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HYMENOPHORE DEVELOPMENT AND EVOLUTION IN *LENTINUS*[1]

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ABSTRACT

Morphological evolution of the *Lentinus* hymenophore was investigated through scanning electron microscopic observations of development in cultured sporocarps of *Lentinus tigrinus, L. crinitus, L. squarrosulus,* and an outgroup *Polyporus arcularius.* Mature *L. sajor-caju* sporocarps from herbarium material were also examined. The early hymenophore of *P. arcularius* was composed of circular pores that became radially elongate and angular as the pileus expanded. The *L. tigrinus* hymenophore was initially composed of irregular to subparallel ridges of hyphae that differentiated into lamellae. Subsequent growth of transverse cross-bridges resulted in a regular, subporoid structure at the base of the hymenophore. The cross-bridges, which are interpreted as homologues of the tangential walls of the *P. arcularius* hymenophore, were absent in *L. crinitus, L. squarrosulus,* and *L. sajor-caju.* By outgroup comparison, cross-bridges at the base of the hymenophore, moderately crowded lamellae, descending hymenophoral trama, lacerate lamella margins, and gymnocarpy are inferred to be plesiomorphic, and absence of cross-bridges, densely crowded lamellae, radiate trama, entire margins, and velangiocarpy are inferred to be derived within *Lentinus.* Nonterminal modifications to developmental programs and lack of correspondence between ontogenetic and phylogenetic polarity were observed. This suggests that use of ontogenetic criteria for assessing polarities and homologies of *Lentinus* hymenophore characters could be misleading.

Key Words: hymenophore, *Lentinus,* ontogeny, phylogeny

Species of *Lentinus* Fr. are wood-decaying basidiomycetes with decurrent lamellae, dimitic sporocarp tissues, hyaline, elliptic to cylindric spores, and, in most species, hyphal pegs (Corner, **1981**; Pegler, **1983**). A close relationship has long been suspected between *Lentinus* and certain polypores (Corner, **1981**; Pegler, **1983**; Singer, **1986**).

The limits of *Lentinus, Panus* Fr., and *Pleurotus* (Fr.) Quél. are controversial. The works by Corner (**1981**), Kühner (**1980**), Pegler (**1975**, **1983**), and Singer (**1986**) all differ significantly in their treatment of these genera. Two brown rot genera, *Neolentinus* Redhead & Ginns and *Heliocybe* Redhead & Ginns, were segregated from *Lentinus* which is otherwise composed of white rot species (Gilbertson, **1980**; Redhead and Ginns, **1985**).

Recent molecular systematic studies have suggested that *Lentinus* subg. *Lentinus sensu* Pegler (**1983**) is monophyletic, but that *Panus, Pleurotus, Neolentinus,* and *Heliocybe* are not in the same lineage as *Lentinus* (Hibbett and Vilgalys, **1991**, **1993**). Here *Lentinus* will be used to mean *Lentinus* subg. *Lentinus sensu* Pegler (**1983**), which is essentially equivalent to *Lentinus sensu* Corner (**1981**).

The molecular studies also upheld the view that *Lentinus* is derived from the polypores (Pegler, **1983**) and suggested that *Polyporus arcularius* Batsch : Fr. is a closely related outgroup (Hibbett and Vilgalys, **1991**, **1993**). *Polyporus arcularius* is a centrally stipitate, relatively ephemeral polypore with radially elongate pores.

Lentinus tigrinus (Bull. : Fr.)Fr. and *P. arcularius* sporocarps have been produced in culture for use in morphological, genetic, and physiological studies (Bobbitt and Crang, **1974**, **1975**; Eul and Schwantes, **1984**; Faro, **1972**; Gibson and Trapnell, **1957**; Kitamoto et al., **1972**, **1974**; Lyman, **1907**; Rosinski and Faro, **1968**; Rosinski and Robinson, **1968**; Snell, **1923**).

The primary objective of this study was to gain insight into the developmental and morphological changes involved in derivation of the lamellate *Lentinus* hymenophore from its poroid ancestors. The secondary objective was to assess the use of ontogenetic criteria for inferring po-

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larity and homology of morphological characters of the *Lentinus* hymenophore. Our observations were interpreted in the context of phylogenetic hypotheses from previous work (Hibbett and Vilgalys, **1991**, **1993**), and thus our study differs from other developmental studies in which ontogenetic data have been used to infer phylogenetic relationships or provide systematic characters (e.g., Reijnders, **1991**; Reijnders and Stalpers, **1992**; Watling, **1985**; and references therein).

MATERIALS AND METHODS

Fungal isolates.—Isolates used in this study and voucher sporocarps produced in culture are deposited in the culture collection and herbarium of the Tottori Mycological Institute (TMI). Fungal isolates and their TMI culture collection numbers are: *P. arcularius.* JAPAN. *Y. Kitamoto 69B* (TMI 32036); *L. crinitus* (Linn.: Fr.)Fr. COSTA RICA. *M. Nuñez 43C* (TMI 32035); *L. squarrosulus* Mont. TAIWAN. Lanyu Island, *E. Nagasawa T27-13* (TMI 31000); THAILAND. *K. M. Graham 100* (TMI 32037); and *L. tigrinus* USA NORTH CAROLINA: Durham, *Vilgalys s.n.* (TMI 32033). *Lentinus sajor-caju* (Fr.)Fr. was represented by mature field-collected sporocarps (TAIWAN. Lanyu Island, *E. Nagasawa T25-1*).

Culture and fruiting conditions.—Cultures were maintained on 1.25% malt-extract agar (MEA) at 4 C.

Polyporus arcularius was fruited on Y2 agar (2.0% glucose, 0.2% yeast extract, 0.2% polypeptone, 0.1% K_2HPO_4, 0.05% KH_2PO_4, 0.05% $MgSO_4$ (Murakami and Takemaru, **1990**). Vegetative growth was at approximately 22 C in darkness. Primordium formation was induced by placing open Y2 plates in a moist chamber in the laboratory at ambient light and temperature (approximately 20–25 C).

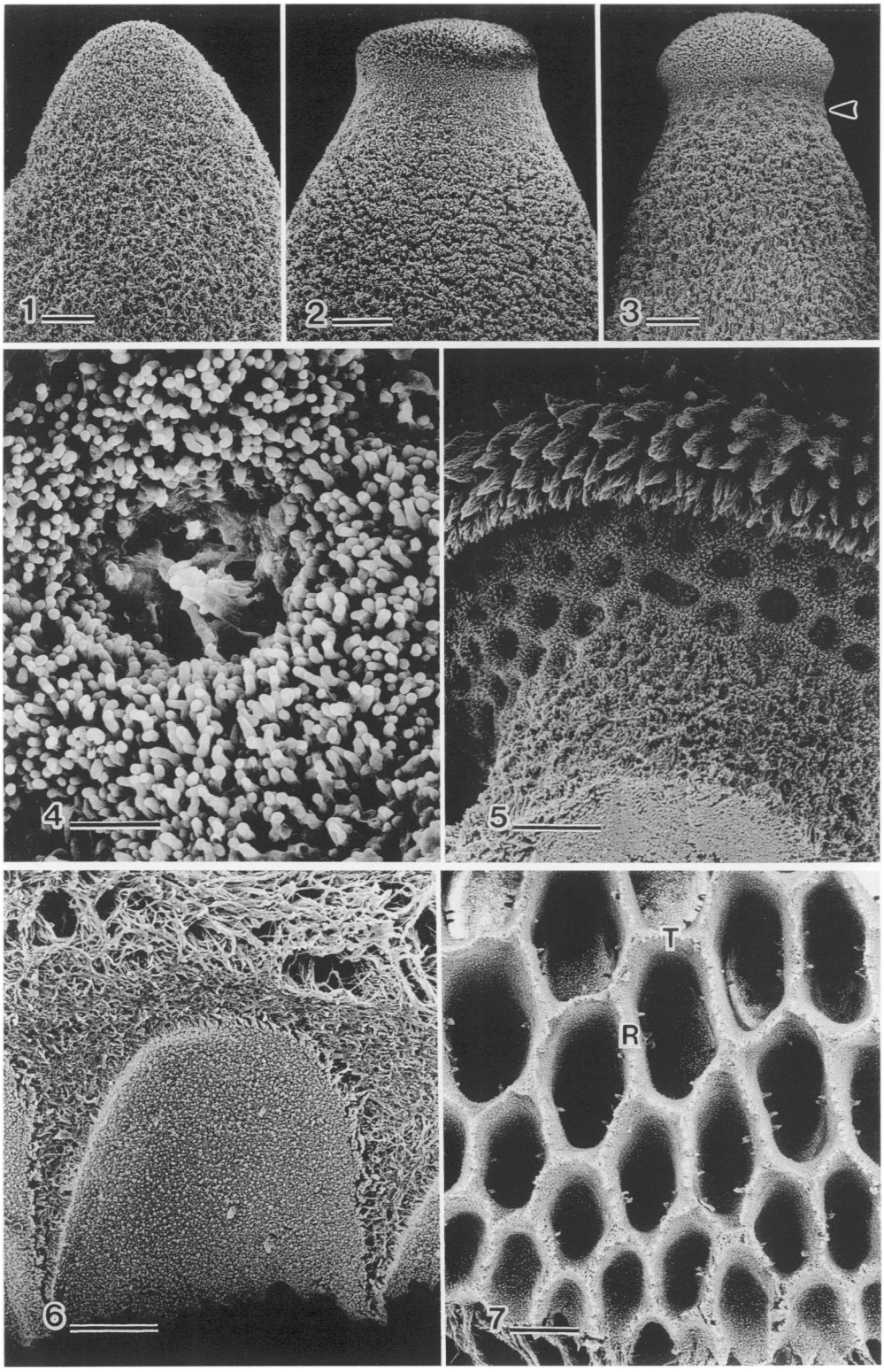
Sawdust-rice bran medium was used for spawn production and fruiting of the *Lentinus* isolates (5:1, *Fagus crenata* Blume sawdust to rice bran by volume, wetted to approximately 65% moisture content and autoclaved). Sawdust medium was placed into 300-ml plastic bottles, packed lightly, provided with a central channel for gas exchange, plugged with cotton batting, and autoclaved. MEA cultures or crumbled sawdust spawn were used as inocula. Spawn run was at approximately 22 C in darkness. To induce primordium formation, sawdust media colonized by *Lentinus crinitus* and *L. squarrosulus* isolates were removed from bottles, immersed in water, transferred to a plastic bag with a cotton batting plug, and placed in a moist 30 C incubator with a 12-h fluorescent light cycle. No special treatment was used to induce *L. tigrinus* to form primordia. After primordia formed, sawdust blocks were removed from bags or bottles and placed in a moist chamber in the laboratory at ambient light and temperature.

Electron microscopy.—Fresh sporocarps (or rehydrated sporocarps of *L. sajor-caju*) were hand-sectioned, washed in 0.066 M PO_4 buffer, pH 7 (Wako, Osaka), under a light vacuum, fixed in 2.5% glutaraldehyde in the 0.066 M PO_4 buffer, soaked overnight in 2% guanidine-2% tannic acid solution at 4 C, rinsed in distilled water and hand-sectioned again. Final sections were postfixed in 2% OsO_4 overnight, rinsed in distilled water, dehydrated in a graded ethanol series followed by an abbreviated amyl acetate series, and critical point dried in a Hitachi HCP-2 unit. Critical point dried materials were mounted on stubs and coated with gold-palladium in a Hitachi E-101 ion sputter coating device. Observations were made on a Hitachi S-800 field-emission scanning electron microscope at 15 kV (unless otherwise noted) and photographed using Fuji Neopan film.

RESULTS

POLYPORUS ARCULARIUS.—Colonies covered Y2 plates within 2–3 wk. Colonies were at first white, with dense aerial mycelium, and later developed a light brown crust. Primordia usually formed at the margin of the colony, by the edge of the plate, or occasionally on the central inoculum plug. Sporocarps developed from clusters of approximately 3–10 primordia, but usually only one to four of them reached maturity. It took 3–5 days for primordia to develop into mature sporocarps and most sporocarps decayed after 8 days. In all the species we studied, rate of sporocarp development and duration of sporocarp life varied from one fruiting to another, apparently in response to laboratory temperature which fluctuated with the weather. The fastest growth and shortest lifespans were observed during warm periods.

Primordia were conical (FIG. 1). Basidia were first observed on 1-day-old primordia, prior to differentiation of the stipe and pileus, about halfway from the top of the primordium. Pileus differentiation began around the second day of growth as the apex of the primordium expanded and became rounded (FIG. 2). Structures of the hymenophore began to be visible along the stipe apex shortly after initiation of pileus development (FIG. 3). No veils were observed. The hymenophore was poroid from the earliest point that it was recognizable (FIGS. 2–4). Initially, the pores were shallow, regularly-spaced, circular depressions with an amorphous extracellular substance lining the base of the pores (FIGS. 4, 5). This substance resembled the extracellular material previously described in developing pores of *Ganoderma lucidum* (W. Curt.: Fr.)Karst. (Mims and Seabury, **1989**). From the second to the third day, the pileus expanded and the hymenophore developed on its underside. During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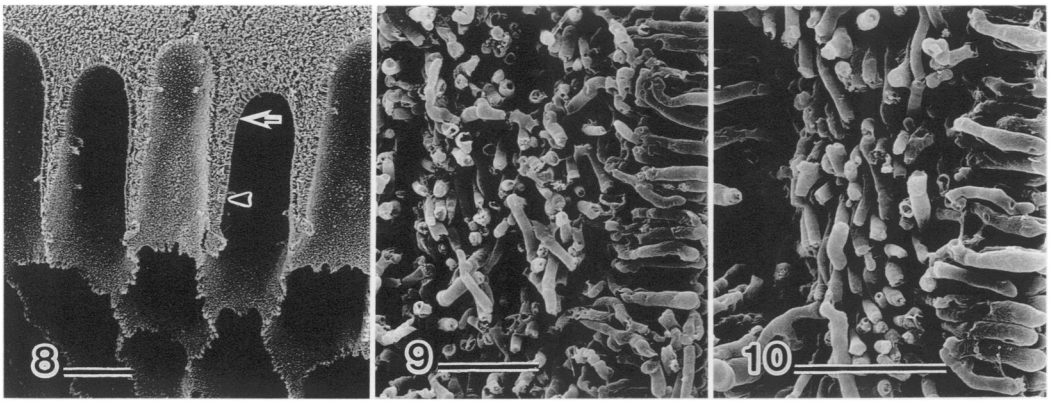

FIGS. 8–10. *Polyporus arcularius* mature hymenophoral trama anatomy. 8. Tangential section through radial walls of hymenophore. Arrow and arrowhead show locations of enlargements in FIGS. 9 and 10, respectively. Note scattered hyphal pegs. 9. Trama at base of hymenophore showing mixture of transversely cut hyphal ends indicating radiate growth, and descending hyphae. 10. Trama near dissepiments showing predominantly descending growth. Scale bars: FIG. 8 = 200 μm; FIGS. 9, 10 = 20 μm.

expansion of the pileus the pores were initially circular (FIG. 5) but later became angular and radially elongate (FIGS. 7, 8). Radial and tangential walls of the hymenophore could be easily distinguished and many pores, especially those near the stipe apex, were nearly rectangular (FIGS. 7, 8). Towards the pileus margin the pores were less elongate and more hexagonal than those near the stipe apex (FIG. 7).

The orientation of hyphae in the earliest-formed hymenophore was more or less perpendicular to the supporting stipe or pileus surfaces (FIG. 4). Soon, the hyphae of the trama began to grow out at oblique angles to the supporting tissues, following the curve of the pileus. With continued growth and broadening of the hymenophore, the angle of the tramal hyphae diverged further from the curve of the pileus, becoming nearly vertical towards the dissepiments and leading to a subregular trama (FIGS. 8–10). In the trama at the base of the hymenophore, many hyphae retained the orientation that characterized earlier growth and were nearly parallel to the underside of the pileus (FIG. 9). In all sections, hyphae of various orientations could be found, leading to an irregular trama (FIGS. 8–10). There was no clear-cut transition between the trama of the base of the hymenophore and the dissepiments. At maturity the dissepiments were lacerate (FIG. 8).

LENTINUS TIGRINUS.—Sawdust medium was fully colonized within 2 wk. Spawn blocks were mostly white, with some light grey to black pigmented areas. Primordia formed in darkness, sometimes before the substrate was fully colonized. Primordia formed on the surface of the spawn blocks, or occasionally formed underneath a surface mycelial mat which was ruptured as the primordia expanded. Sporocarps were usually numerous, in dense cespitose clusters, but occasionally only a few sporocarps were formed. Timing and rate of growth were similar to those of *P. arcularius* and sporocarps persisted for 5–9 days.

Primordium morphology was similar to *P. arcularius* (FIGS. 11, 12) and basidia were observed on otherwise undifferentiated primordia. Basidia on undifferentiated *L. tigrinus* primordia were also reported by Bobbitt and Crang (**1975**) and

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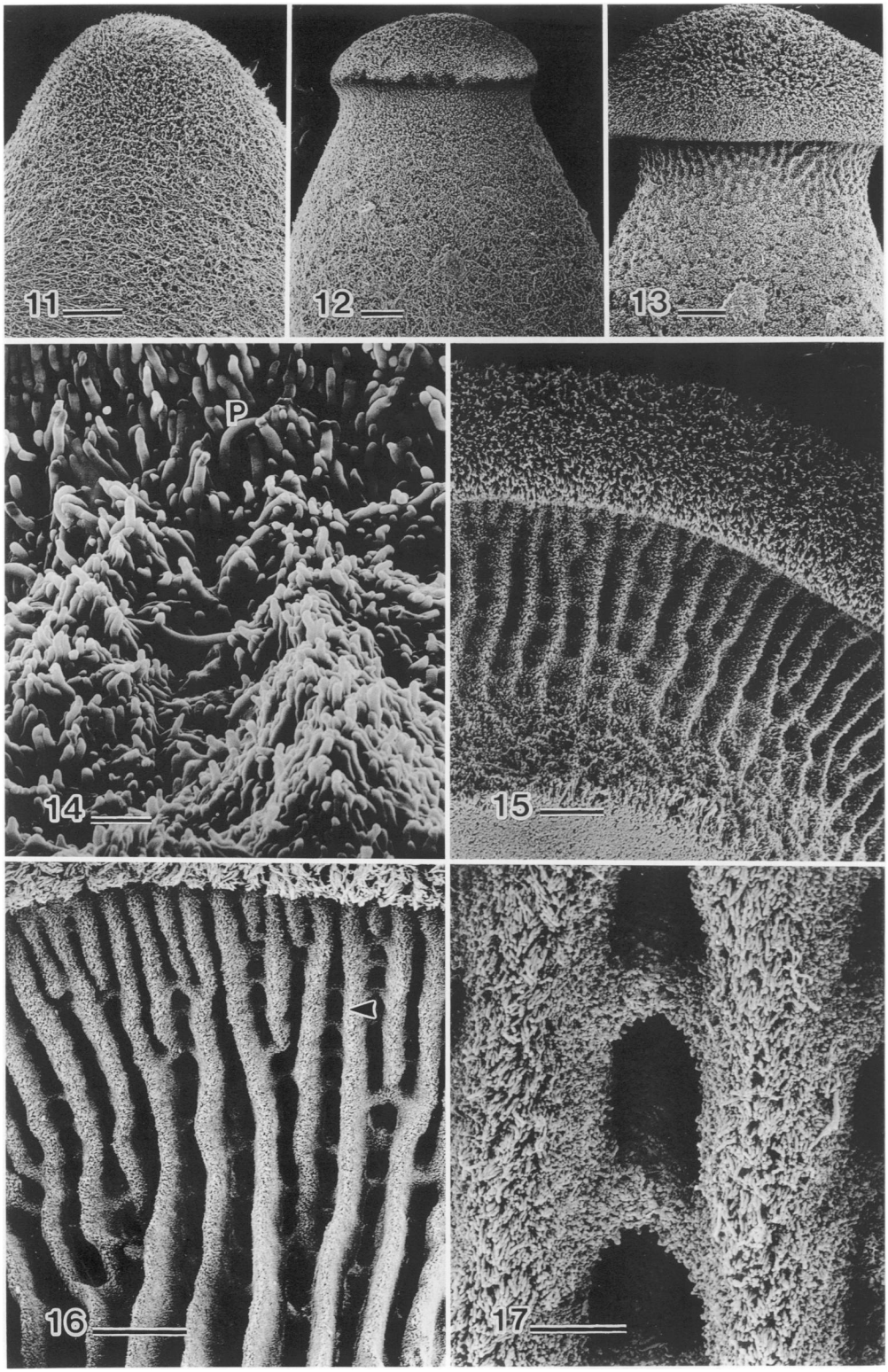
FIGS. 1–7. *Polyporus arcularius* hymenophore development. 1. Primordium. 2, 3. Initiation of pileus and hymenophore differentiation. Young pores are visible as circular depressions on stipe apex (FIG. 3, arrowhead). 4. Detail of young pore showing extracellular material lining base of pore. 5. Underside of immature pileus with circular pores. 6. Radial section through tangential walls of mature hymenophore; note lacerate dissepiments. 7. Mature hymenophore near pileus margin showing tangential (T) and radial (R) walls of angular, radially elongate pores. Scale bars: FIGS. 1–3, FIGS. 5–7 = 200 μm; FIG. 4 = 25 μm.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are known from other agarics (e.g., Miller, **1971**). Pileus initiation was similar to *P. arcularius* and no veil was observed (FIGS. 12, 13). Hymenophore development began on the stipe apex, below the expanding pileus (FIG. 13). The earliest-formed hymenophore structures were narrow, irregular to subparallel ridges of appressed hyphae (FIGS. 13, 14; these were termed "outfoldings" by Bobbitt and Crang, **1975**). Within 2–3 days, the ridges of hyphae became distinct, parallel lamellae with some anastomosis and reticulation, especially over the stipe apex (FIGS. 15, 16). Transverse cross-bridges developed between the lamellae at their base (FIGS. 16, 17). Cross-bridges could be easily differentiated from lamellar anastomoses because the cross-bridges were narrower than the lamellae and intersected the lamellae at right angles (FIGS. 17, 24). The cross-bridges continued to grow but never reached the width of the lamellae. At maturity, the lamellae and cross-bridges formed a regular, subporoid network composed of more or less rectangular, concave chambers at the base of the hymenophore (FIGS. 24, 25). In radial section, the cross-bridges of *L. tigrinus* were triangular, as were the tangential walls of the *P. arcularius* hymenophore (FIGS. 6, 25).

Development of the hymenophoral trama was also similar to *Polyporus arcularius*. Growth of the initial ridges of the hymenophore was perpendicular to the supporting tissues (FIG. 14). Subsequent growth of the trama was at oblique angles to the curve of the pileus. Consequently, tangential sections of young hymenophores showed transversely cut hyphae when taken near the stipe, and a regular, parallel trama when taken near the pileus margin (FIGS. 18–20). At maturity, the trama at the base of the hymenophore was subparallel to the pileus surface whereas near the margin the trama was more regular and nearly parallel (FIGS. 21–23). Mature lamellae were up to 3–4 mm broad, and moderately crowded, and became lacerate with age (FIG. 21).

LENTINUS CRINITUS.—Sawdust medium was colonized in 3–4 wk. Spawn blocks developed a darkly pigmented brown to black crust. After 5–7 wk, scattered clumps of two to five primordia formed on the surface of the spawn block. Sporocarps developed to maturity in 4–6 days and usually persisted for about 9 days.

Morphology of the primordia and early differentiation of the pileus and stipe were similar to those of *L. tigrinus* (FIGS. 26–28). However, no basidia were observed on the undifferentiated primordia. From the primordium stage onwards, *L. crinitus* was more hispid than *L. tigrinus* because of fascicles of agglutinated hyphae on the surface of the sporocarp (FIGS. 26–29).

Hymenophore differentiation began along the stipe apex and spread to the underside of the pileus as it expanded (FIGS. 28–30). No veils were observed. The early hymenophore was composed of ridges of hyphae similar to those observed in *L. tigrinus* (FIGS. 28, 29). Subsequent hymenophore development was similar to that of *L. tigrinus*, but no cross-bridges formed at the base of the lamellae (FIGS. 30–32). There were some lamellar anastomoses, mostly over the stipe apex (FIG. 30), but the hymenophore remained entirely lamellate at maturity (FIGS. 31, 32).

Development and anatomy of the hymenophoral trama was similar to that of *L. tigrinus*. Hyphae at the base of the hymenophore were almost parallel to the underside of the pileus whereas towards the margin the trama was more regular (FIGS. 33, 34). The lamellae were not as broad as in *L. tigrinus* (up to 1.5 mm), and the margins were entire to denticulate (FIG. 32). At maturity the lamellae were densely crowded (FIG. 31).

LENTINUS SQUARROSULUS.—Time for colonization and fruiting was similar to that of *L. crinitus*. Spawn blocks developed a dark brown rind on which scattered primordia formed singly or in clusters that gave rise to up to five cespitose spo-

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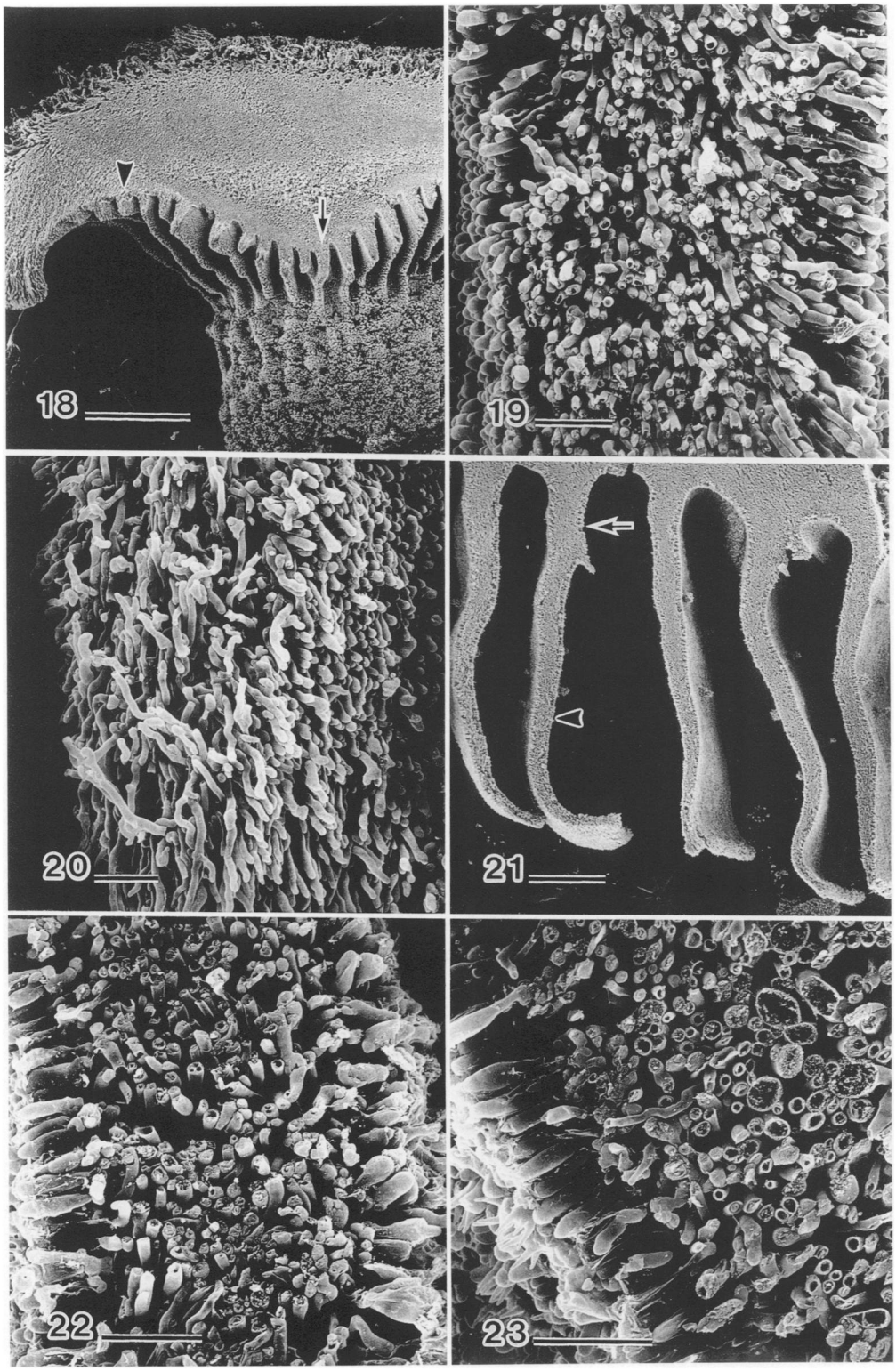
FIGS. 11–17. *Lentinus tigrinus* early hymenophore development. 11. Primordium. 12, 13. Initiation of pileus and hymenophore development. Early hymenophore is formed of irregular ridges of hyphae (FIG. 13). 14. Detail of ridges of appressed hyphae of early hymenophore on stipe, looking up towards underside of young pileus (P). 15, 16. Later stages of hymenophore development. Ridges have differentiated into distinct lamellae. Cross-bridges are first visible as faint transverse bands in FIG. 15. Arrowhead in FIG. 16 indicates location of enlargement in Fig. 17 (2 kV). 17. Detail of cross-bridges at base of young lamellae (2 kV). Scale bars: FIGS. 11, 12, 15 = 200 μm; FIGS. 13, 16 = 400 μm; FIG. 14 = 25 μm; FIG. 17 = 100 μm.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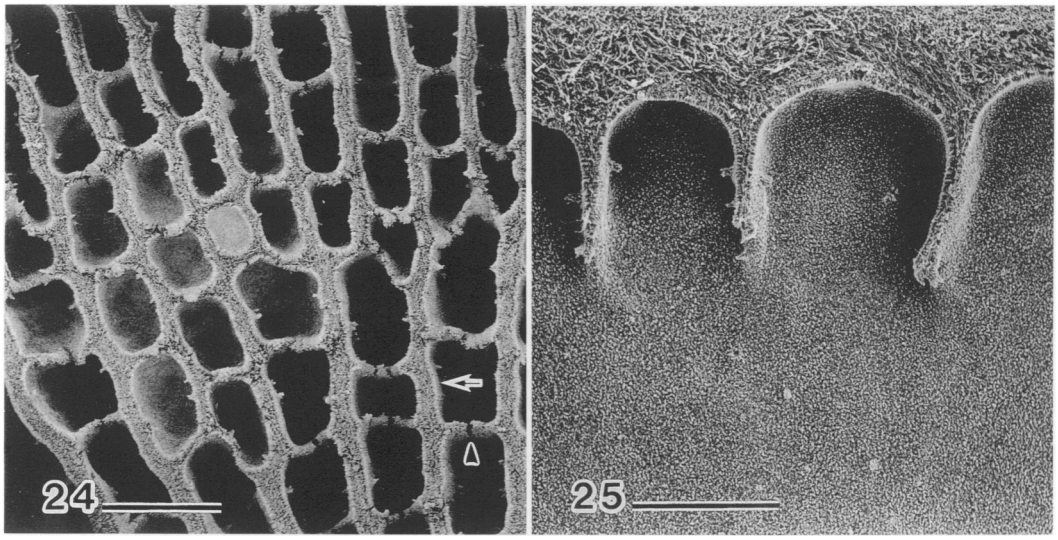

FIGS. 24, 25. *Lentinus tigrinus* mature hymenophore. 24. Scalp section showing subporoid arrangement of lamellae (arrow) and cross-bridges (arrowhead; 2 kV). 25. Radial section through cross-bridges. Scale bars = 500 μm.

rocarps. Two to three days were required for primordia to develop into mature sporocarps. Sporocarps decayed rapidly and rarely persisted for more than 6 days. These observations agree with a previous account of the growth of *L. squarrosulus* on natural substrates by Corner (**1981**).

The primordium and young sporocarp were enclosed by an ephemeral universal veil composed of interwoven hyphae (FIGS. 35, 36). No basidia were observed on the veil. As the pileus expanded the veil became cortinoid (FIG. 37). The veil obscured surface views of the developing hymenophore, but in sections of young sporocarps the lamellae could be seen developing in longitudinal cavities underneath the veil tissue (FIGS. 37, 38). The lamellae eventually ruptured the veil, remnants of which remained as scattered clumps or lines on the sides of the lamellae and the stipe apex (FIG. 39). In other regards, development of the lamellae resembled that of *L. crinitus*. No cross-bridges were formed, and except for some slight anastomosing, mostly over the stipe apex, the hymenophore was completely lamellate at maturity (FIG. 40).

Growth and anatomy of the hymenophoral trama were similar to *L. crinitus* (FIGS. 41, 42). The margins of the lamellae were entire (FIG. 41). The width and degree of crowding were similar to *L. crinitus*.

LENTINUS SAJOR-CAJU.—Lamellae of mature sporocarps were narrow (up to 1 mm) and densely crowded, with few anastomoses and no cross-bridges (FIGS. 43, 44). The hymenophoral trama was densely woven and parallel to the underside of the pileus, even at the margins of the lamellae

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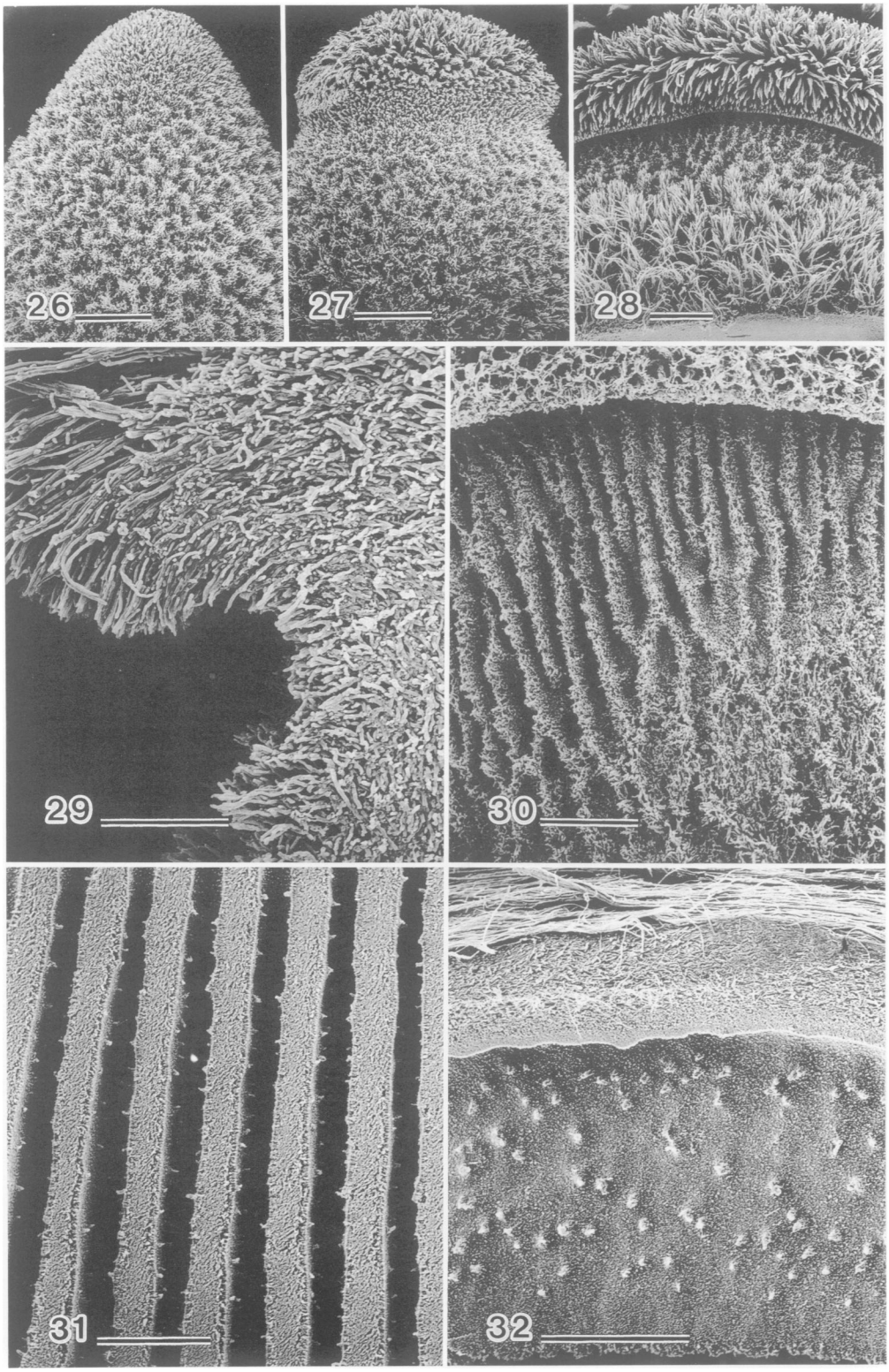
FIGS. 18–23. *Lentinus tigrinus* hymenophoral trama development. 18–20. Tangential sections of young sporocarp at approximately same stage as FIGS. 16 and 17. 18. Arrow and arrowhead indicate locations of enlargements in FIGS. 19 and 20, respectively. 19. Section close to stipe apex. Hyphae are growing out at oblique angles to the stipe surface, resulting in transversely-cut hyphal ends. 20. Section close to pileus margin. Hyphae are curving down from pileus undersurface, resulting in descending trama. 21–23. Mature sporocarp sections. 21. Tangential section. Arrow and arrowhead indicate location of enlargement in FIG. 22 and approximate depth of section in FIG. 23, respectively. Note scattered hyphal pegs. 22. Tangential section near base of lamella with transversely-cut hyphae, indicating radiate trama. 23. Scalp section near lamella margin showing transversely-cut hyphae, indicating descending growth. Scale bars: FIG. 18 = 1 mm; FIGS. 19, 20, 22, 23 = 20 μm; FIG. 21 = 150 μm.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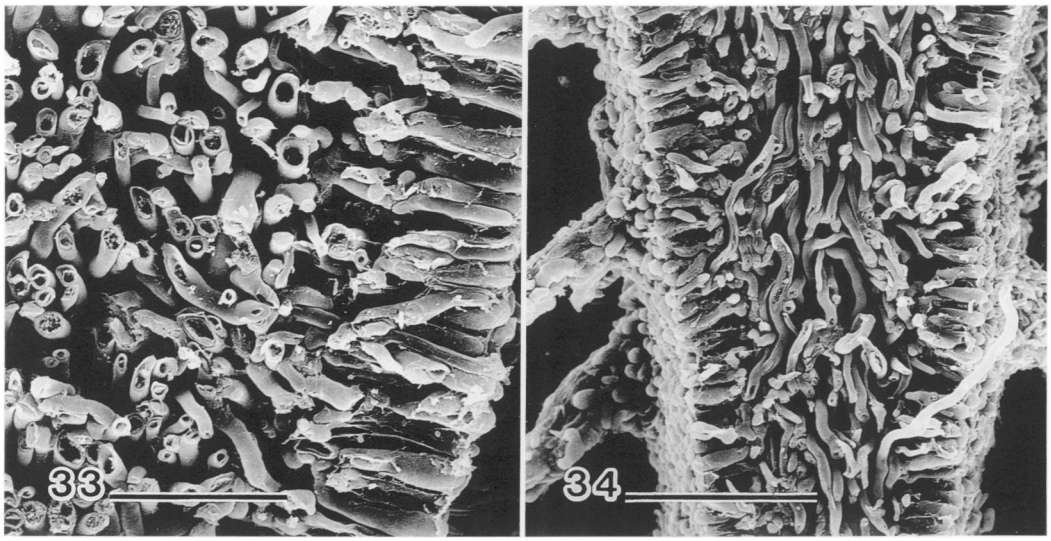

FIGS. 33, 34. *Lentinus crinitus* mature hymenophoral trama. 33. Tangential section near base of lamella showing transversely-cut hyphae, indicating radiate growth. 34. Same section near margin showing mostly descending growth. Note hyphal pegs. Scale bars: FIG. 33 = 20 μm; FIG. 34 = 40 μm.

(FIGS. 45, 46). In all tangential sections only transversely-cut hyphal ends were visible (FIGS. 45, 46).

DISCUSSION

On the basis of prior phylogenetic hypotheses (Hibbett and Vilgalys, **1991**, **1993**), we divided the organisms studied here into an ingroup *Lentinus* and an outgroup *P. arcularius*. *Lentinus squarrosulus* and *L. sajor-caju* were not included in the previous molecular studies. Their inclusion in the ingroup is based on a morphological genus concept (Corner, **1981**). Ontogenies and morphological features were treated as phylogenetic characters of the organisms and were polarized using the outgroup criterion (Maddison et al., **1984**). Ontogenetic and morphological features that most resembled those of *P. arcularius* were therefore designated plesiomorphic. This approach allowed us to make the following inferences about evolutionary changes in developmental programs and morphology:

Derivation of lamellae from pores.—Transverse walls at the base of the lamellae were noted by Corner (**1981**) and Pegler (**1983**) in certain *Lentinus* species, especially sect. *Tigrini* Pegler, which includes *L. tigrinus*. Pegler suggested that the subporoid construction of some species was, "possibly indicating a polypore ancestry" (Pegler, **1983**, p. 4). We also suggest that the cross-bridges of *L. tigrinus* are homologous to the tangential pore walls of *P. arcularius* (FIGS. 6–8, 24, 25). The regular spacing of the cross-bridges in *Lentinus tigrinus* is similar to the arrangement of the tangential walls of the *P. arcularius* hymenophore (FIGS. 7, 24). The lamellae of *L. tigrinus* are moderately crowded and in this regard resemble the radial walls of the large *P. arcularius* pores, whereas the *Lentinus* species that lack cross-bridges have more crowded lamellae. Both

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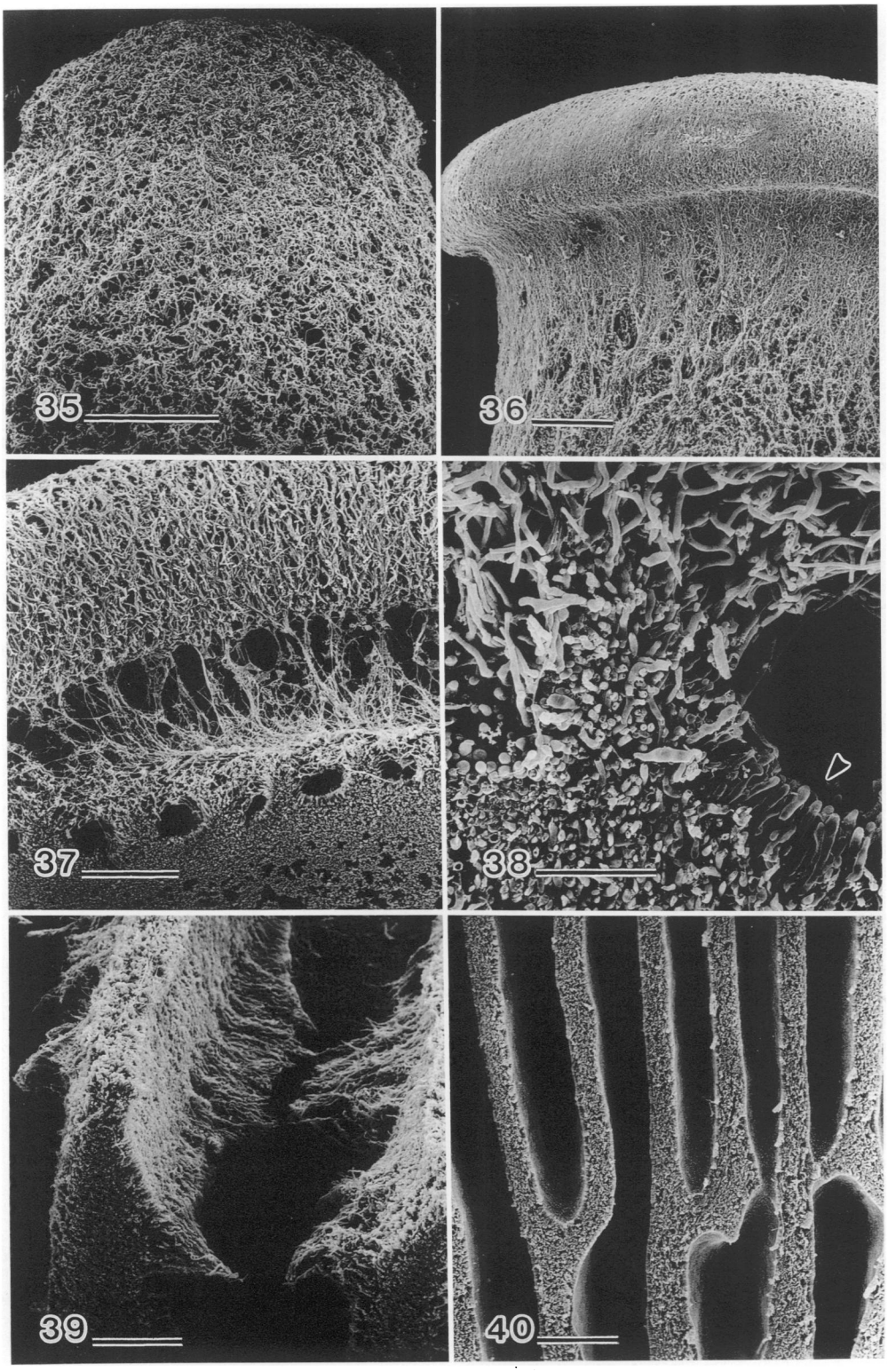
FIGS. 26–32. *Lentinus crinitus* hymenophore development. 26. Primordium. 27, 28. Initiation of pileus and hymenophore differentiation. Young hymenophore (FIG. 28) is composed of irregular ridges of hyphae similar to those of *L. tigrinus*. 29. Longitudinal section at approximately same stage as FIG. 28. Surface hyphae in region of developing hymenophore are growing out at right angles to stipe surface. 30. Later hymenophore development with some anastomosing, but no cross-bridge formation (2 kV). 31. Scalp section of mature hymenophore showing absence of cross-bridges. Note hyphal pegs (and in FIG. 32). 32. Radial section of mature hymenophore showing finely denticulate margin. Scale bars: FIGS. 26–28, 30–32 = 400 μm; FIG. 29 = 100 μm.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the cross-bridges and tangential walls have a triangular profile in radial section and have a predominantly descending trama (FIGS. 6, 25).

An alternate interpretation of the cross-bridges is that they are a uniquely evolved autapomorphy of *L. tigrinus,* not a homologue of the tangential walls of the *P. arcularius* hymenophore. However, this is an unparsimonious interpretation of hymenophore evolution. The hypothesis that cross-bridges and tangential pore walls are homologous requires only one evolutionary transformation: loss of cross-bridges. The hypothesis that cross-bridges are an autapomorphy requires a minimum of two evolutionary transformations: loss of tangential walls, and gain of cross-bridges. To assert that cross-bridges and tangential pore walls are not homologous, one must invoke an evolutionary character reversal by which parallel evolution has occurred. Homology of the cross-bridges and tangential pore walls is therefore supported by parsimony as well as morphological similarity.

Derivation of the lamellate *Lentinus* hymenophore can be interpreted as the result of reduction of tangential elements of the hymenophore. *Lentinus tigrinus,* with its subporoid hymenophore, is therefore interpreted as a plesiomorphic intermediate between the polypore outgroup and the other, more derived *Lentinus* species that lack cross-bridges. In terms of a change in development, the reduction seems to involve a delay in the onset of growth of the tangential walls, perhaps coupled with a reduction in their growth rate, or early cessation of growth. These are heterochronic changes because they affect the relative rate and timing of developmental events (Gould, **1977**; McKnight, **1992**, for another basidiomycete example; Raff and Wray, **1989**). In this case, the affected developmental events are growth of tangential and radial elements of the hymenophore. The developmental changes have the consequence that the *L. tigrinus* hymenophore initially appears lamellate and only becomes distinctly subporoid in later ontogenetic stages (FIGS. 15, 24). Thus, in *L. tigrinus,* the apparent ontogenetic polarity is lamellate to subporoid whereas the putative phylogenetic polarity is poroid to lamellate (Hibbett and Vilgalys, **1991**, **1993**; Pegler, **1983**). Using ontogeny alone to polarize the pore-gill transition would therefore be misleading.

We had expected that the early morphologies of the *P. arcularius* and *Lentinus* hymenophores would be similar, with later deviations in development accounting for the differences in adult morphologies. This would have been consistent with von Baer's law, which implies that early development of related organisms should be similar (Gould, **1977**; Kluge, **1985**). Moore (**1987**) also expressed the opinion that fungal ontogenies are evolutionarily conserved when, drawing a parallel between mammalian embryology and fungal development, he wrote, "it is inconceivable that agaric fungi would use a multitude of morphogenetic processes to arrive at the same mushroom body plan." However, morphology of the early hymenophore of *P. arcularius* was strikingly different from that of *L. tigrinus* and *L. crinitus* (FIGS. 3–5, 13–15, 28–30; it was not observable in *L. squarrosulus* because of the veil). Contrary to our speculation, hymenophores of *P. arcularius* and *L. tigrinus* came to resemble each other most in mature stages, after the elongation of the pores in *P. arcularius* and the development of cross-bridges in *L. tigrinus* (FIGS. 6–8, 24, 25). The hymenophores of *P. arcularius* and *Lentinus* are presumably homologous, and yet their morphologies differ at early developmental stages. Other examples of putative homologues that have dissimilar ontogenetic origins were discussed by Roth (**1988**).

The relationship between ontogeny and phylogenetic inference has been debated at length elsewhere (Alberch, **1985**; Kluge, **1985**; Kluge and Strauss, **1985**; Mabee, **1989**; Mishler, **1988**; Nelson, **1978**; Patterson, **1982**; de Queiroz, **1985**;

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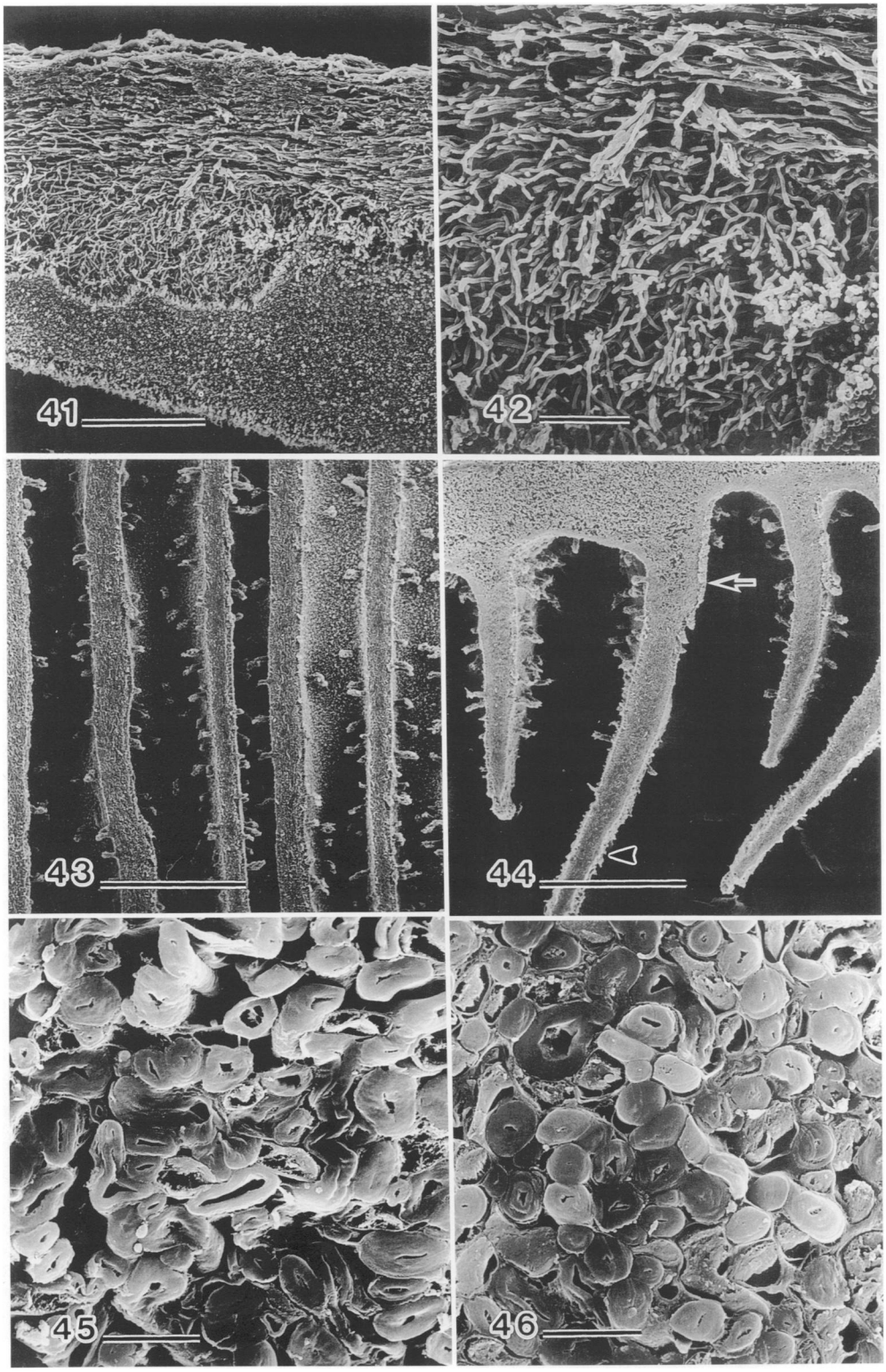
FIGS. 35–40. *Lentinus squarrosulus* (TMI 32037) hymenophore and veil development. 35. Primordium covered by interwoven hyphae. Outline of young pileus is visible. 36. Early pileus expansion. Hymenophore is not visible because of veil. 37. Later developmental stage. Veil is becoming cortinoid as pileus expands. Lamellae can be seen developing under veil in transverse section. 38. Detail from FIG. 37 showing lamellae and young hymenium (arrowhead) forming under veil. 39. Mature lamellae near stipe apex showing remnants of ruptured veil. 40. Scalp section of mature hymenophore near stipe apex showing lamellar anastomoses. Scale bars: FIGS. 35, 36, 40 = 400 μm; FIG. 37 = 200 μm; FIG. 38 = 40 μm; FIG. 39 = 100 μm.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Stevens, **1980**; and others). Our results suggest that application of ontogenetic criteria for assessing evolutionary polarity and homology of *Lentinus* and *P. arcularius* hymenophore characters could be misleading. It would be premature to generalize these results to all fungi. Nevertheless, other workers have suggested that paedomorphosis, which is retention of juvenile features of ancestors in their mature descendants, has been important in evolution of certain groups of fungi (Bruns et al., **1989**; Kreisel, **1991**; Thiers, **1984**) and this would also cause problems for applications of ontogenetic criteria in character analysis (Kluge, **1985**).

Evolution of the hymenophoral trama and gill margin.—Development and anatomy of the hymenophoral trama in *Lentinus* has been described by Chang (**1965**, as cited *in* Corner, **1981**, and Pegler, **1983**), Corner (**1981**), Pegler (**1983**), and Pegler and Young (**1983**). These authors described two ways that the gills of *Lentinus* can develop: 1) descending growth, in which the hyphae in the trama grow down at right angles to the pileus surface; and, 2) radiate growth, in which the hyphae in the trama are radially aligned and parallel to the surface of the pileus. In tangential sections, purely descending growth yields a regular trama whereas purely radiate growth yields transversely cut hyphal ends (Corner, **1981**; Pegler, **1983**; Pegler and Young, **1983**). Intermediate types of construction that are difficult to classify as either descending or radiate growth have been described in *Lentinus* (Corner, **1981**; Pegler, **1983**; Pegler and Young, **1983**).

Corner (**1981**) and Pegler and Young (**1983**) suggested that descending growth is typical of polypore development. In *P. arcularius* we observed an irregular trama with a mixture of radiate and descending growth (FIGS. 8–10). *Lentinus tigrinus, L. crinitus,* and *L. squarrosulus* had a similar mixed, irregular trama, but there was variation in the degree of descending versus radiate growth. *Lentinus tigrinus* had the most strongly developed descending growth, followed by *L. crinitus,* and *L. squarrosulus* (FIGS. 21–23, 33, 34, 41, 42). The distinction between the last two species was subtle. In contrast, *L. sajor-caju* had a distinctive, strongly-developed radiate construction (FIGS. 45, 46). Based on comparison to the outgroup, the hymenophoral trama types that we observed in *Lentinus* can be arranged in a polarized morphocline from the plesiomorphic, mixed descending-radiate *L. tigrinus* type (Pegler and Young, **1983**), to the derived, strongly radiate *L. sajor-caju* type.

Corner (**1981**), Pegler (**1983**), and Pegler and Young (**1983**) noted that uneven attenuation of descending growth leads to a lacerate margin. By extension, our results suggest that the lacerate margin is a plesiomorphic character in *Lentinus* that is a consequence of extensive descending growth. The dissepiments of *P. arcularius* and the lamella margin of *L. tigrinus* become lacerate with age, whereas the other *Lentinus* species, with narrower lamellae, all have denticulate to entire margins.

Our anatomical observations essentially agree with descriptions by Corner (**1981**), Pegler (**1983**), and Pegler and Young (**1983**). However, Reijnders and Stalpers (**1992**) doubted the existence of the radiate type of construction in *Lentinus.* The discrepancy between the reports illustrates the difficulty of using hymenophoral tramal anatomy as a systematic character. As noted by Reijnders and Stalpers (**1992**), meaningful comparisons require equivalent sections from comparable developmental stages, preferably from fresh material. Early growth patterns can be obscured by later irregular, invasive growth of hyphae (Corner, **1981**; Pegler, **1983**; Pegler and Young, **1983**).

Of the six lentinoid species that Reijnders and Stalpers (**1992**) examined, only two, *L. suavis-*

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FIGS. 41–46. *Lentinus squarrosulus* (TMI 3100) and *L. sajor-caju* mature hymenophores. 41. *Lentinus squarrosulus* radial section with part of hymenium chipped away after critical point drying; stipe is to the right of the frame. 42. Detail from FIG. 41 showing radiate growth at the base of the hymenophore and in the pileus context, and mixed descending-radiate growth towards the margin. 43–46. *Lentinus sajor-caju* mature hymenophore. 43. Scalp section showing absence of cross-bridges. Note numerous hyphal pegs (and in FIG. 44). 44. Tangential section. Arrow and arrowhead show locations of enlargements in FIGS. 45 and 46, respectively. 45. Densely packed hymenophoral trama at base of lamella with purely radiate growth. 46. Hymenophoral trama at margin of lamella with purely radiate growth. Scale bars: FIG. 41 = 200 μm; FIG. 42 = 50 μm; FIGS. 43, 44 = 400 μm; FIGS. 45, 46 = 5 μm.

simus Fr. and *L. tigrinus,* are members of *Lentinus* as applied here. The others are members of *Neolentinus* (Redhead and Ginns, **1985**) and *Panus sensu strictu.* In our observations of *L. tigrinus,* only the base of the mature lamellae retained the radiate construction and the rest of the trama was irregular to descending (FIGS. 21–23). It is therefore not surprising that Reijnders and Stalpers (**1992**, p. 23) interpreted the trama at the base of the lamellae in *L. tigrinus* as, "densely interwoven."

Evolution of veils.—Except for *L. squarrosulus,* all the species whose ontogeny we observed, including the outgroup, were gymnocarpic (Watling, **1985**). Kühner (**1925**) and Pegler (**1983**) reported that *L. tigrinus* has a cortinoid veil but we did not observe a veil in *L. tigrinus* (FIG. 13), nor did Bobbitt and Crang (**1975**) in their developmental studies. Veils or other protective structures are reported for six *Lentinus* species besides *L. tigrinus* and *L. squarrosulus* in three different sections (Pegler, **1983**). There is also a North American secotioid-like variety of *L. tigrinus* that has a membranous tissue covering the hymenophore (Pegler, **1983**; Rosinksi and Robinson, **1968**). Our results suggest that veils are derived features within *Lentinus.*

Because of its limited taxonomic sampling, this study cannot address the full range of morphological and developmental diversity in *Lentinus.* Developmental observations of other *Lentinus* species are needed, but they alone will not be sufficient to test the hypotheses about morphological and developmental evolution presented here. For that, it will be necessary to combine ontogenetic data with phylogenetic trees derived from nonmorphological characters.

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