Open access • Journal Article • DOI:10.3852/MYCOLOGIA.97.1.269

Perithecial ascomycetes from the 400 million year old Rhynie chert: an example of ancestral polymorphism — Source link

Thomas N. Taylor, Hagen Hass, Hans Kerp, Michael Krings ...+1 more authors

Institutions: University of Kansas, University of Münster, University of Georgia

Published on: 01 Jan 2005 - Mycologia (Mycological Society of America)

Topics: Rhynie chert, Ascocarp, Asteroxylon and Paraphyses

Mycologia, 97(1), 2005, pp. 269–285.

Perithecial ascomycetes from the 400 million year old Rhynie chert: an example of ancestral polymorphism

Editor's note: Unfortunately, the plates for this article published in the December 2004 issue of Mycologia 96(6):1403–1419 were misprinted. This contribution includes the description of a new genus and a new species. The name of a new taxon of fossil plants must be accompanied by an illustration or figure showing the essential characters (ICBN, Art. 38.1). This requirement was not met in the previous printing, and as a result we are publishing the entire paper again to correct the error. We apologize to the authors.

T.N. Taylor[1]

Department of Ecology and Evolutionary Biology, and Natural History Museum and Biodiversity Research Center, University of Kansas, Lawrence, Kansas 66045

H. Hass
H. Kerp

Forschungsstelle für Paläobotanik, Westfalische Wilhelms-Universität Münster, Germany

M. Krings

Bayerische Staatssammlung für Paläontologie und Geologie, Richard-Wagner-Straße 10, 80333 München, Germany

R.T. Hanlin

Department of Plant Pathology, University of Georgia, Athens, Georgia 30602

Abstract: We describe a perithecial, pleomorphic ascomycetous fungus from the Early Devonian (400 mya) Rhynie chert; the fungus occurs in the cortex just beneath the epidermis of aerial stems and rhizomes of the vascular plant *Asteroxylon*. Perithecia are nearly spherical with a short, ostiolate neck that extends into a substomatal chamber of the host plant; periphyses line the inner surface of the ostiole. The ascocarp wall is multilayered and formed of septate hyphae; extending from the inner surface are elongate asci interspersed with delicate paraphyses. Asci appear to be unitunicate and contain up to 16 smooth, uniseriate-biseriate ascospores. The method of ascospore liberation is unknown; however, the tip of the ascus is characterized by a narrow, slightly elevated circular collar. Ascospores appear 1–5 celled, and germination is from one end of the spore. Also present along the stems and interspersed among the perithecia are acervuli of conidiophores that are interpreted as the anamorph of the fungus. Conidiogenesis is thallic, basipetal and probably of the holoarthric-type; arthrospores are cube-shaped. Some perithecia contain mycoparasites in the form of hyphae and thick-walled spores of various sizes. The structure and morphology of the fossil fungus is compared with modern ascomycetes that produce perithecial ascocarps, and characters that define the fungus are considered in the context of ascomycete phylogeny.

Key words: anamorph, arthrospores, ascomycete, ascospores, conidia, fossil fungi, Lower Devonian, mycoparasite, perithecium, Rhynie chert, teleomorph

INTRODUCTION

Among the true fungi the Ascomycota is the largest group, containing more than 3000 genera and approximately 32 000 species, and includes a variety of associations with plants, animals, green algae and cyanobacteria. The principal morphological feature that distinguishes ascomycetes from other fungi is the sac-like structure termed the ascus in which the sexual ascospores are produced. Although historically there have been several major classifications of ascomycetes (Hawksworth et al 1995), the recent use of gene sequence data has resulted in the recognition of three major groups (e.g. Liu et al 1999). These include the Archiascomycetes, or the unicellular yeast-like forms, the Ascomycetous yeasts or Saccharomycetales and the Euascomycetes or filamentous forms that enclose their asci in or on an ascoma (Alexopoulos et al 1996). Among this latter group, the discomycetes, loculoascomycetes, pyrenomycetes and plectomycetes form a well-supported monophyletic group (Berbee and Taylor 2001). The pyrenomycetes and plectomycetes historically are distinguished based on features of the ascoma (Barr 2001). Plectomycetes include fungi with nonostiolate cleistothecia that contain multiple layers of asci, and asco-

Accepted for publication April 27, 2004.
[1] Corresponding author. E-mail: tntaylor@ku.edu

spores are typically unicellular. The pyrenomycetes are characterized generally by flask-shaped, ostiolate perithecia that produce ovoid to cylindrical persistent asci. Asci are produced in a hymenium that also may contain sterile hyphae. Ascospores often are discharged forcibly and may be one to several celled.

As a result of a renewed interest in the Lower Devonian Rhynie chert, numerous fungi have been identified that include several members of the Chytridiomycetes (Taylor et al 1992b), including a blastocladialean (Remy et al 1994a). Also present are several examples of mycoparasites (Hass et al 1994), parasites (Taylor et al 1992a), a glomeromycete including the formation of endomycorrhizae in the land plant *Aglaophyton* (Remy et al 1994b, Taylor et al 1995), and a lichen formed by a cyanobacterium and a fungus (Taylor et al 1997). Well preserved fungal remains within the Rhynie chert can be identified as pyrenomycetes that contain asci and ascospores (Taylor et al 1999). Also present on the same host are conidiophores. These teleomorphic and anamorphic forms, together with various stages in the development of the fungus, provide the opportunity to characterize a new fossil in the Early Devonian ecosystem that can be compared with certain modern filamentous ascomycetes. It is the intent of this paper to describe a perithecial ascomycete from the Rhynie chert containing exceptionally well preserved asci and ascospores (Taylor et al 1999), in addition to the anamorphic state of the fungus.

MATERIALS AND METHODS

The Rhynie chert site consists of more than 10 plant-bearing beds that are represented as siliceous sinters (Trewin and Rice 1992). Information on the geology and setting of Rhynie chert can be found in Rice et al (2002). The age of the chert generally is considered to be Pragian (Early Devonian) based on palynomorph assemblages (Richardson 1967) and radiometric dating (Rice et al 1995). The perithecia occur in the cortex of the land plant *Asteroxylon mackiei* and were studied by means of petrographic thin sections prepared by cementing a small piece of the chert containing the fungus to a microscope slide and grinding the chert to a thickness of approximately 50–150 μm. Observations and micrographs were prepared using oil immersion objectives directly on the polished rock surface without cover glasses. Slides are deposited in the Paleobotanical Collection in the Forschungsstelle für Paläobotanik, Westfälische Wilhelms-Universität, Münster, Germany. Acquisition numbers and types are noted in the figure descriptions and in the diagnosis.

Twelve morphological characters from Barr (2001) for nine ascomycete taxa and *Paleopyrenomycites* were analyzed under maximum parsimony using PAUP 4.0. The analysis was performed using exhaustive search. A total of 64 most parsimonious trees were obtained, each 12 steps long. The analysis did not include anamorphic features.

TAXONOMY

Paleopyrenomycites Taylor, Hass, Kerp, Krings et Hanlin gen. nov.

Generic diagnosis. Ascocarp of globose, nearly spherical perithecia with short neck positioned beneath host stoma; perithecium wall of two layers of septate hyphae; ostiole lined with periphyses, hymenium of elongate, unitunicate asci and paraphyses, ascospores uni- to perhaps multicelled, elongate with monopolar germination; conidiophores unbranched as acervuli; conidiogenesis thallic, basipetal, and possibly holoarthric; arthrospores cube-shaped.

P. devonicus Taylor, Hass, Kerp, Krings et Hanlin

Specific diagnosis. Perithecia beneath epidermis in outer cortical tissues of host, up to 400 μm diam with short (50 μm) neck; perithecium wall multilayered with an inner zone of large (5–9 μm), irregularly oriented hyphae and outer, slightly thicker zone of small (3–5 μm), shorter hyphae that tend to parallel surface of perithecium wall; hymenium extending to just below level of ostiole and formed of intermixed elongate asci and paraphyses, asci nonsynchronous in development, 40–50 μm long and 10–15 μm wide, clavate with a narrow base, ascus tip operculate with a narrow collar; paraphyses thin-walled and extending upward from inner wall to a level slightly above asci; up to 16 ascospores per ascus in both uniseriate and biseriate arrangement, ascospores unornamented, smooth, up to 10 μm long, 1–5 (?) celled, germination at narrow end forming narrow unbranched hypha; conidiophores up to 600 μm long and 10 μm diam, septate, arthrospores 4 × 5 μm diam.

Holotype. Specimen in petrographic thin section slide PB 3411 in the W. Remy Collection permanently deposited in the Forschungsstelle für Paläobotanik, Westfälische Wilhelms-Universität, Münster; FIGS. 2, 7, 12, 22–24 in this paper.

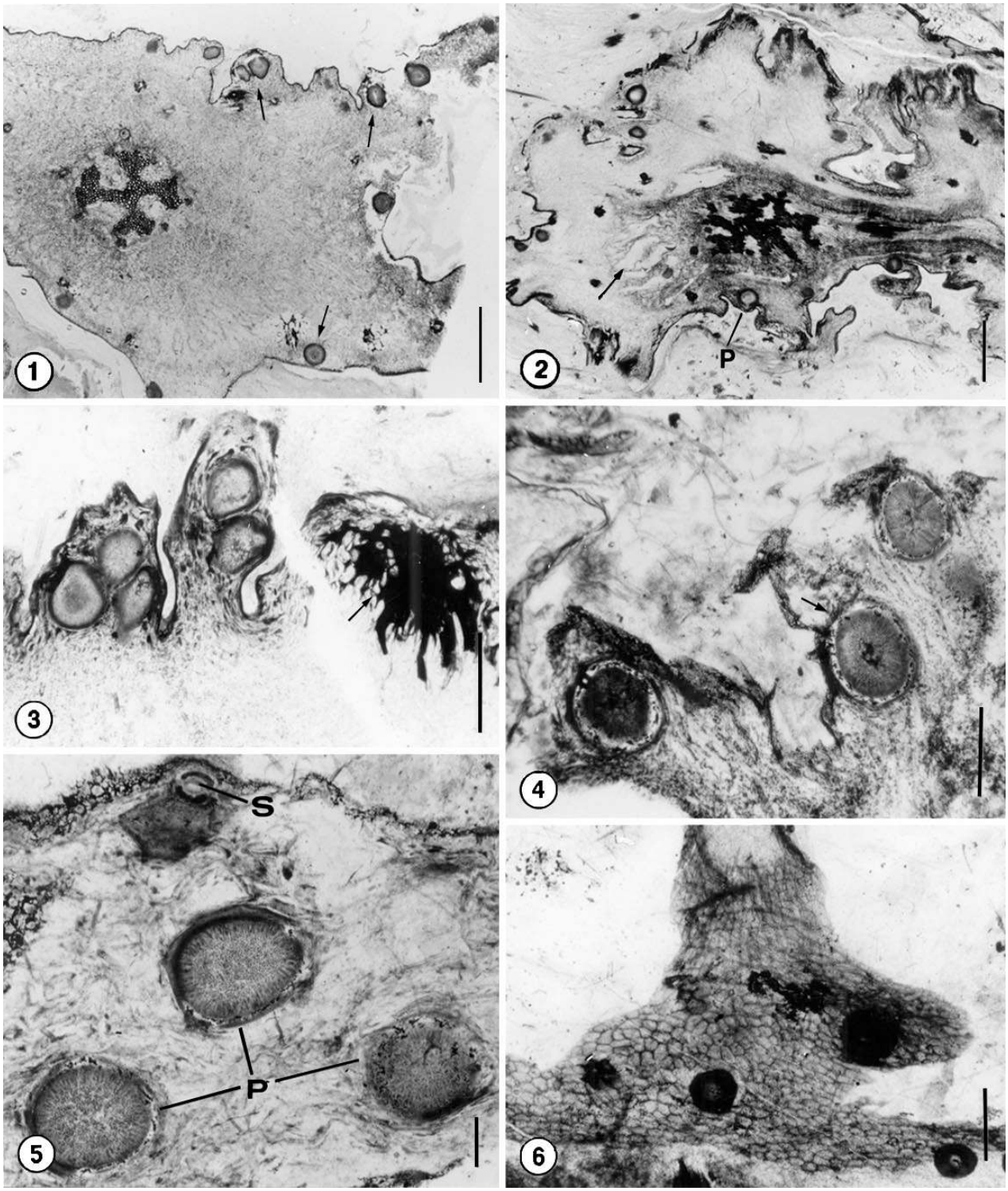
Paratypes. Perithecia present in slides PB3401, 3404, 3409, 3410, 3412, 3413, 3414, 3416, 3417, 3418, 3433, 3436, 3437, 3441, 3445, 3469 in the above collection; FIGS. 1, 3–6, 8–11, 13–21, 25–45 in this paper.

Type locality. Rhynie, Aberdeenshire, Scotland. National Grid Reference NJ 494276 (Edwards 1986).

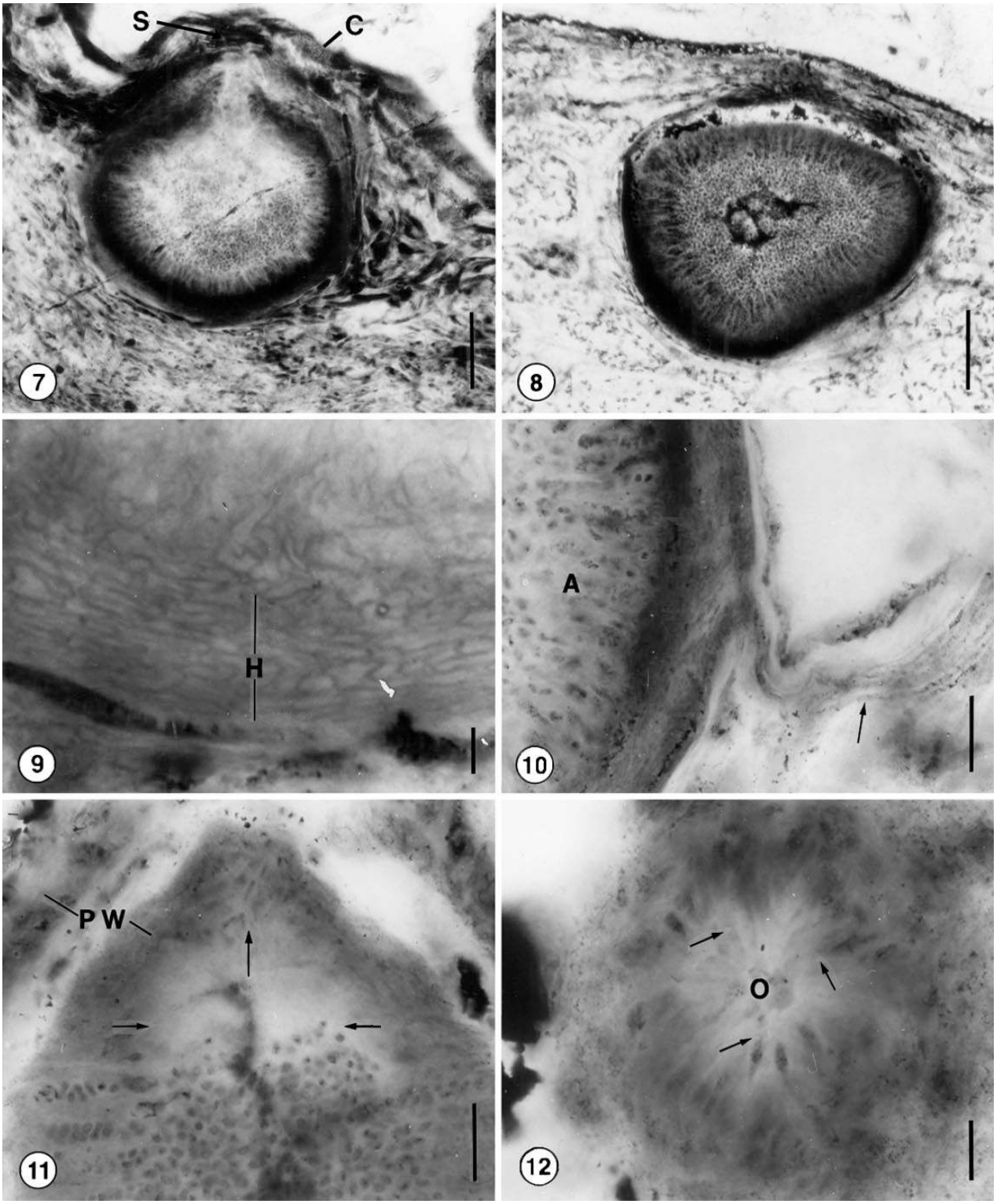
Age. Early Devonian.

Stratigraphic position. Pragian.

Etymology. The generic name is *Paleopyrenomycites* is proposed as a combination of *palaios,* ancient, and the informal class of ascomycetes, pyrenomycetes; the ending *ites* is used to designate a fossil taxon as sug-



FIGS. 1–6. *Paleopyrenomycites devonicus*. 1. Transverse section of *Asteroxylon* rhizome with numerous perithecia (arrows) in cortex just beneath epidermis. Slide 3404, Bar = 1.0 mm. 2. Older aerial stem at transition level with cortical trabeculae (arrow). Perithecium (P) the same as that in FIG. 7. Slide 3411, Bar = 1.0 mm. 3. Several closely associated perithecia. Note necrotic area (arrow) in cortex. Slide 3409, Bar = 0.5 mm. 4. Partially decayed stem showing three perithecia; wall of perithecium disassociated (arrow). Slide 3433, Bar = 250 μm. 5. Cluster of mature perithecia (P) in cortex. Note oblique section of perithecium just beneath thickened guard cells and stoma (S). Slide 3437, Bar = 100 μm. 6. Base of enation with several perithecia. Slide 3469, Bar = 0.5 mm.



FIGS. 7–12. *Paleopyrenomycites devonicus.* 7. Medial longitudinal section of perithecium showing central cavity and ostiole. Note relationship with stoma (S) and cuticle (C) of host. Slide 3411, Bar = 100 μm. 8. Transverse section of perithecium showing asci and free ascospores in central cavity. Slide 3410, Bar = 100 μm. 9. Detail of perithecium wall showing outer horizontal layer (H) and inner zone of more vertically oriented, shorter hyphae. Slide 3416, Bar = 5 μm. 10. Mature perithecium showing compressed nature of wall hyphae and delicate hyphae (arrow) extending in to cortex of host. Note mature asci (A). Slide 3437, Bar = 25 μm. 11. Longitudinal section showing position of ostiole (arrow) and decayed perithecium wall. Note that the upper level within the perithecium is devoid of asci (horizontal arrows). Slide 3437, Bar = 25 μm. 12. Transverse section through perithecium neck showing ostiole (O) surrounded by periphyses (arrows). Slide 3411, Bar = 10 μm.

gested by Pirozynski and Weresub (1979). The specific epithet *devonicus* refers to the geologic age of the Rhynie chert.

RESULTS

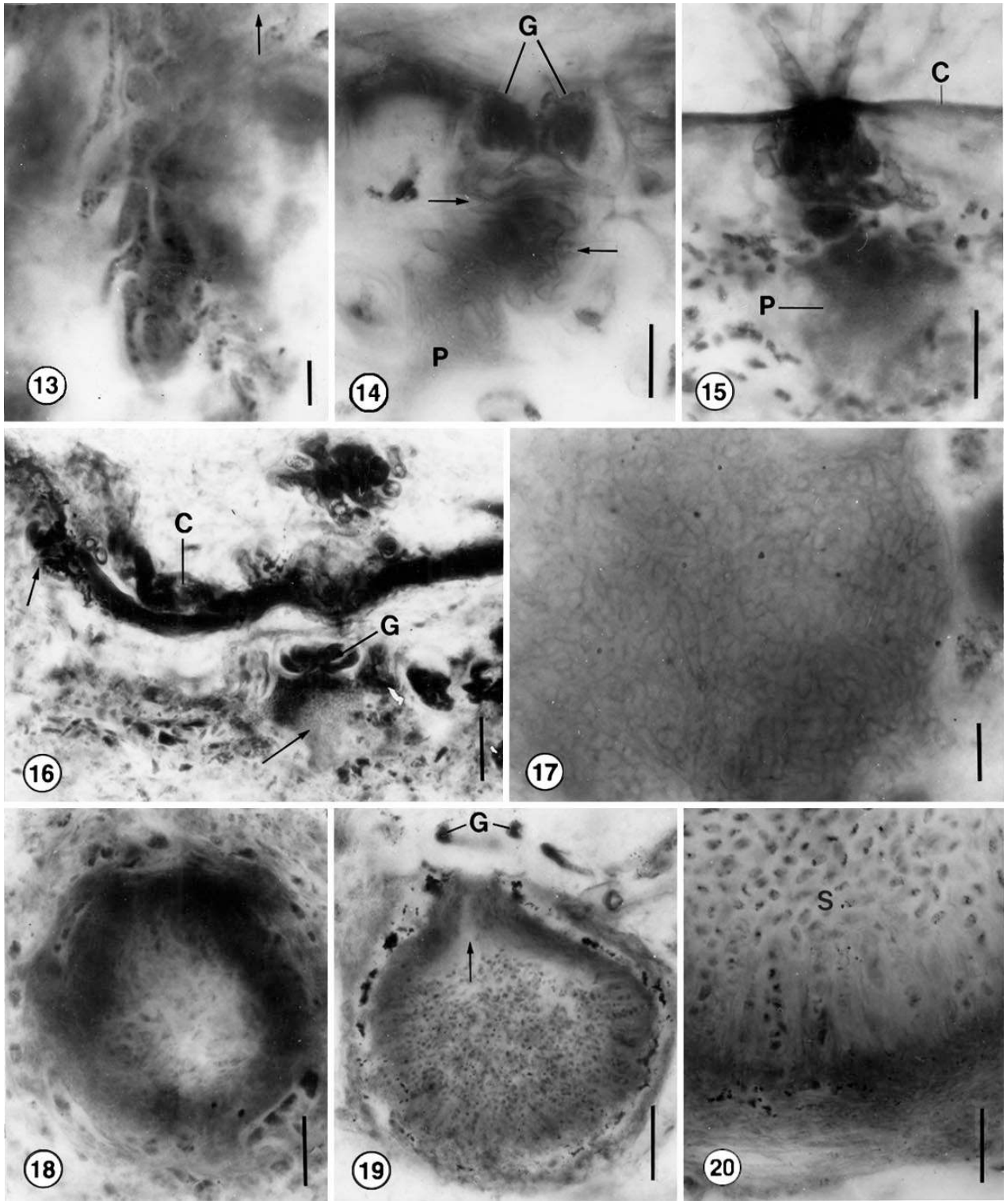
General morphology.—Sections of the lycopod *Asteroxylon mackiei* contain numerous perithecia randomly scattered in the cortical tissues just beneath the epidermis on aerial stems and rhizomes. On the rhizomes they tend to be clustered near the bases of enation-bearing stems and are especially numerous on the aerial stems in the transition region just below the level of the enations. When associated with enations the perithecia are most numerous at the bases (FIGS. 3, 4), however, a few extend out onto the lamina a short distance (FIG. 6); a few immature perithecia are close to the distal margin. Perithecia typically are solitary but some may occur in loosely defined clusters within the cortical tissues (FIG. 5). In some stems the cortical tissues of the host in the region of the perithecia appear necrotic with no clearly defined cell walls visible; sometimes the wall of the perithecium has been partially decayed (FIG. 4). In other instances the cortical tissues are replaced by opaque material (FIG. 3). These regions appear similar to cortical tissues in some extant host plants infected by perithecial fungi. There does not appear to be any difference in the distribution of ascocarps in either large, older stems as defined by axis diameter and presence of cortical trabeculae (FIG. 2), or narrow, less mature axes. Stems with disrupted, necrotic cortical parenchyma contain mature perithecia only in which most of the ascospores appear to have been released.

Perithecia are globose to nearly spherical or sometimes slightly elongate due to crowding when found in clusters (FIG. 3). Mature perithecia are up to 400 μm diam, and characterized by a slightly elongate neck up to 50 μm long through which mature ascospores are released. The neck typically is associated closely with the substomatal chamber of the host plant and often positioned just beneath the guard cells (FIGS. 5, 7). In many specimens the development of the perithecium results in the displacement of cuticle in the region of the guard cells (FIGS. 5, 7, 19).

Extending through the neck of the perithecium is a narrow ostiolar canal approximately 20 μm diam (FIG. 7). In a mature perithecium the canal is slightly tapered distally so that the ostiole is relatively narrow at the distal end of the neck (FIG. 12). Lining the inner surface of the canal are numerous, short, thin-walled periphyses (FIG. 12). They measure approximately 2 μm diam and up to 15 μm long. Near the neck of the perithecium the periphyses appear to be slightly directed upward toward the ostiole (FIG. 11). Periphyses appear to be adpressed to the wall of the ostiolar canal (FIG. 19) in more mature perithecia based on the extent of ascus development.

The wall of the mature perithecium is up to 50 μm thick and constructed of two distinct layers. In section view the perithecia often appear angled, with protrusions where the ascocarp is pushed up against cells in the host cortex (FIG. 8). The outer pseudoparenchymatous layer is approximately 20–30 μm thick and consists of tightly packed, parallel, septate hyphae that are aligned with the surface of the perithecium (FIG. 9). Hyphae of this layer often appear brick-like in organization with the individual hyphae 3–5 μm diam; some of these cells are swollen. To the inside of this zone is a slightly thinner (15–20 μm thick) region of larger (5–9 μm), more irregular hyphae (FIG. 17). This layer decreases distally and is absent in the neck of the perithecium. As asci mature and ascocarp development continues, the outer zone of the wall becomes disassociated, often appearing as opaque bands on the periphery of the hymenium (FIGS. 8, 19). FIGURE 10 shows several delicate hyphae (each approximately 1 μm diam) separated from the outer wall of the perithecium. The outermost zone of the ascocarp is irregular and might represent some type of melanized layer.

The large number of perithecia in axes of *Asteroxylon,* often at different stages of development, afford some insight into the development of the ascocarp in *Paleopyrenomycites.* What we interpret as an early stage in the formation of the perithecium consists of a partially coiled cluster of interwoven hyphae, some with swollen regions (FIG. 13). Hyphae that make up this aggregate are approximately 2 μm diam., tightly intertwined and distinct from the larger hyphae of the conidiophores that may be in the same region (FIG. 15). As ascocarp development continues vegetative hyphae fill the substomatal chamber of the host (FIG. 16). At this stage the perithecium is less than 100 μm diam, about one-fifth the diameter of a mature ascocarp. There is no recognizable differentiation of the centrum at this stage (FIG. 15), although in some perithecia a few asci are present and these contain a single nucleus. There are also no well defined wall layers or outer boundary layer in immature perithecia. What is interpreted as an early stage in the formation of the centrum is illustrated (FIG. 18). Expansion and disintegration of the pseudoparenchyma cells ultimately form the perithecium wall, and further growth of hyphae in the apical region result in the formation of the neck. As development continues the central region of the perithe-



FIGS. 13–20. *Paleopyrenomycites devonicus*. 13. Tangled hyphae of early stage of perithecium. Arrow indicates surface of host. Slide 3410, Bar = 5 μm. 14. Large hyphae of conidiophore in substomatal chamber beneath guard cells (G) and possible early stage of perithecium (P). Slide 3445, Bar = 25 μm. 15. Conidial stage extending through cuticle(C) of host and immature perithecium (P). Slide 3441, Bar = 50 μm. 16. Immature perithecium (arrow) beneath guard cells (G). Host tissue is poorly preserved except for cuticle (C). Arrow at left identifies another stoma. Slide 3417, Bar = 50 μm. 17. Oblique section of perithecium wall showing organization of short, irregular hyphae. Slide 3441, Bar = 10 μm. 18. Perithecium with differentiated wall and central lumen, but lacking asci. Slide 3436, Bar = 50 μm. 19. Mature perithecium beneath guard cells (G). Note decayed wall, ostiole (arrow), and asci. Slide 3436, Bar = 50 μm. 20. Detail of mature perithecium showing asci and paraphyses. Note free spores (S) in the central region. Slide 3437, Bar = 20 μm.

cium becomes more conspicuous because paraphyses and asci are easier to distinguish (FIG. 19).

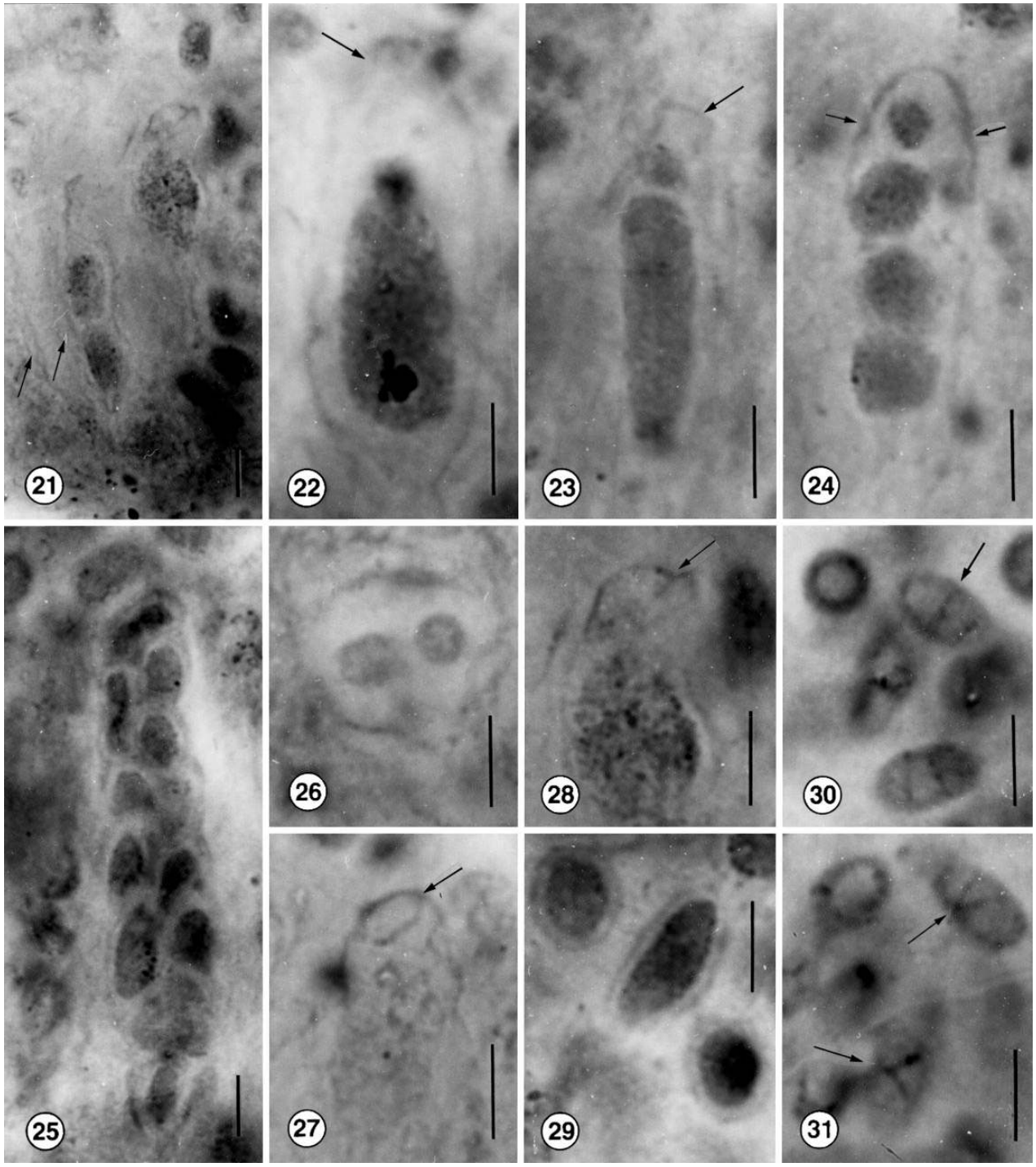
The centrum consists of asci and sterile hair-like paraphyses that line the inner surface of the hymenium except in the region of the slightly extended neck. Paraphyses are scattered among asci and arise from the inner wall of the perithecium (FIG. 9). They generally are about the same length as the asci. While it is difficult to distinguish paraphyses from immature asci, paraphyses do not appear truncated at the base. Paraphyses have variable diameter (up 15 μm), unbranched, aseptate and up to 50 μm long (FIG. 21). The tips are tapered, and there is no suggestion that they fuse to form an epithecium. Some appear twisted due to compaction among asci or in those asci that have released spores. Irregular fragments between mature asci in the perithecium suggest that paraphyses might have deliquesced as the asci matured. In some perithecial ascomycetes the deliquescence of paraphyses and asci serves as an aid in dispersal.

Asci.—Asci in *Paleopyrenomycites* do not appear to be synchronous in development because both mature and immature asci are closely associated in the perithecium (FIG. 7). In early stages of development the ascus is spherical (approximately 10 μm diam) and sometimes contains a large, dense inclusion. Mature asci are approximately 50 μm long, cylindrical to slightly clavate, sometimes appearing widest at the midlevel. At the base is a narrow, short stalk. The changes that occur in the shape of the ascus are consistent with reports on modern ascomycetes in which the highly flexible ascus wall changes to accommodate the enlarging ascospores and as a result of compaction due to the closely spaced asci and paraphyses. Raju (2002) suggests that in some modern species of *Neurospora* ascus shape is controlled genetically and linked to dispersal adaptations.

Asci in *Paleopyrenomycites* appear to be most similar to the unitunicate type, characterized by a single, uniformly thickened wall and distinct pore at the distal end (Luttrell 1951). In early stages of development the tip of the ascus is differentiated into a small (2 μm diam) protrusion that extends up from the margin of the ascus approximately 3 μm. This structure appears to be consistent in immature asci (FIGS. 22, 23). In mature asci this region becomes differentiated into a narrow collar that surrounds a circular, rounded cap (FIG. 28). FIGURE 27 shows the operculum and slightly thickened ring where the cap was attached to an ascus through which the ascospores have already been released. Some ascomycetes possess a refractive ring or thickening in the apical region of the ascus (Wong et al 1999). In modern ascomycetes this structure has been useful as a taxonomic feature; however, ultrastructural studies of ascus development indicate that many ascus types exist, including intermediate forms (Read and Beckett 1996). There is only a slight suggestion of an apical thickening in the ascus tip of the fossil. Perhaps in *Paleopyrenomycites* the original cap dehisced or deliquesced to release spores. One immature ascus that contains four spores also shows a slight invagination (FIG. 24, arrows) in the ascus tip and might represent an early stage of ascus differentiation leading to the formation of the distal collar.

Ascospores.—Immature asci contain slightly elongate structures that we interpret as the remains of cytoplasm; in some of these are more opaque structures that might be remnants of nuclei (FIG. 22). As ascus development continues there is generally a uniform separation in the cytoplasm (FIG. 21). In other asci, however, the cytoplasm becomes more elongate with the material at the distal end separated to form a spore-like aggregation (FIG. 23). At the base of this ascus the cytoplasm contains a dense region of opaque material suggestive of a nucleus (FIG. 23). The ascus contains four ascospores that are aligned in a linear arrangement (FIG. 24). Three have uniform diameter (5 μm), and the distal one is slightly smaller because of the plane of section. The number of ascospores produced in each mature ascus is difficult to determine in thin section preparations, but 16 per ascus appears to be the upper limit, with some arranged in either a uniseriate or biseriate pattern (FIGS. 11, 25). FIGURE 26 is a section through the approximate midlevel of an ascus showing two ascospores in a biseriate arrangement. Numerous free ascospores are within the cavity of the perithecium, suggesting that the spores were released passively into the cavity of the perithecium (FIGS. 19, 20). The spore aggregation perhaps then was exuded in a mass through the ostiole.

Immature ascospores typically are circular to globose, while mature spores range from fusiform to elongate, often with slightly rounded ends. Mature spores generally are circular in transverse section and up to 10 μm long (FIG. 29). Well preserved forms possess an outer, uniform zone 0.6 μm thick that surrounds the spore body (FIG. 29). This layer generally is absent in spores that have undergone septation and is interpreted as a mucilage sheath or gelatinous coating (Read and Beckett 1996). None of the spores appears united by this sheath-like coating. Ascospores of *Paleopyrenomycites* lack any distinctive pattern of ornament; the opaque, often granular appearance of the surface in some spores (FIG. 29) is interpreted as the result of the cell contents.



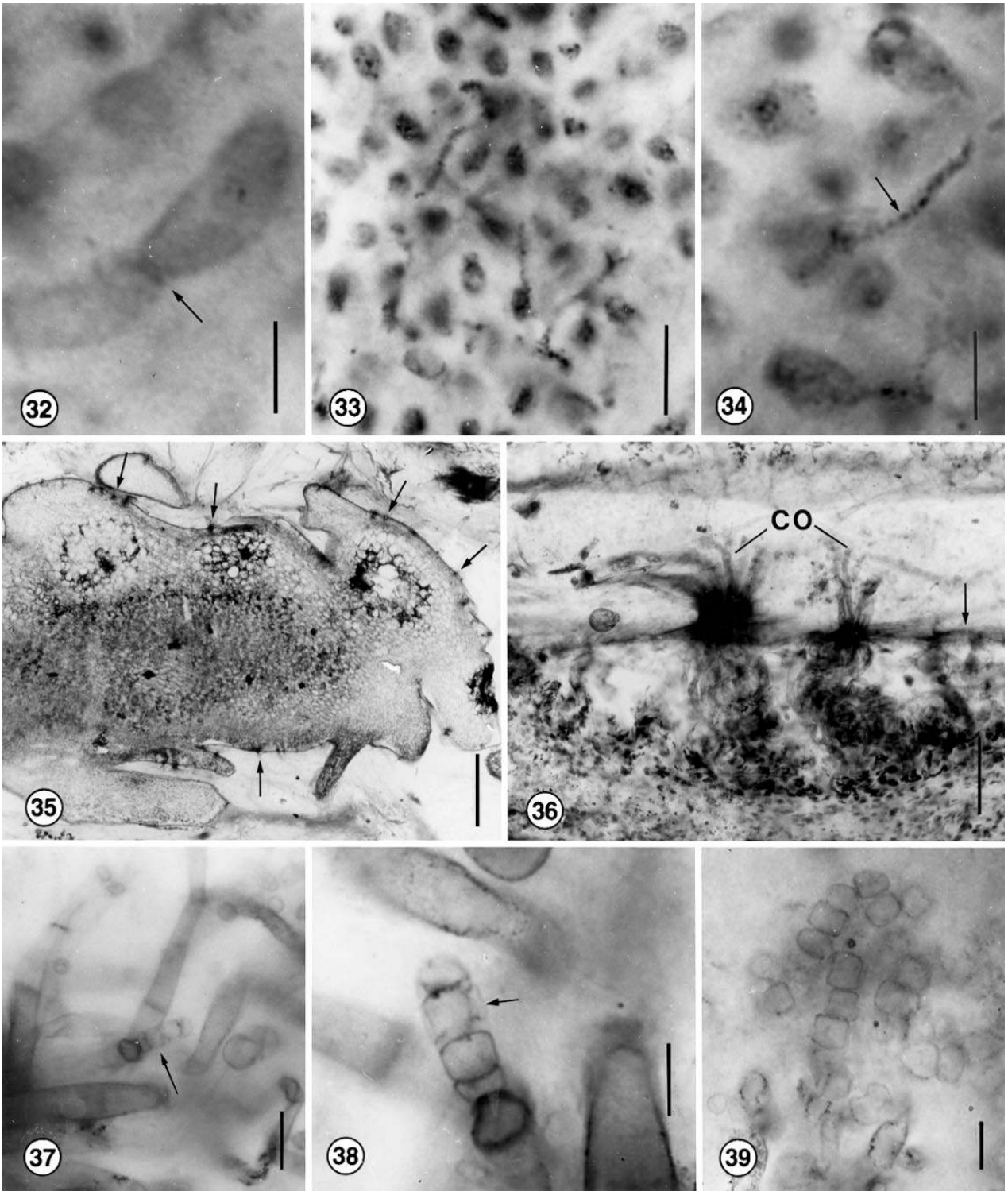
FIGS. 21–31. *Paleopyrenomycites devonicus*. 21. Two immature asci, one with binucleate ascus; the other a single nucleus. Arrows indicate delicate paraphyses. Slide 3441. 22. Uninucleate ascus. Arrow indicates collar of operculate ascus tip. Slide 3411. 23. Binucleate ascus with operculate tip (arrow). Slide 3411. 24. Four-nucleate (?) stage in immature ascus. Note slight invagination at ascus tip (arrows) that may represent an early stage in the development of the collar. Slide 3411. 25. Mature ascus showing biseriate arrangement of ascospores. Slide 3418. 26. Transverse section of ascus showing arrangement of two spores. Slide 3436. 27. Ascus tip with operculum removed (arrow). Slide 3436. 28. Immature ascus showing collar (arrow) and operculum. Slide 3441. 29. Mature ascospores with thick wall. Slide 3418. 30. Ascospores with transverse septations (arrow). Slide 3436. 31. Ascospores showing oblique cross walls (arrows). Slide 3433. All bars = 5 μm.

Ascospores within asci generally are unicellular, but a few appear multiseptate. Within the cavity of the perithecium and the matrix surrounding the fossils, 1–5 septate-like forms are common (FIG. 30). In these ascospore septation typically is uniform, in which the septum divides the spore into nearly equal segments (FIG. 30). In some ascospores, however, septa appear to be obliquely positioned, resulting in the production of cuneate cells (FIG. 31), or with walls formed at right angles to the secondary wall, resulting in the formation of dictyospores. Along some spore walls are minute, spherical, opaque bodies that are conspicuous in transmitted light (FIGS. 30, 31). We are uncertain whether these represent refraction properties of the fossilized spore wall or some cytoplasmic component such as guttules. Another interpretation of the multiseptate spores is that the septations are an artifact in which cytoplasm has condensed between oil droplets in the spores. None of the ascospores shows evidence of a specialized germ pore or slit at either end. In some perithecia and within the matrix aggregations of ascospores have germinated (FIG. 33). In the earliest stage of germination a small bulb-like protrusion extends from one end of the spore. This is followed by the development of a narrow (0.4 μm), generally unbranched germ tube that may be up to 10 μm long. A small number of germ tubes show a single dichotomy at the tip (FIG. 33), and dark granules often are conspicuous along the length of the germ tube (FIG. 34). One end of the spore is flattened slightly and appears attached to an adjacent spore-like structure (FIG. 32). The diffuse nature of the wall of the second ''spore'' suggests that this material might represent an expanded strand of mucilage extruded from a spore body that functioned to hold an aggregate of spores together, perhaps in dispersal from the perithecium (Ingold 1933). It also might represent an early stage in the germination of the ascospore or a young ascus with a central, diffuse diploid nucleus before the stage illustrated in FIG. 22.

Anamorph.—Scattered along the axes of *Asteroxylon,* and intermingled with immature perithecia, are small tufts of conidiophores (FIG. 35). Based on the close and constant physical association with the teleomorph, we suggest that these represent the anamorph of *Paleopyrenomycites.* Conidiophores occur as acervuli that arise from subcuticular, tightly aggregated masses of somatic hyphae and rupture the cuticle of the host plant. Somatic hyphae associated with the conidiophores just beneath the cuticle appear to have short segments of 4–8 μm (FIG. 15). Some of these hyphae possess enlarged regions and have more narrow segments suggestive of chlamydospores from which possibly additional acervuli were produced. As a result of the growth of somatic hyphae and the formation of conidiophores, a shallow cavity is formed beneath the cuticle of the host or the cuticle becomes markedly separated from the host epidermis to form a shallow depression (FIG. 36). Only a few conidiophores appear to be associated with stoma of the host; most of them push through and rupture the cuticle (FIG. 36). No conidiophores appear to be associated with mature perithecia. Acervuli consist of a few to more than 20 conidiophores, which extend 150–600 μm up from the cuticle of the host. Conidiophores are smooth and only rarely branch. They are narrow (3–4 μm) at the base and expand distally (5–10 μm). Septation of conidiophores occurs at intervals of 50–100 μm, except at the base where segments are shorter (about 20 μm). Two spores (one below the cuticle, the other above) are associated with hyphae that extend below the cuticle of the host (FIG. 36). These might represent ascospores or, more likely, swollen arthrospores just before germination.

Conidial development in *Paleopyrenomycites* appears to be thallic, in which there is septation of a conidiophore into distinct conidia (Hennebert and Sutton 1994). This may involve the tip of the conidiophore (FIG. 39) or a side branch (FIG. 38). Development of conidia appears to be holoarthric and basipetal with the oldest conidium at the tip (Alexopoulos et al 1996). Another possible interpretation is that conidiogenesis in *Paleopyrenomycites* is enteroblastic phialidic like that in the modern fungus *Chalara.* In a few conidiophores an outer wall is suggested (FIG. 38, arrows), which would imply enteroarthric conidiogenesis (Cole 1981, Ulloa and Hanlin 2000). Mature arthrospores have slightly rounded ends, are nearly cube-shaped and 4×5 μm diam. Despite the fact *Paleopyrenomycites* is pleomorphic, the organization and morphology of the anamorphic stage provides no obvious clues as to the relationship of the fossil with modern groups.

Mycoparasites.—A variety of mycoparasites have been identified among Rhynie chert organisms and include epibiotic, interbiotic and endobiotic forms (Hass et al 1994). Within several perithecia of *Paleopyrenomycites,* or associated with them, are other fungal hyphae and spores that are interpreted as mycoparasites (FIGS. 41–44). These include tightly coiled hyphae that surround ascospore-containing perithecia, in which the wall of the ascocarp is degraded extensively (FIG. 41). Other perithecia with dispersed ascospores contain smooth, septate hyphae that ramify throughout the perithecial locule and extend out from the ostiole (FIG. 42). These hyphae are 2–4 μm



FIGS. 32–39. *Paleopyrenomycites devonicus*. 32. Possible ''double'' ascospore showing incomplete wall formation (arrow). Slide 3401, Bar = 5 μm. 33. Several ascospores with germ tubes. Slide 3433, Bar = 10 μm. 34. Germ tube (arrow) extending from end of ascospore. Slide 3437, Bar = 5 μm. 35. Oblique section of *Asteroxylon* stem at transition level showing numerous conidiophores (arrows) erupting from surface. Slide 3445, Bar = 1.0 mm. 36. Detail of conidiophores (CO) on surface of stem in FIG. 35. The cuticle (arrow) has been separated from the host tissue by the extensive development of somatic hyphae. Slide 3445, Bar = 100 μm. 37. Conidiophore branches (arrow). Slide 3445, Bar = 20 μm. 38. Conidiophore side branch in early stage of holothallic conidia development. The arrow indicates possible outer wall of conidiophore. Slide 3445, Bar = 10 μm. 39. Arthric conidia showing disarticulation. Slide 3445, Bar = 10 μm.

wide and often are characterized by short lateral branches that arise at right angles. Small spores (chlamydospores?) occasionally are present within the perithecia and interspersed among mature asci. These spores are up to 20 μm diam; they have a thin, multilayered wall and typically possess a central, opaque inclusion approximately 4 μm diam (FIG. 44).

Also present in the host plant are larger septate hyphae (10–12 μm diam) with oblique lateral branches (FIG. 45). They are approximately the same size as the extraradical hyphae of *Glomites rhyniensis,* an arbuscular mycorrhiza that colonizes axes of *Aglaophyton,* another Rhynie chert plant (Taylor et al 1995). Numerous chlamydospores also are present in the cortical tissues of the host (FIG. 40). These have a range of 240–360 μm diam and fall within the size and morphology of the morphotype *Palaeomyces* (Kidston and Lang 1921). In cursory examination at low magnifications it might be difficult to distinguish between the chlamydospores and perithecia in sections of host axes because both are approximately the same diameter. This might explain in part why perithecial ascomycetes have not been identified previously from Rhynie chert plants. However, the chlamydospores generally are imbedded more deeply within the host, while perithecia tend to be distributed just beneath the epidermis (FIG. 40).

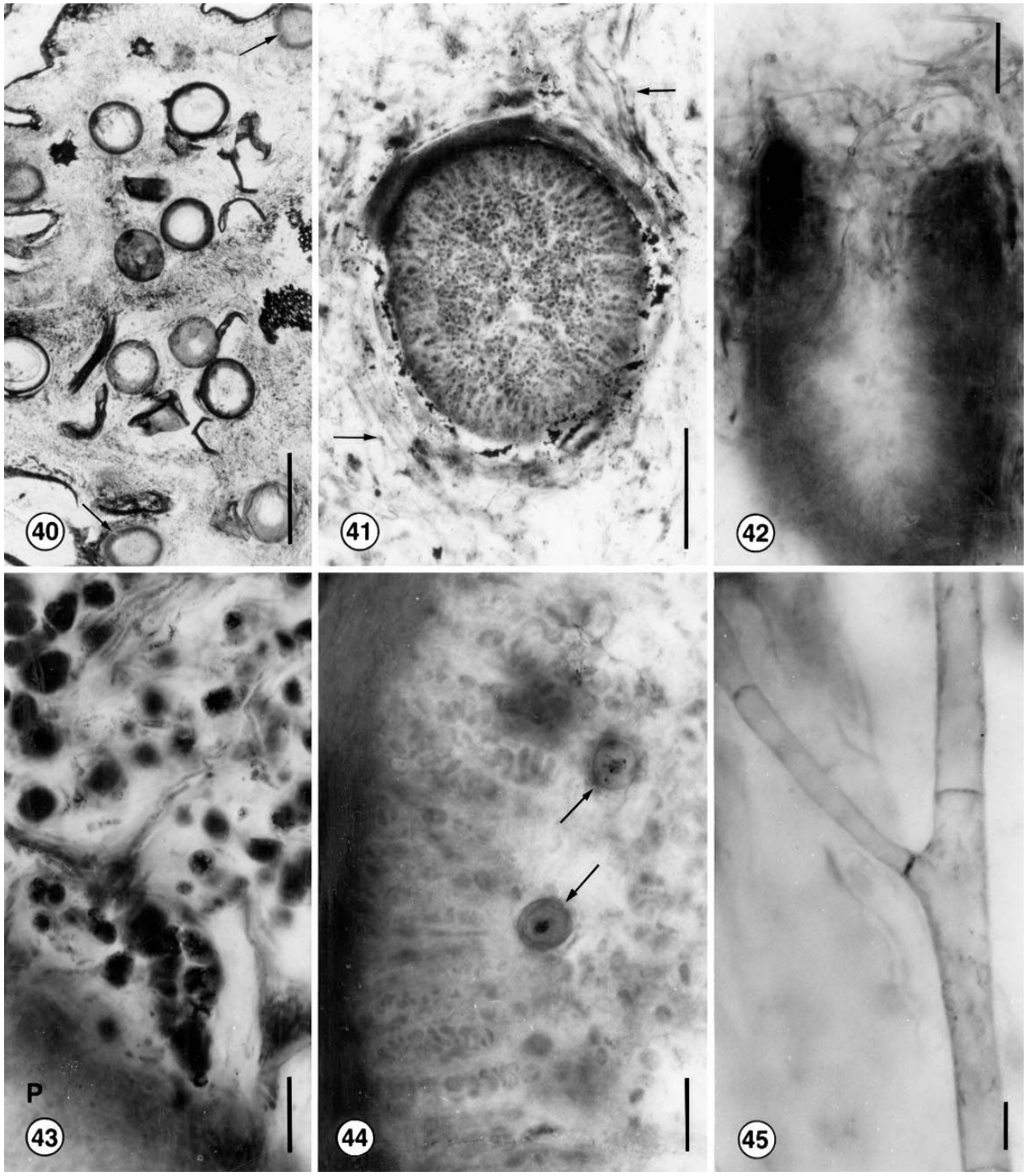
DISCUSSION

The fossil history of ascomycetes remains poorly understood (Taylor 1994). The earliest fossils appearing to have affinities with the ascomycetes come from the mid–late Silurian and consist of chains of septate spores with scars such as those produced by certain conidial fungi (Sherwood-Pike and Gray 1985). Also present are hyphae with perforate septa, including some bearing short branches that resemble conidiophores that produce phialides. Because the fossils were discovered by digesting rock fragments, nothing is known about the host of these fungi. Ascomycetes also have been reported from the Lower Devonian in the form of elliptical structures interpreted as thyriothecia (Krassilov 1981) and small spore-like bodies considered to represent ascomata (Pons and Locquin 1981). However, none of these studies reports evidence of asci containing ascospores.

Several spore-like structures termed sporocarps are known from the Carboniferous. They consist of a wall of interlaced hyphae that surrounds a central cavity containing thin-walled spores, some of which contain still smaller spores (Stubblefield and Taylor 1983). Although such sporocarps initially were interpreted as cleistothecia containing spherical asci and ascospores, these fossils now are believed to be the fruiting bodies of a zygomycetous fungus in which the smallest spores are interpreted as mycoparasites (Taylor 1994). Another problematic Carboniferous fungus with potential ascomycetous affinities is *Palaeosclerotium,* a sporocarp that contains asci and ascospores. This sporocarp is associated with a basidiomycete that also might constitute a mycoparasitic association (Rothwell 1972). Several cleistothecium-like fossils from the Triassic are interpreted as ascomycetes (White and Taylor 1988); however, all of these lack clear evidence of asci and septate hyphae. From the Cretaceous onward scientists have found numerous examples of microthyriaceous fungi, some of which are identical to modern ascomycetes (e.g. Alvin and Muir 1970, Daghlian 1978).

Numerous attempts have been made to classify the Ascomycota, using a full range of available techniques and character states (Barr 2001). Until the advent of molecular approaches, the principal features used to define the major groups of Euascomycetes were the morphology and structure of the ascocarp, including the organization of the ascus, development and septate mycelium. Although several recent reports underscore the monophyletic nature of the group with support at 99% (Berbee and Taylor 2001), bootstrap support is weak for the branching order along the backbone of the tree (Tehler et al 2000). However, two groups of ascomycetes are regarded as monophyletic based on trees constructed from rDNA (Samuels and Blackwell 2001) and the nuclear gene RPB2 (Liu et al 1999). These include the pyrenomycetes and plectomycetes. Ascomycetes with unitunicate asci produced within a perithecial ascocarp historically have been included in the formal taxonomic group Pyrenomycetes (Alexopoulos et al 1996), while those with closed ascocarps were placed in the Plectomycetes. However, pyrenomycetes have been used more recently in a descriptive sense for fungi with perithecial ascocarps and unitunicate asci (Samuels and Blackwell 2001). Within the group are endophytes, parasites of plants, mammals, fungi, saprobes, and various symbionts of arthropods that can be found in an extensive array of ecosystems (Alexopoulos et al 1996). The presence of a perithecial ascocarp and apparently unitunicate ascus suggest that the closest affinities of *Paleopyrenomycites* might lie with the pyrenomycetes.

The development of the centrum and features associated with the ascus also have been used as taxonomic characters to help define major groups within the pyrenomycetes (Luttrell 1951). Of these, five centrum types possess unitunicate asci. To a large extent the centrum types relate to sterile tissues that occupied the perithecial cavity in which asci developed.



FIGS. 40–45. *Paleopyrenomycites devonicus*. 40. Axis containing numerous thick-walled chlamydospores, probably of *Glomites*. Arrows indicate perithecia of about the same size. Slide 3412, Bar = 0.5 mm. 41. Mature perithecium with decayed wall. Arrows indicate closely associated hyphae of mycoparasite. Slide 3437, Bar = 100 μm. 42. Longitudinal section of perithecium and tangled network of hyphae. Slide 3414, Bar = 50 μm. 43. Portion of perithecium (P) with very small hyphae of associated external fungus. Slide 3413, Bar = 25 μm. 44. Section of perithecium showing asci and mycoparasites (arrows). Slide 3410, Bar = 20 μm. 45. Somatic hyphae associated with chlamydospores in several axes of *Asteroxylon* containing perithecia. Slide 3410, Bar = 10 μm.

Using these centrum features, two types appear most closely associated with the mature perithecium of *Paleopyrenomycites*. One of these, the Xylaria-type, contains numerous spherical asci and paraphyses that develop from the inner surface of the base and sides of the perithecium and, as a result, expand the ascocarp to form the central cavity. Hyphal growth at the apex results in the formation of an ostiolate neck containing periphyses. Asci develop upward over an extended period among the paraphyses, resulting in a continuous hymenium of asci and paraphyses (Luttrell 1951). Ascospores may be symmetric to asymmetric and uni-bicelled. Some authors include some Diatrypales and Amphisphaeriales within the Xylariales.

In the Diaporthe-type the expansion and breakdown of a group of pseudoparenchymatous cells results in the formation of the perithecial cavity embedded in the host tissue. Paraphyses that typically deliquesce at maturity are scattered among asci. Uecker (1994) notes that the Xylaria and Diaporthe-types may be distinguished by the expanded subhymenial pseudoparenchyma and ephemeral paraphyses in the Diaporthe-type, whereas in Xylaria-type any subhymenial pseudoparenchyma present is not expanded and paraphyses are persistent. The Sordaria-type has been suggested as an intermediate pattern between Xylaria and Diaporthe forms because paraphyses are present (Huang 1976); however, Parguey-Leduc and Janex-Favre (1981) discount the importance of paraphyses in establishing this centrum pattern. While *Paleopyrenomycites* provides some information about early stages in centrum development, all the features used to characterize a single pattern cannot be documented conclusively. The limited sections that are available suggest that the fossil has more in common with centrum development of the Xylaria-type, but as noted by some authors, centrum development ultimately might prove to be a highly variable character and of little systematic importance (Lumbsch 2000). Where known, the anamorphic stage of these extant families is of the blastic type while in the fossil conidiogenesis appears to be thallic. Rossman (1993) indicates that within the modern pyrenomycetes a larger number of the anamorphic forms have been connected with the Diaporthales and Hypocreales than the Ophiostomatales and Xylariales.

Samuels and Blackwell (2001) list two principal life history types among pyrenomycetes. In one the fungus produces ascospores for a short duration at either end of the growing season, with most of the reproductive effort directed at the formation of conidia. A second type, found in saprobes and mycoparasitic forms, is characterized by the germination of conidia or ascospores to form a mycelium and then the rapid production of ascospores. While it remains impossible to comment on seasonality within the Rhynie chert ecosystem or the timing in the life history, the presence of both conidia and ascospores within the same host tissue at the time of preservation suggests that the *Paleopyrenomycites* life history might have been closer to the second type. The presence of necrotic areas in the *Asteroxylon* axes adds support to the hypothesis that *Paleopyrenomycites* may have functioned as a pathogen.

Another group of filamentous ascomycetes that superficially bear some morphological resemblance to *Paleopyrenomycites* are members of the Loculoascomycetes (Barr and Huhndorf 2001). In these fungi asci are produced in cavities or locules whose wall consists of stromal tissue. Two additional characters that tend to define the group are bitunicate asci and a knob-like base that attaches the ascus to the ascocarp wall. However, the primary character used to distinguish members of the group is developmental and includes the ascomata forming before nuclear pairing in the dikaryon, rather than pairing and then ascomata formation. Although we have commented to a limited extent on the possible development of the ascocarp in *Paleopyrenomycites,* we have insufficient evidence that could be used to define the point of nuclear pairing. It appears that the asci in the fossil are of the unitunicate-type, although admittedly it is difficult to distinguish between a laminated single wall in the fossil and two individual walls such as those found in bitunicate asci.

Cladistic analysis.—It is difficult to accurately link fossil and modern fungi, but the use of cladistic analyses at times has been powerful in focusing attention on both character states and presumably closely related groups. Certain diagnostic characters that are sufficiently well preserved in the fossil provide the opportunity to assess potential relationships with modern forms and at the same time establish a benchmark that can be used to polarize features. Although there is still no universal agreement as to the higher level taxonomic categories of fungi possessing most pyrenomycete features (e.g. Eriksson and Hawksworth 1993, Spatafora 1995, Hamamoto and Nakase 2000), both morphological and molecular characters have been useful in broadly defining several higher-order groups (Alexopoulos et al 1996). In attempting to place *Paleopyrenomycites* within a phylogenetic context we used the 12 character states identified by Barr (2001). Of these, the eight pertaining to ascus shape, opening, arrangement and ascospore symmetry easily can be evaluated in the fossil. Type and position of the ascoma and some features of the hamathecium also can be inferred from the fossil perithecia. Tro-

phic condition, habit and especially the origin of the ascoma, however, remain equivocal. We used nine of these characters scored for the fossil in a data matrix together with eight representative pyrenomycete orders taken from Samuels and Blackwell (2001) and Barr and Huhndorf (2001). The data matrices were analyzed cladistically and comparisons made, using the Pezizales as outgroup. The 50% majority-rule consensus tree did not resolve the relationship of *Paleopyrenomycites* to other groups of fungi.

Evolution of ascomycetes.—The pleomorphic nature of the biology of ascomycetes makes them especially challenging organisms with which to work (Seifert and Gams 2001). This aspect of fungal life history biology also presents a major obstacle because it is difficult to equate the more common anamorphic states to the teleomorphs, although molecular techniques might help to remedy this situation. The oldest evidence of putative ascomycetes to date are phialides (elongate-shaped conidiogenesis cells that produce blastic conidia) reported from the Early Silurian of Virginia (Pratt et al 1978) and Late Silurian of Sweden (Sherwood-Pike and Gray 1985). In *Paleopyrenomycites* conidia are of the thallic-type, in which the conidium is formed by the transformation of the existing cell in a conidiophore. Although fungal conidiophores should be preserved in fossil assemblages, they would be difficult to identify and thus have been reported infrequently except when found in association with other components of the anamorphic stage. For example, Tertiary arthroconidia have been reported from several specimens preserved in amber (e.g. Stubblefield et al 1985, Ting and Nissenbaum 1986). If the Silurian fossils are correctly interpreted as phialides of ascomycetes then the blastic pattern of conidiogenesis predates the thallic forms found in Rhynie chert fungi by approximately 40 mya.

The continued analysis of characters, broader sampling of taxa and revised hypotheses have pushed back the hypothetical divergences times of major fungal lineages. Berbee and Taylor (2001) suggest that Ascomycota and Basidiomycota diverged approximately 390 mya. These authors initially used 1.0% as the relationship between geologic time and nucleotide substitution in their molecular clock (Berbee and Taylor 1993) but later revised that number to 1.26% (Berbee and Taylor 2001). This pushes the divergence of the ascomycetes from the basidiomycetes to about 500 mya. Using the initial report of the Lower Devonian perithecial ascomycete described here (Taylor et al 1999), Heckman et al (2001) more recently extended the divergence back to at least 670 mya. While the use of molecular clocks has not been accepted universally (e.g. Rodriguez-Trelles et al 2002), discoveries in the fossil record in some instances are helping to focus evolutionary events, using a combination of morphological and molecular datasets. In other cases molecular data as yet have not provided the resolving power necessary to determine relationships among the earliest ascomycetes. For example, information determined from molecular sequences suggests that the pyrenomycetes occur at the base of the Euascomycetes but the tree generated is not well supported (Berbee and Taylor 2001). Most pyrenomycetes possess elongate asci that forcibly eject ascospores, while in *Paleopyrenomycites* ascospores appear to have been dispersed passively. Based on the nuclear gene RPB2 elongate asci and forcibly ejected ascospores are hypothesized to have appeared early in the evolution of the Ascomycota (Liu et al 1999) but more recent molecular data suggest that several features associated with the ascus might have appeared and then were lost several times (Liu 2004). Shape of the perithecium with a short ostiolate neck also is correlated with spore discharge and also is present in the fossil. One order of filamentous ascomycetes that receives greater support as being ancestral based on molecular data is the Pezizales (Lumbsch et al 2000), a group sometimes termed the operculate discoascomycetes. It is worth noting that these fungi produce asci and paraphyses in an open ascoscarp, rather than the closed ascoscarp found in *Paleopyrenomycites*. The discovery of *Paleopyrenomycites* from rocks dated at 400 mya underscores that, if apothecia and operculate unitunicate asci represent the ancestral condition as postulated based on the nuclear gene RPB2 (Liu 2004), then this divergence is far more ancient than might have been predicted. To more accurately resolve the base of the filamentous ascomycetes clade, Berbee et al (2000) statistically examined the amount of sequence information that would be required to confirm whether the basal position of the Pezizales is accurate and hypothesized that three times as much data would be required to make this determination. Using this as a starting point these authors postulate that seven times more information would be necessary to resolve the divergence of the next group.

It initially was hypothesized that a fungus like *Taphrina* possessed features that would make it an excellent example of a common ancestor between the ascomycetes and basidiomycetes (Savile 1968). With the addition of sequence data the Archiascomycetes was proposed as the basal group of ascomycetes that included forms with a sexual state, but which lack ascogenous hyphae (Kurtzman and Sugiyama 2001). Ascocarps are not produced, but some forms possess clavate asci that forcibly liberate ascospores. Of note, two of the orders (Protomycetales and Taphrinales)

include taxa that are parasitic on ferns. However, Prillinger et al (2002) more recently support three classes of ascomycetes (Hemiascomycetes, Euascomycetes, Protomycetes) based on 18S rDNA sequence data, quantitative and qualitative monosaccharide patterns of purified cell wall, the ultrastructure of septal pores and urease activity. However, their analysis suggests a basal position for the Hemiascomycetes, with the Protomycetes and Euascomycetes as sister groups. While much remains to be learned about the Archiascomycetes (= Protomycetes), based on molecular phylogenetic analysis (Tanabe et al 2004) there can be little doubt that the features found in *Paleopyrenomycites* currently make it an unlikely candidate for inclusion in the group. If the divergence time estimates that have been suggested between the ascomycetes and basidiomycetes have validity, then the characters seen in the fossil suggest a very rapid evolution for the filamentous ascomycetes with perithecia.

Although the diversity of morphological features present in the Euascomycetes makes it difficult to generalize about which character states might be primitive in modern groups, some interesting features are present in *Paleopyrenomycites* that have a bearing on this question. Other features that have been used to relate modern taxa, but which are not present in the fossil, or are impossible to resolve, include color of the perithecia, presence or absence of amyloid apical ring on the ascus, ascospore color and a variety of developmental characters. In spite of this, several features present in *Paleopyrenomycites* are recognized in some modern orders. For example, a number of features are shared by *Paleopyrenomycites* and *Glomerella,* a parasite of flowering plants. Both are characterized by an ostiolate perithecium and unicellular, hyaline ascospores. Conidiophores occur as acervuli and are included in the anamorph *Colletotrichum*.

The Rhynie chert represents a snapshot of fungal diversity in an Early Devonian ecosystem. This is especially interesting because macroplants from the same deposit demonstrate early stages in the evolution of terrestrial plant structures and physiological adaptations necessary to exist in an aerial environment while the fungi that appear in the same ecosystem morphologically are identical to their counterparts in modern ecosystems (Taylor et al pers comm). Moreover several of the Rhynie chert fungi already had entered into symbioses with land plants and cyanobacteria and had demonstrated a wide range of parasitic interactions. How long did it take for these interactions to evolve? And do they support or refute the rapid divergence of fungi as suggested by the Rhynie chert ecosystem? ''Finding evidence of rapid increase in numbers and diversity of fossilized conidia, fruiting bodies, and lichens should contribute to reconstructing early evolution among these fungi,'' said Berbee and colleagues (2000). We anticipate that the report of *Paleopyrenomycites* from the Lower Devonian Rhynie chert should help to focus efforts on combining molecular and morphological datasets and to formulate and test new hypotheses that address this intriguing challenge.

ACKNOWLEDGMENTS

This study was supported in part by the National Science Foundation DEB-9815699 to TNT and OPP-0003620 to ELT and TNT. We thank Drs Matias Cafaro and Mark Mort for helpful advice and discussion regarding cladistic analyses.

LITERATURE CITED

Alexopoulos CJ, Mims CW, Blackwell M. 1996. Introductory Mycology. 4th ed. New York: Wiley. p 868.

Alvin KL, Muir MD. 1970. An epiphyllous fungus from the Lower Cretaceous. Biol J Linn Soc 2:55–59.

Barr ME. 2001. Ascomycota. In: McLaughlin DJ, McLaughlin EG, Lemke PJ, eds. The Mycota VII Part A. Springer-Verlag. p 161–177.

———, Huhndorf SM. 2001. Loculoascomycetes. In: McLaughlin DJ, McLaughlin EG, Lemke PA, eds. The Mycota VII Part A. Springer-Verlag. p 283–305.

Berbee ML, Taylor JW. 1993. Dating the evolutionary radiations of the true fungi. Can J Bot 71:1114–1127.

———, ———. 2001. Fungal molecular evolution: gene trees and geologic time. In: McLaughlin DJ, McLaughlin, Lemke PA, eds. Mycota-Systematics and Evolution VII. Springer-Verlag. p 229–245.

———, Carmean DA, Winka K. 2000. Ribosomal DNA and resolution of branching order among the Ascomycota: how many nucleotides are enough? Mol Phylo Evol 17: 337–344.

Cole GT. 1981. Conidiogenesis and conidiomatal ontogeny. In: Cole GT, Kendrick B, eds. Biology of Conidial Fungi. Vol. 2. New York: Academic Press. p 271–327.

Daghlian CP. 1978. A new melioloid fungus from the early Eocene of Texas. Palaeontology 21:171–176.

Edwards DS. 1986. *Aglaophyton major,* a non-vascular landplant from the Devonian Rhynie chert. Bot J Linn Soc 93:173–204.

Eriksson OE, Hawksworth DL. 1993. Outline of the Ascomycetes—1993. Syst Ascomyc 12:51–257.

Hamamoto M, Nakase T. 2000. Phylogenetic relationships among fungi inferred from small subunit ribosomal RNA gene sequences. In: Priest FG, Goodfellow M, eds. Applied Microbial Systematics. Kluwer Academic Publ. p 57–71.

Hass H, Taylor TN, Remy W. 1994. Fungi from the Lower Devonian Rhynie chert: mycoparasitism. Amer J Bot 81: 29–37.

Hawksworth DL, Sutton BC, Ainsworth GC. 1995. Ainsworth

& Bisby's Dictionary of the Fungi. 8th ed. International Mycological Institute, Cambridge, CAB Int. Cambridge.

Heckman DS, Geiser DM, Eidell BR, Stauffer RL, Kardos NL, Hedges SB. 2001. Molecular evidence for the early colonization of land by fungi and plants. Science 293: 1129–1133.

Hennebert GL, Sutton BC. 1994. Unitary parameters in conidiogenesis. In: Hawksworth DL, ed. Ascomycete Systematics: Problems and Perspectives in the Nineties. New York: Plenum. p 65–76.

Huang LH. 1976. Developmental morphology of *Triangularia backusii* (Sordariaceae). Can J Bot 54:250–267.

Ingold CT. 1933. Spore discharge in the Ascomycetes. I. Pyrenomycetes. New Phytol 32:175–196.

Kidston R, Lang WH. 1921. Old Red Sandstone plants showing structure, from the Rhynie chert bed, Aberdeenshire. Part 5. The Thallophyta occurring in the peat-bed; the succession of the plants throughout a vertical section of the bed, and the conditions of accumulation and preservation of the deposit. Trans Roy Soc Edinburgh 52:855–902.

Kurtzman CP, Sugiyama J. 2001. Ascomycetous yeasts and yeastlike taxa. In: McLaughlin DC, McLaughlin EG, Lemke PA, eds. The Mycota. Systematics and Evolution, Part A. Berlin: Springer-Verlag. p 179–200.

Krassilov V. 1981. *Orestovia* and the origin of vascular plants. Lethaia 14:235–250.

Liu YJ, Whelen S, Hall BD. 1999. Phylogentic relationships among Ascomycetes: evidence from an RNA polymerse II subunit. Mol Biol Evol 16:1799–1808.

———. 2004. Body plan evolution of ascomycetes, as inferred from ab RNA polymerase II phylogeny. Proc Nat Acad Sci USA 101:4507–4512.

Lumbsch HT. 2000. Phylogeny of filamentous ascomycetes. Naturwiss 87:335–342.

———, Lindermuth R, Schmitt I. 2000. Evolution of filamentous ascomycetes inferred from LSU rDNA sequence data. Plant Biol 2:525–529.

Luttrell LS. 1951. Taxonomy of the pyrenomycetes. Univ Missouri Studies 24:1–120.

Parguey-Leduc A, Janex-Favre MC. 1981. The ascocarps of ascohymenial pyrenomycetes. In: Reynolds DR, ed. Ascomycete Systematics—The Luttrellian Concept. Springer-Verlag. p 102–123.

Pirozynski KA, Weresub LK. 1979. The classification and nomenclature of fossil fungi. In: Kendrick B, ed. The Whole Fungus, the Sexual-Asexual Synthesis. Vol. 2. Proc. 2nd Intl. Mycol. Conf., Univ. Calgary, Kananaskis, Alberta Natl. Mus. Nat. Sci., Natl. Mus. Canada and Kananaskis Foundation, Ottawa, Canada. p 417–739.

Pons D, Locquin MV. 1981. *Mycokidstonia sphaerialoides* Pons & Locquin, gen. et sp. nov., Ascomycètes fossile Dévonien. Cahiers de Micropaléontologie 1:101–104.

Pratt LM, Phillips TL, Dennison JM. 1978. Evidence of nonvascular land plants from the Early Silurian (Llandoverian) of Virginia, U.S.A. Rev Palaeobot Palynol 25: 121–149.

Prillinger H, Lopandic K, Schweigkofler W, Deak R, Aarts HJM, Bauer R, Sterflinger K, Kraus GF, Maraz A. 2002. Phylogeny and systematics of the fungi with special reference to the Ascomycota and Basidiomycota. In: Breitenbach M, Crameri R, Leher SB, eds. Fungal Allergy and Pathogenicity. Chem. Immunol. Basel, Karger, p 207–295.

Raju NB. 2002. Meiosis and ascospore development in nonlinear asci of *Neurospora pannonica*. Mycologia 94:99–104.

Read ND, Beckett A. 1996. Ascus and ascospore morphogenesis. Mycol Res 100:1281–1315.

Remy W, Taylor TN, Hass H. 1994a. Early Devonian fungi: a blastocladalean fungus with sexual reproduction. Amer J Bot 81:690–702.

———, ———, ———, Kerp H. 1994b. 400 million year old vesicular arbuscular mycorrhizae (VAM). Proc Nat Acad Sci USA 91:11841–11843.

Rice CM, Ashcroft WA, Batten DJ, Boyce AJ, Caulfield JBD, Fallick AE, Hole MJ, Jones E, Pearson MJ, Rogers G, Saxton JM, Stuart FM, Trewin NH, Turner G. 1995. A Devonian auriferous hot springs system, Rhynie, Scotland. J Geol Soc London 152:229–250.

———, Trewin HH, Anderson LI. 2002. Geological setting of the Early Devonian Rhynie cherts, Aberdeenshire, Scotland: an early terrestrial hot spring system. J Geol Soc London 159:203–214.

Richardson JB. 1967. Some British Lower Devonian spore assemblages and their stratigraphic significance. Rev Palaeobot Palynol 1:111–129.

Rodriguez-Trelles F, Tarrio R, Ayala FJ. 2002. A methodological bias toward overestimation of molecular evolutionary time scales. Proc Natl Acad Sci 99:8112–8115.

Rossman AY. 1993. Holomorphic hypocrealean fungi: *Nectria sensu stricto* and teleomorphs of *Fusarium*. In: Reynolds DR, Taylor JW, eds. The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics. International Mycological Inst. CAB International. p 149–160.

Rothwell GW. 1972. *Palaeosclerotium pusillum* gen. et sp. nov., a fossil eumycete from the Pennsylvanian of Illinois. Can J Bot 50:2353–2356.

Samuels GJ, Blackwell M. 2001. Pyrenomycetes—fungi with perithecia. In: McLaughlin DL, McLaughlin EG, Lemke PA, eds. The Mycota—Systematics and Evolution VII Part A. Springer-Verlag. p 221–255.

Savile DBO. 1968. Possible interrelationships between fungal groups. In: Ainsworth GC, Sussman AS, eds. The Fungi, an Advanced Treatise. Vol. III. Academic Press. p 649–675.

Seifert KA, Gams W. 2001. The taxonomy of anamorphic fungi. In: McLaughlin DJ, McLaughlin EG, Lemke PA, eds. The Mycota—VII. Systematics and Evolution. Part A Springer-Verlag. p 307–347.

Sherwood-Pike MA, Gray J. 1985. Silurian fungal remains: probable records of the class Ascomycetes. Lethaia 18: 1–20.

Spatafora JW. 1995. Ascomal evolution of filamentous ascomycetes: evidence from molecular data. Can J Bot 73(1):S811–S815.

Stubblefield SP, Taylor TN. 1983. Studies of Paleozoic fungi. I. The structure and organization of *Traquairia* (Ascomycota). Amer J Bot 70:387–399.

———, Miller CE, Taylor TN, Cole GT. 1985. *Geotrichites glaesarius,* a conidial fungus from Tertiary Dominican amber. Mycologia 77:11–16.

Tanabe Y, Saikawa M, Watanabe MM, Sugiyama J. 2004. Molecular phylogeny of Zygomycota based on the EF-1 and RPB1 sequences: limitations and utility of alternate markers to rDNA. Mol Phylog Evol 30:438–449.

Taylor TN. 1994. The fossil history of ascomycetes. In Hawksworth DL, ed. Ascomycete Systematics—Problems and Perspectives in the Nineties. Plenum Press. p 167–174.

———, Hass H, Remy W. 1992a. Devonian fungi: interactions with the green alga *Palaeonitella*. Mycologia 84: 901–910.

———, ———, Kerp H. 1997. A cyanolichen from the Lower Devonian Rhynie chert. Amer J Bot 84:992–1004.

———, ———, ———. 1999. The oldest fossil ascomycetes. Nature 399. 648.

———, Remy W, Hass H. 1992b. Fungi from the Lower Devonian Rhynie chert: Chytridiomycetes. Amer J Bot 79:1233–1241.

———, ———, ———, Kerp H. 1995. Fossil arbuscular mycorrhizae from the Early Devonian. Mycologia 87:560–573.

Tehler A, Farris JS, Lipscomb DL, Källersjö M. 2000. Phylogenetic analyses of the fungi based on large rDNA data sets. Mycologia 92:459–474.

Ting WS, Nissenbaum A. 1986. Fungi in the Lower Cretaceous amber from Israel. Spec. Publ. Explor and Devl. Research Center. Chinese Petrolem Corp. p 27.

Trewin NH, Rice CM. 1992. Stratigraphy and sedimentology of the Devonian Rhynie chert locality. Scot J Geol 28: 37–47.

Uecker FA. 1994. Ontogeny of the ascoma of *Glomerella cingulata*. Mycologia 86:82–88.

Ulloa M, Hanlin RT. 2000. Illustrated dictionary of mycology. APS Press. p 448.

White JF Jr, Taylor TN. 1988. Triassic fungus from Antarctica with possible ascomycetous affinities. Amer J Bot 75:1495–1500.

Wong S-W, Hyde KD, Gareth Jones EB, Moss ST. 1999. Ultrastructural studies on the aquatic ascomycetes *Annulatascus velatisporus* and *A. triseptatus* sp. nov. Mycol Res 103:561–571.