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BRIEF COMMUNICATION

IS *PHELLINITES DIGIUSTOI* THE OLDEST HOMOBASIDIOMYCETE?[1]

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The oldest generally accepted fossil of mushroom-forming fungi (homobasidiomycetes) is *Phellinites digiustoi,* from the Jurassic of Patagonia. The next-oldest homobasidiomycete fossil does not occur until about 70 million years later, in the mid-Cretaceous. The goal of this project was to reassess the identity of *Phellinites* and refine the minimum-age estimate for the origin of homobasidiomycetes. We examined the holotype of *Phellinites digiustoi* and a second collection from the type locality. Thin sections were prepared from both collections. *Phellinites* has a cellular structure, confirming that it is biological in origin, but there are no hyphae, spores, or other fungal structures. Rather, *Phellinites* appears to be the outer bark (rhytidome) of a conifer. Fossils of Araucariaceae are common in the region where *Phellinites* was found, and so it is plausible that *Phellinites* is the bark of an *Araucaria*-like tree. The minimum age of the mushroom-forming fungi, based on direct fossil evidence, is revised to the mid-Cretaceous.

Key words: Araucariaceae; basidiomycetes; Cerro Cuadrado; fossil; paleomycology; periderm; *Phellinites digiustoi.*

Mushroom-forming fungi (homobasidiomycetes) influence the growth and distribution of plants through their activities as saprophytes, mycorrhizal symbionts, and pathogens. Homobasidiomycetes are also of direct importance to animals, which graze upon hyphae and fruiting bodies, disperse spores, and enter into fungal symbioses. Although the importance of homobasidiomycetes in contemporary ecosystems is well documented, little is known of their functioning in ancient ecosystems or their significance for the evolution of plants and animals (cf. Taylor and Osborn, 1996). To assess the impact that homobasidiomycetes may have had on the evolution of the terrestrial biota, it is necessary to know the approximate age of their origin. Few fossils are available to assist in such efforts, and so it is imperative that all reported homobasidiomycete fossils be rigorously identified. The goal of the present study was to reassess the identity of the oldest generally accepted homobasidiomycete fossil, *Phellinites digiustoi,* and refine the minimum age estimate for the origin of the homobasidiomycetes.

Homobasidiomycete taxonomy is based primarily on the morphology of fruiting bodies, which tend to be soft, ephemeral structures and are rare in the fossil record (Hibbett, Grimaldi, and Donoghue, 1997). Microfossils of hyphae with clamp connections are known from as far back as the Pennsylvanian (300 Mya, Dennis, 1970), but these are not necessarily evidence of homobasidiomycetes (cf. Hibbett, Grimaldi, and Donoghue, 1997). Clamped hyphae are formed not only by homobasidiomycetes, but also by heterobasidiomycete "jelly fungi," such as Auriculariales, Dacrymycetales, and Tremellales. Clamp connections have also been reported in certain Ustilaginales (basidiomycete "smuts"; Roberson and Luttrell, 1989) and are considered to be homologous to the croziers of ascomycetes (but see Tehler, 1988), which are the sister group to the basidiomycetes. Thus, the occurrence of clamped hyphae in the Pennsylvanian does not provide a minimum age estimate for the homobasidiomycete clade. The characters needed to identify a fossil as a homobasidiomycete include basidial morphology, septal pore ultrastructure, DNA sequences, or fruiting body morphology.

The oldest reported homobasidiomycete fruiting body is a putative bracket fungus, *Eopolyporoides kuklei,* from the Middle Triassic Moolayember Formation of Queensland, Australia (Playford, Rigby, and Archibald, 1982, as cited in Truswell, 1996, pp. 329–331). No anatomical details or hymenophore structures were reported from *Eopolyporoides,* which casts doubt on its identity as a fungus. *Eopolyporoides* has been overlooked in the paleomycological literature, and it should be reexamined. Two other fossils that were initially interpreted as polypores, *Polyporites stevensoni* (Brown, 1936) and *P. browni* (Wieland, 1934), both from the Cretaceous, were later determined to be a coral and a lungfish dental plate, respectively (Brown, 1938; cf. Tiffney and Barghoorn 1974).

Phellinites digiustoi was found in the petrified forests of Santa Cruz, Patagonia, and was interpreted as the fruiting body of a wood-decaying polypore (Singer and Archangelsky, 1958). The name *Phellinites* alludes to the extant genus *Phellinus* (Hymenochaetaceae), which includes tough, perennial bracket fungi. The petrified forests of Santa Cruz were estimated to date from the "Matilda Formation of the Upper Mesojurassic", thus making *Phellin-*

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ites about 160 Mya in age (Singer and Archangelsky, 1958). Fossils of Araucariaceae are common in this region (Wieland, 1935; Singer and Archangelsky, 1958; Stockey, 1977). Although *Phellinites* was not found attached to fossilized wood, it was presumed to have been growing on the trunk of an *Araucaria*-like tree (Singer and Archangelsky, 1958).

The next-oldest homobasidiomycete fossils after *Phellinites* are all gilled mushrooms: *Archaeomarasmius leggetti,* (mid-Cretaceous, 90 Mya; Hibbett, Grimaldi, and Donoghue, 1995, 1997), *Protomycena electra* (Miocene, 15–30 Mya; Hibbett, Grimaldi, and Donoghue, in press), and *Coprinites dominicana* (Miocene, 15–30 Mya; Poinar and Singer, 1990). *Archaeomarasmius, Protomycena,* and *Coprinites* are beautifully preserved in amber, and there can be little doubt that they are homobasidiomycetes. In contrast, the one published photograph of *Phellinites* (which is silicified) does not strongly suggest a fungal fruiting body (Singer and Archangelsky, 1958, fig. 2). Nevertheless, the identification of *Phellinites* as a homobasidiomycete has never been challenged. Tiffney and Barghoorn (1974, p. 22), in their monumental review of the fungal fossil record, listed *Phellinites* as "accepted," and this may account for the acceptance of this fossil in later works (see also Taylor, 1993). For example, *Phellinites* was cited as a "convincing silicified shelf fungus" and used as a calibration point for molecular clock dating in the 18S rDNA study of Berbee and Taylor (1993, p. 1125). In addition, the recent mycology textbook by Alexopoulos, Mims, and Blackwell (1996, table 3-1) lists a "shelf fungus" in the Jurassic, which presumably is *Phellinites.* Finally, in our own work (Hibbett, Grimaldi, and Donoghue, 1995, p. 377), we cited *Phellinites* as "the oldest clearly holobasidiomycetous fossil."

Despite its broad acceptance, the evidence that *Phellinites* is a homobasidiomycete is weak. In addition to the one photograph, the description of *Phellinites* was accompanied by two anatomical drawings made from a section "about 350 μ thick," which was cut in an orientation thought to be transverse to the pores. One is a low-magnification view of the supposed pores, and the other is a high-magnification sketch showing five club-shaped structures that were interpreted as sterile cells of the hymenium (Singer and Archangelsky, 1958, figs. 3–4). Tramal hyphae were described, but not sketched. There were no spores, basidia, or clamp connections reported.

We examined two specimens of *Phellinites,* which are housed in the paleobotanical collections of the Fundación Miguel Lillo (Tucumán, Argentina): ARGENTINA. SANTA CRUZ. Estancia Bella Vista, Cerro Tortuga, 1956, *De Giusto s.n.*, (HOLOTYPE: Fundación Miguel Lillo, LIL Pb No. 66, includes slide); same general location, 1958, *Archangelsky-Herbst s.n.*, (Fundación Miguel Lillo, LIL Pb No. 4070). There were no annotation labels inside either packet, suggesting that we are the first workers to have examined this material since it was deposited.

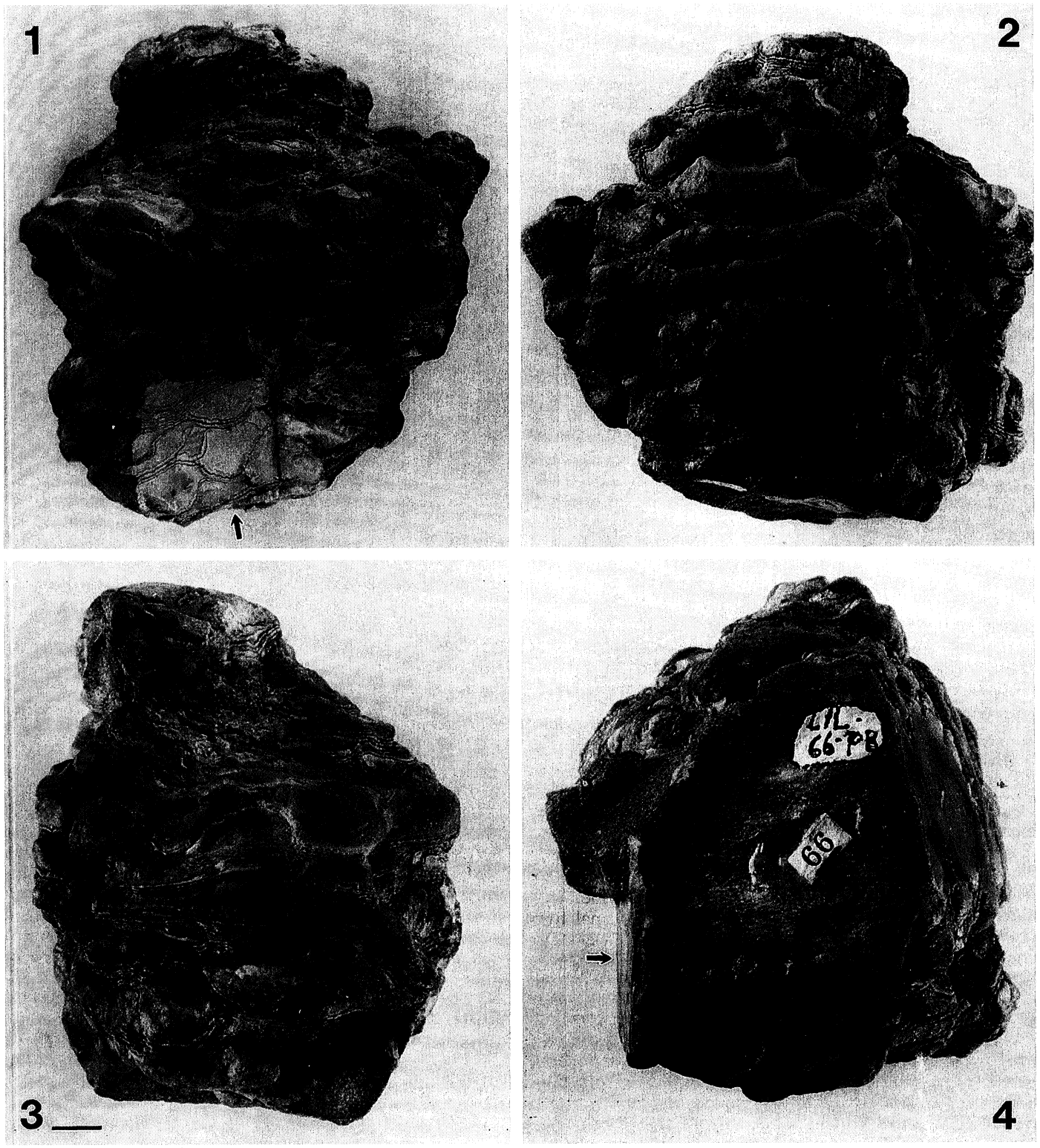
The holotype of *Phellinites digiustoi* (LIL Pb No. 66) is roughly ungulate, 50 × 55 × 40 mm. The piece is composed of alternating layers of smooth stone ~ 3–8 mm wide, separated by bands of stone with a striated or layered appearance, ~ 2–6 mm wide (Figs. 1–4). One side of the piece, parallel to the layers, is more or less smooth. From the cut surface (where the section was made), it is apparent that the striated layers cut off irregular, lens-shaped sections of the interior (Fig. 1).

The second collection (LIL Pb No. 4070) has not been described previously. According to the label data, it was determined as *Phellinites digiustoi* by S. Archangelsky, and was collected in the same general location as the holotype (LIL Pb No. 66). LIL Pb No. 4070 is roughly rectangular, 115 × 55 × 30 mm. As in the holotype, one side is more or less smooth, although in LIL Pb No. 4070 it is interrupted by shallow cracks and is distinctly concave. As in the holotype, alternating bands of smooth and striated stone are present in LIL Pb No. 4070, with the layers more or less parallel to the smooth side of the piece (Figs. 5–6).

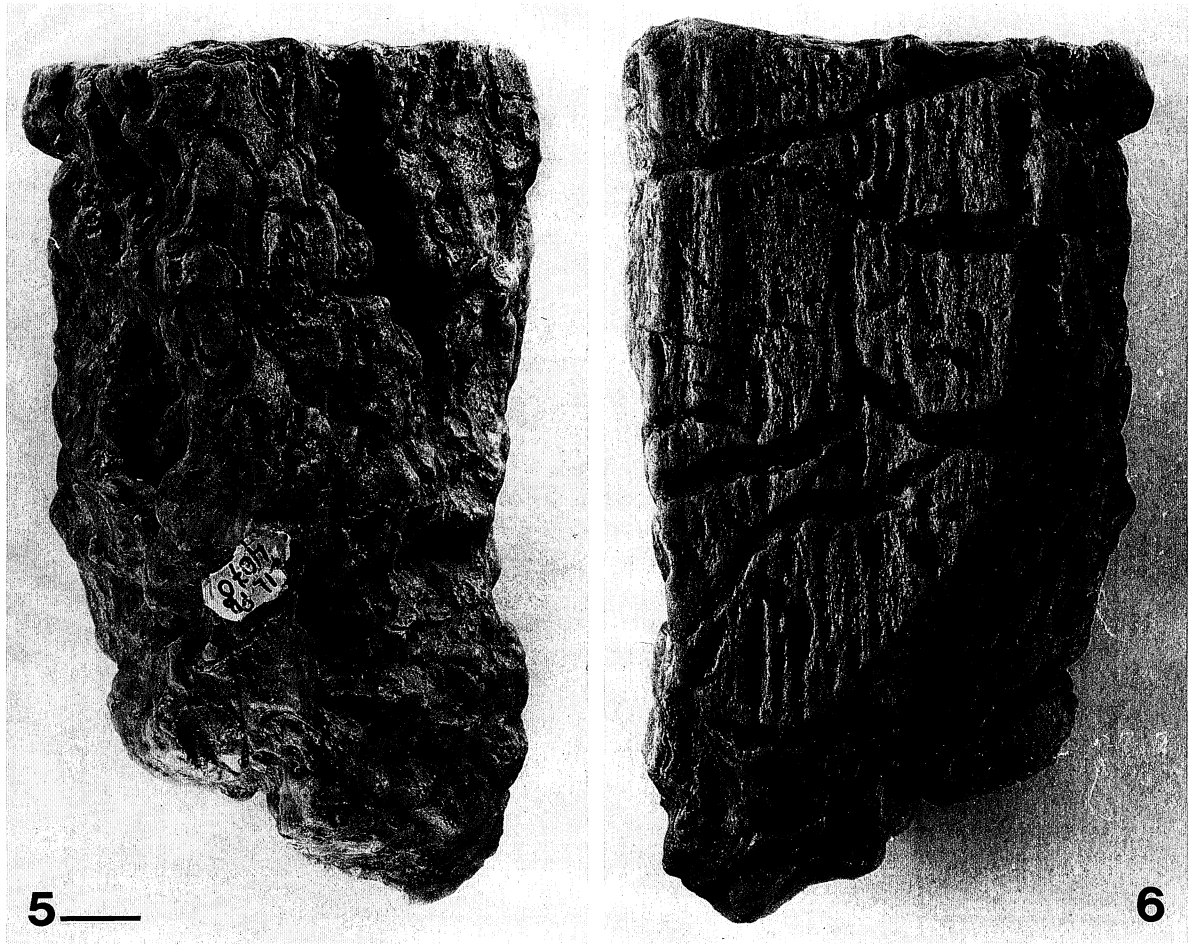
The original slide from the holotype is still intact, although the coverslip has cracked and the mounting medium has yellowed. We examined the slide under oil immersion but were unable to observe the structures interpreted by Singer and Archangelsky as sterile hymenial elements (1958, fig. 4). New sections ~ 30 μm thick were cut perpendicular to the plane of the layers (Spectrum Petrographics, Winston, Oregon). The section of the holotype was made parallel and immediately adjacent to the original section. The sections were photographed with dark-field (Figs. 7–8) or bright-field illumination (Figs. 9–14).

The holotype and LIL Pb No. 4070 share a similar cellular microstructure (Figs. 7–8). The striated regions are composed of alternating layers of dark and light cells (Figs. 9–13). The layers of dark cells are interrupted by files of light, thin-walled cells (Figs. 11–13). Cells in the striated layers are more or less rectangular in cross-section and arranged in regular files (Fig. 13). In certain locations the dark layers of cells taper down tangentially to fine points (Figs. 12–13). The lens-shaped areas delimited by the striated layers are composed of cells that are thin-walled and more or less round in cross section. Scattered throughout these areas are open spaces, which could be canals or clusters of cells (Figs. 11–12).

The cellular structure of *Phellinites* indicates that it is biological in origin, but there is no evidence of hyphae or other fungal structures. The anatomy of *Phellinites* suggests that it is a plant fossil, and agrees well with the structure of the outer bark (rhytidome) of woody plants (Chang, 1954a, b; Esau, 1960). As the stems of woody plants grow, the first-formed periderm is displaced outward and sequent periderms arise within the secondary phloem, which is cut off in sections. Depending on the species, the sections of secondary phloem delimited by sequent periderms may be irregularly lens-shaped, or may form concentric rings around the stem. We interpret the striated layers of *Phellinites* as sequent periderms, and the lens-shaped areas as regions of secondary phloem and other tissues of secondary origin. The open spaces scattered throughout the lens-shaped areas of secondary phloem could be resin canals or the remains of sclereid clusters. The cell shapes and arrangements in these tissues agree well with those in extant plants (rectangular periderm cells in regular ranks, and more or less circular secondary phloem cells). The smooth surfaces of both pieces (Figs. 4, 6) are interpreted as the inner surfaces, possibly the border between the living secondary phloem and the outer bark. If our interpretation is correct, then judging by the numerous layers of periderm and the cur-



Figs. 1–4. *Phellinites digiustoi* holotype, LIL Pb. No. 66. **1.** View showing cut face of transverse section (arrow). Striated periderm layers are visible on surface as well as in sectioned area. **2.** Rotated 180° horizontally, relative to Fig. 1. **3.** Rotated 90° to the right, relative to Fig. 1. Scale bar for all figures = 5 mm. **4.** Rotated 90° vertically (upwards), then 90° counterclockwise, relative to Fig. 1. Smooth inner surface, possibly border of live secondary phloem and rhytidome. Arrow shows cut face of transverse section (compare to Fig. 1 for orientation).

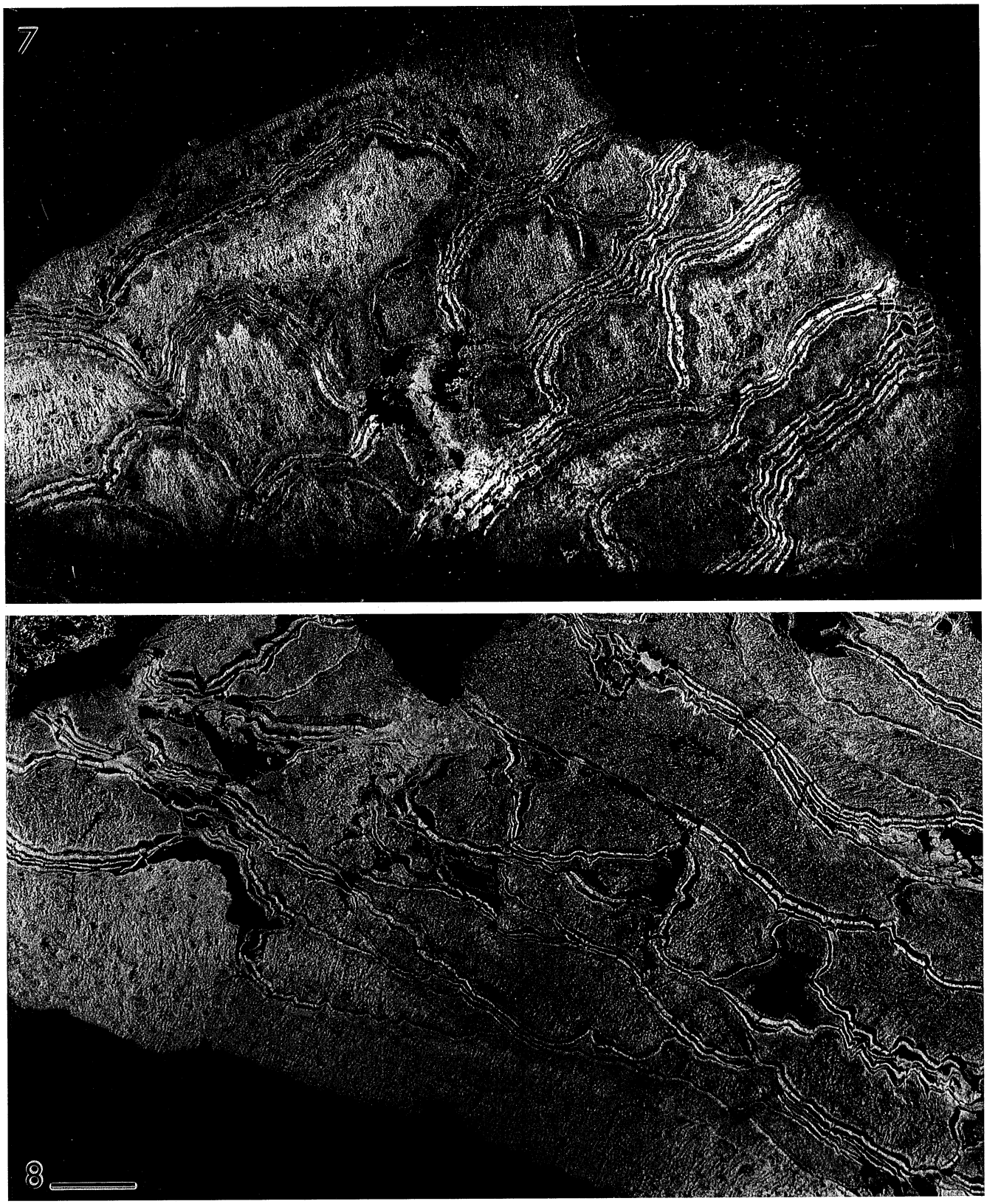


Figs. 5–6. *Phellinites digiustoi,* LIL Pb. No. 4070. **5.** Outer surface. Note striations, similar to holotype. Scale bar for both figures = 1 cm. Scale bar is perpendicular to vertical axis. **6.** Smooth inner surface. Note shallow curvature of surface.

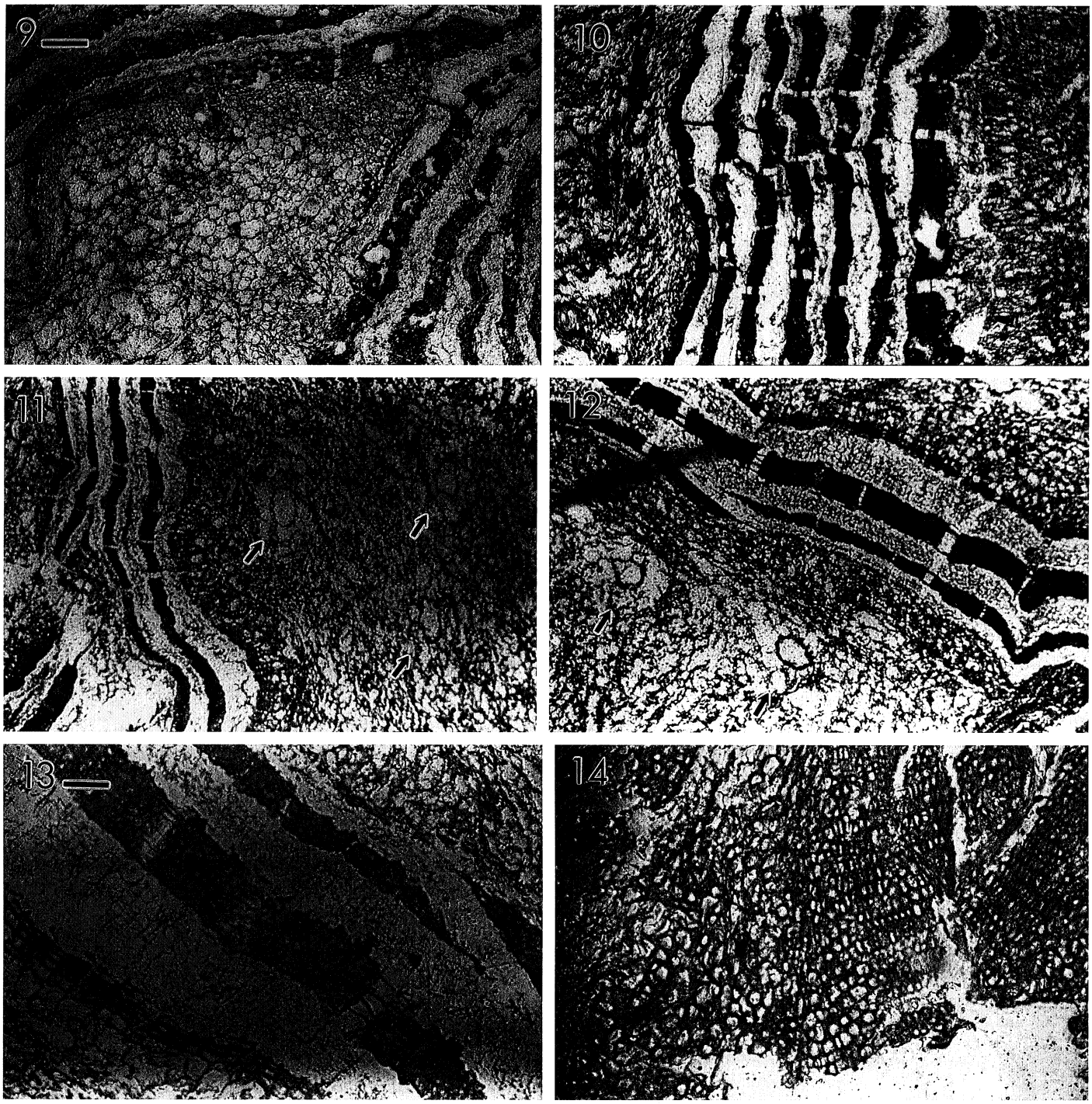
vature of the concave, inner surface of LIL Pb. 4070, the plant that produced *Phellinites* was probably a large tree.

The anatomy of bark varies considerably among woody plant taxa, but there have been surprisingly few comparative studies. The studies with the greatest taxonomic breadth that we are aware of are those by Chang (1954a, b), who compared bark anatomy of 15 genera of conifers (actually 16, if *Sequoiadendron* is segregated from *Sequoia*) and ten genera of angiosperms from North America. Many of the species studied by Chang form sequent periderms that cut off lens-shaped sections of secondary phloem, as in *Phellinites.* The periderms of *Phellinites* are composed of alternating light and dark bands (Figs. 9–13), which are similar to the conspicuous alternating layers of light, thin-walled and dark, thick-walled cells in the sequent periderms of *Larix, Picea,* and *Pinus* (Chang, 1954a, figs. 17, 26, table 1). This type of structure is also reported to be "present occasionally" in *Abies, Pseudotsuga, Chamaecyparis, Cupressus,* and *Libocedrus* (Chang, 1954b, table 1). Thus, based on this one character, *Phellinites* agrees with Chang's descriptions of Pinaceae, Taxaceae, Taxodiaceae, or Cupressaceae.

The Cerro Cuadrado petrified forests of Santa Cruz are composed of the trunks and seed cones of Araucariaceae (Wieland, 1935; Singer and Archangelsky, 1958; Stockey, 1977). It is therefore plausible that *Phellinites* is the periderm of Arauacariaceae. Unfortunately, we have been unable to locate published studies of *Araucaria* bark anatomy (because Chang limited his studies to North American taxa, he did not include *Araucaria*). To evaluate the possibility that *Phellinites* could be a member of Araucariaceae, we examined bark anatomy in *Araucaria* specimens in the wood collection of the Bailey-Wetmore Laboratory of Plant Anatomy and Morphology of Harvard University (Aw). Free-hand or freezing microtome transverse sections (considerably thicker than the sections of the fossil) were made from *Araucaria angustifolia* (Aw 28396) and *A. cunninghamii* (Aw 30546). Both species have a conspicuously banded periderm structure (Figs. 15–17), which is similar to *Phellinites* (Figs. 11–12) and the conifers studied by Chang (1954a, b). This type of periderm appears to be typical of conifers in general. In both species of *Araucaria,* the dark bands of cells in the periderm taper down tangentially to fine points that overlap each other, as in *Phellinites* (Figs. 12–13, 16–17). The secondary phloem of *A. cunninghamii* (Fig. 15) also contained open spaces (possibly sclereid clusters), which are similar to those in the secondary phloem of *Phellinites* (Figs. 11–12). A feature of *Phellinites* not shared by the



Figs. 7–8. *Phellinites digiustoi,* dark-field. Conspicuously layered sequent periderms delimit regions of nonliving secondary phloem, which have scattered openings that may be resin ducts or sclereid clusters. **7.** Holotype, LIL Pb. No. 66. **8.** LIL Pb. No. 4070. Scale bar for both figures = 2.5 mm.



Figs. 9–14. *Phellinites digiustoi,* bright-field. **9.** Holotype, LIL Pb. No. 66. Sequent periderms bordering lens-shaped region of secondary phoem. Scale bar for Figs. 9–12 = 300 μm. **10.** Holotype, LIL Pb. No. 66. Periderm with alternating light and dark bands of cells. Note files of light cells interrupting dark layers. **11.** LIL Pb. No. 4070. Periderm and secondary phloem with open spaces (arrows) that may be resin canals or sclereid clusters. **12.** LIL Pb. No. 4070. Periderm and secondary phloem with open spaces, possibly resin canals or sclereid clusters. Note that two of the dark layers of cells taper down tangentially to fine points (compare to Figs. 16–17). Dark bands in periderm are interrupted by single files of light, thin-walled cells. **13.** LIL Pb. No. 4070. Detail from Fig. 12 showing regular files of rectangular cells in periderm, with dark bands interrupted by single ranks of light, thin-walled cells. Scale bar for Figs. 13–14 = 120 μm. **14.** LIL Pb. No. 4070. Thick-walled, rectangular cells in regular ranks, possibly phellem.

Figs. 15–17. *Araucaria* periderm anatomy. **15.** *Araucaria cunninghamii* (Aw 30456). Multiple layers of periderm with fragments of secondary phloem (compare to Fig. 10). Open space (arrow), possibly resin canal or sclereid cluster, is similar to open spaces in secondary phloem in *Phellinites* (compare to Figs. 11–12). Scale bar for Figs. 15–16 = 250 μm, for Fig. 17 = 100 μm. **16.** *Araucaria angustifolia* (Aw 28396). Periderm with secondary phloem. Dark layers taper down tangentially to fine points, as in *Phellinites,* but are not interrupted by ranks of thin-walled cells (compare to Figs. 12–13). **17.** *Araucaria angustifolia,* detail of periderm from Fig. 16 showing regular arrangement of more or less rectangular cells (compare to Fig. 13).

conifers studied by Chang (1954a, b) or the *Araucaria* species that we examined is that the dark bands of periderm are interrupted by single files of light, thin-walled cells (Figs. 10–13). This distinctive character may eventually provide clues to the precise identity of *Phellinites.* In the meantime, we interpret *Phellinites* as the outer bark of a conifer, possibly related to Araucariaceae. The minimum age estimate for the homobasidiomycetes, based on direct fossil evidence (Hibbett, Grimaldi, and Donoghue, 1995, 1997), must therefore be revised from the Jurassic to the mid-Cretaceous.

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