was variable, with the majority of the spores produced in an isobilateral arrangement. In order to examine a taxon which consistently produces tetraspores in tetrahedral tetrads, it was necessary to examine a member of the Rhodophycophyta. One alga which meets this criterion is *Polysiphonia* (Bold and Wynne, 1985).

The tetraspore wall of *Dictyota dichotoma* (Fig. 10) ranges from 0.5–6.0 µm in thickness depending on the stage of development. The majority of the spore wall stains uniformly, although the inner and outermost portions of the wall may be slightly more darkly stained. The wall is coarsely fibrillar and unstratified. The wall of *Dictyota dichotoma* bears no structural resemblance to that seen in *Protosalvinia,* nor is it resistant to acetolysis.

Phaeophycean eggs have no distinct walls. The structure which encloses them prior to fertilization is known as a mesochite (Pollock, 1970). The mesochite surrounds the entire packet of eggs and unites them in a sac-like
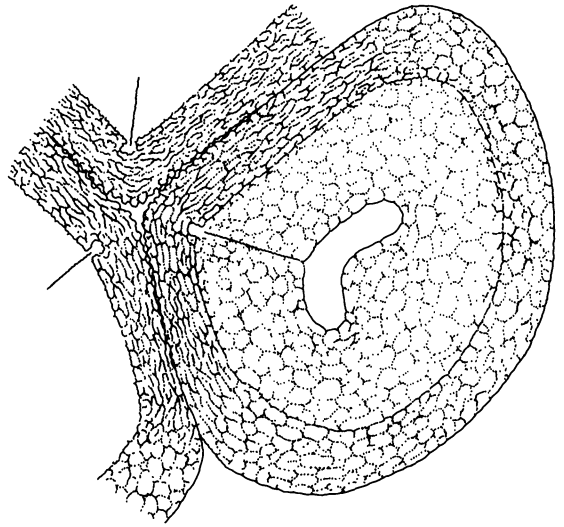


Fig. 13. Section of *Protosalvinia* spore showing the lateral distribution of the various wall layers. Wall thickness and lumen size are not drawn to scale.

organization. In *Ascophyllum nodosum* the mesochite consists of two layers (Fig. 11): an inner, fibrillar, unlaminated zone (ca. 500 nm thick), and an outer, more finely fibrillar, faintly laminated layer (ca. 2.5 µm thick). Ultrastructurally, the mesochite of this alga bears little resemblance to any of the structures associated with *Protosalvinia.*

The mesochite of *Fucus* L. (Fig. 12) differs markedly from that of *Ascophyllum.* It is thinner (ca. 1.25 µm) and has a substructure which stains lightly, is loosely fibrillar, and is distinctly layered in some regions. Incorporated in this loose matrix are darkly staining granules which are largest (ca. 75 nm) at the surface of the egg, and smallest (ca. 40 nm) where the layer contacts the cytoplasm. Based on centrifugal settling characteristics, Pollock (1970) suggested that these dark particles are composed of polyphenolics or tannins which were extruded from the eggs prior to fixation.

There is a gradual decrease in unit size in the outer zone of both the fucalean mesochite and the outer wall of *Protosalvinia* spores. The

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(upper arrow), and the appearance of the inner wall region. ×15,000. **8.** Contact region of two adjacent spores showing the transition zone from lamellae to globular units. This area forms the curvaturae perfectae seen in transmitted light. The surface layer on the outside of the tetrad is also shown (arrow) ×10,000. **9.** Detailed view of protrusion of inner layer (2) illustrated in Fig. 4. ×30,000. **10.** *Dictyota dichotoma.* Tetraspore wall with fibrillar construction and lack of prominent laminations. The outer surface of the spore is at the top of the micrograph. ×18,000. **11.** *Ascophyllum nodosum.* Two layered mesochite with faint lamination of the outer layer (A) and fibrillar construction of both layers. ×18,000. **12.** *Fucus* sp. Mesochite showing weakly staining fibrillar framework and darkly staining particles embedded within. Note the decrease in unit thickness toward the inner surface (bottom of micrograph). ×40,000.

thickness of these two structures is also comparable. However, the fundamental way in which these two layers are distributed—around the entire egg packet in *Fucus,* and around each individual spore in *Protosalvinia*—makes it unlikely that the similarities are anything but coincidental.

Tetraspores of *Polysiphonia* did not withstand acetolysis, nor were their walls visible when prepared by omitting this procedure. These observations are corroborated by the results of a recent investigation of tetrasporogenesis in the red alga *Chondria tenuissima,* in which the staining of the tetraspore wall was very light (Tsekos, Schnepf and Makrantonakis, 1985).

DISCUSSION—Gray and Boucot (1979) refer to *Protosalvinia* as an emergent aquatic plant. One of their arguments is the suggestion that *Protosalvinia* possesses durable-walled meiotic structures with a permanent triradiate scar. Throughout the course of this investigation, no convincing triradiate marks were observed, primarily due to the lack of success in separating the members of the tetrad. This difficulty is probably the result of a meshing of the corrugations in the surface layers of adjacent spores. Ultrastructural examination reveals, however, that there is a pre-formed germinal suture in spores of *Protosalvinia.* This establishes the meiotic nature of these structures.

Citing personal communications with several phycologists, Gray and Boucot (1977) argue convincingly that no extant alga possesses resistant-walled meiotic structures like those seen on *Protosalvinia.* Spores produced by representatives of the red and brown algal groups commonly cited in the literature as producing trilete spores, resemble neither those of vascular plants or of *Protosalvinia* when examined at the ultrastructural level.

The presence of a highly resistant thallus and spore covering is an issue which must be addressed when attempting to evaluate the life strategy of *Protosalvinia.* The hypothesis advanced by Schopf and Schwietering, while providing a credible explanation for the geographic and stratigraphic distribution, fails in this regard. Conversely, Gray and Boucot's arguments rest largely on the desiccation resistant nature of the spores, but do not adequately address the distributional anomalies. Reports of outcrop and subsurface occurrences of *Protosalvinia* in North America are primarily from the westernmost and deepest portions of the Appalachian basin (Schopf and Schwietering, 1970), and the southeastern portions of the Illinois basin (Cross and Hoskins, 1950; Kep-

ferle, 1981). This geographic range has been broadened by the discovery of *Protosalvinia* in the Michigan basin (Matthews, 1983), and a new locality in West Virginia (Schwietering and Neal, 1978). Niklas, Phillips, and Carozzi (1976) report on occurrences of this genus in the central Amazon Basin of Brazil. The fossil is almost exclusively found in organic rich shales which are regarded by many to have been deposited in deep water (Gray and Boucot, 1977). It is difficult to envision a mode of seaward transport which could result in a pattern of occurrences restricted to mid-basin sediments, particularly in light of the singularly resistant nature of the fossils. Such mechanisms require that the fossils be selectively removed from (or not be deposited in) fluvial and nearshore sediments.

In spite of the minor weaknesses of Gray and Boucot's arguments, the information presented here firmly supports the contention that the spores of *Protosalvinia* compare favorably in their durable nature with those that presently are produced exclusively by land plants. Structurally, however, the wall architecture of *Protosalvinia* is quite unlike anything yet reported, fossil or living.

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