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SPOROCARP ONTOGENY IN *PANUS* (BASIDIOMYCOTINA): EVOLUTION AND CLASSIFICATION¹

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Ontogenies of cultured *Panus conchatus*, *P. rudis*, and *P. fulvus* sporocarps were observed macroscopically and with scanning electron microscopy. Hymenophore differentiation in *Panus* involves periclinal growth of context hyphae below a closed surface palisade of hymenial elements, resulting in a cantharelloid appearance and radiate trama. This pattern is qualitatively different from that in *Lentinus* s. str., which suggests that lamellae of *Panus* and *Lentinus* are not homologous. *Panus conchatus* and *P. rudis* sporocarps have short stipes, develop directly from the mycelium, and mature in 5–10 d. *Panus fulvus* sporocarps have an elongate stipe, develop from a pseudosclerotium, and mature in about 3 wk, the first approximately 15 d of which involve apical elongation of a stipelike primordium that is able to dedifferentiate and regenerate cut apices. *Panus conchatus* and *P. rudis* sporocarps lacked regeneration ability. *Panus conchatus* sporocarps developed an ephemeral partial veil that was obliterated during sporocarp expansion. Outgroup comparison suggests that evolutionary changes in developmental programs in *Panus* have included: 1) delay in offset of primordium growth, with a corresponding increase in primordium size and time to maturation (hypermorphosis); 2) insertion of the pseudosclerotial stage in ontogeny; 3) gain of ability for dedifferentiation and regeneration; and 4) nonterminal gain or loss of veil tissue.

The generic limits and phylogenetic relationships of *Panus* Fr. and *Lentinus* Fr. are controversial (Corner, 1981; Hibbett and Vilgalys, 1993; Pegler, 1983). Both genera include wood-decaying basidiomycetes that have a tough dimitic construction, decurrent lamellae, and cylindrical to ellipsoid hyaline spores. *Panus* and *Lentinus* are found on all continents except Antarctica, and reach their greatest density and species diversity in the tropics (Corner, 1981; Pegler, 1983).

Major treatments for *Panus* and *Lentinus* include those of Corner (1981), Kühner (1980), Pegler (1983), and Singer (1986). Each is unique in its delimitation of *Panus* and *Lentinus*. Pegler and Corner both strongly emphasized the system of sporocarp hyphal analysis that was developed by Corner (1932). Pegler (1983) included *Panus* as a subgenus of *Lentinus* whereas Corner (1981) maintained *Panus* as a distinct genus, but their classifications are otherwise essentially parallel. In contrast, Singer (1986) and Kühner (1980) emphasized the anatomy of the hymenophoral trama and the number of nuclei per spore in their treatments of *Panus*, *Lentinus*, and other lentinioid-pleurotoid fungi. Consequently, even though there are significant differences between the Singer and Kühner classifications, they are more similar to each other than either is to the Pegler or Corner classifications. The discrepancies between the Corner-Pegler and Singer-Kühner treatments are exacerbated by disagreements about the type species of *Panus* and *Lentinus*.

The Corner, Kühner, Pegler, and Singer classifications were constructed without consideration of wood decay

chemistries. In basidiomycetes, two major classes of wood decay type are recognized: 1) white rot, in which both lignin and cellulose are degraded; and 2) brown rot, in which lignin is not appreciably degraded. In 1985, Redhead and Ginns segregated the brown rot species of *Panus* and *Lentinus* as *Neolentinus* Redhead and Ginns and *Heliocybe* Redhead and Ginns. *Neolentinus* and *Heliocybe* correspond to parts of *Lentinus* subg. *Panus* sensu Pegler, *Panus* sensu Corner, and *Lentinus* sensu Singer and Kühner (this distribution illustrates the conflict between the previous classifications).

Recently, molecular characters have been applied to systematics of *Panus* and *Lentinus* (Hibbett and Vilgalys, 1991, 1993). These studies included distance-based analyses of restriction fragment length polymorphisms in ribosomal DNA (rDNA, Hibbett and Vilgalys, 1991), and cladistic analyses of rDNA sequence data, alone or in conjunction with morphological characters (Hibbett and Vilgalys, 1993). The rDNA data supported the monophyly of *Lentinus* sensu Corner (1981), *Neolentinus* (in large part), and a restricted concept of *Panus*. In the cladistic analyses of rDNA sequence data, *Panus* s. str. was represented by *P. conchatus* (Bull.: Fr.) Fr., which is the type species of *Panus* (Corner, 1981; Pegler, 1983), *P. rudis* Fr., and *P. fulvus* (Berk.) Pegler and Rayner. The monophyly of the clade containing these species was strongly supported by both bootstrap (Felsenstein, 1985) and decay index (Mishler, Donoghue, and Albert, 1991) measures of topological robustness.

Some polypores and bracket fungi are anatomically similar to *Panus* and *Lentinus* (Corner, 1981; Pegler, 1983). Consequently, some authors place *Panus* and *Lentinus* in the Polyporaceae, despite the fact that *Panus* and *Lentinus* are gilled mushrooms (e.g., Singer, 1986). The rDNA studies supported the view that *Lentinus* s. str. is derived from *Polyporus* Fr., but did not indicate that *Panus* is derived from polypore fungi. This suggests that the lamellae of *Panus* and *Lentinus* are not homologous, but rather are products of convergent evolution.

We have previously investigated ontogeny of the *Lentinus* s. str. hymenophore using scanning electron mi-

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croscopy (SEM) of cultured sporocarps (Hibbett, Murakami, and Tsuneda, 1993). In the present paper, we report developmental observations of three species of *Panus* s. str. Our primary objective in this work was to determine if there are developmental differences between the putatively convergent *Panus* and *Lentinus* hymenophores. Such differences would corroborate our previous molecular phylogenetic hypotheses, and support the taxonomic segregation of *Panus* and *Lentinus*.

We were also interested in comparing sporocarp ontogenies within *Panus*. *Panus conchatus* and *P. rudis* have short lateral to excentric (occasionally central) stipes, but *P. fulvus* has a slender, elongate central stipe whose length far exceeds pileus diameter. In addition, *P. fulvus* sporocarps develop from a pseudosclerotium (Petch, 1915; Corner, 1981; Pegler, 1983) that is lacking in *P. conchatus* and *P. rudis*. (Pseudosclerotia are composed of wood substrates infiltrated by hyphae, whereas true sclerotia are composed only of hyphae.) Our second objective was therefore to understand the modifications to developmental programs in *Panus* that are responsible for these striking morphological differences. Finally, we were interested in the implications of patterns of developmental evolution in *Panus* for the use of ontogenetic data as criteria for assessing evolutionary polarity of morphological characters in fungi.

MATERIALS AND METHODS

Fungal isolates fruited in this study and voucher sporocarps are deposited in the culture collection and herbarium of the Tottori Mycological Institute (Table 1). *Panus rudis* and *P. conchatus* were represented by four isolates each, and *P. fulvus* was represented by a single isolate.

Cultures were maintained on 1.25% malt extract agar (MEA) at 4 C. Sawdust medium consisting of 5:1 *Fagus crenata* Blume sawdust to rice bran by volume, wetted to approximately 65% moisture content, was used for fruiting. Sawdust medium was packed into 300- or 800-ml plastic bottles, provided with a central channel for gas exchange, plugged with cotton batting, and autoclaved. MEA cultures or crumbled, colonized sawdust medium (spawn) was used as inoculum.

Prior to fruiting, *P. conchatus* and *P. rudis* spawn was removed from the plastic bottle (or the top of the bottle was cut off), immersed in water, and transferred to a moist chamber in the laboratory at ambient light and temperature conditions. Some *P. conchatus* and *P. rudis* spawn blocks were removed from their bottles, crumbled, packed into plastic bags with a cotton plug, and allowed to knit back together into a solid block prior to fruiting. The spawn blocks were misted daily with water.

Panus fulvus was fruited from pseudosclerotia that were produced inside colonized spawn. Pseudosclerotia were removed from spawn 3–4 mo after inoculation, washed, and either stored at 4 C for 1–3 wk, or fruited directly. To induce primordium formation, pseudosclerotia were placed in a 30 C incubator with a 12-hr fluorescent light cycle. After primordium initiation the pseudosclerotia were transferred to the moist chamber in the laboratory.

Preparation of materials for SEM, SEM observations, photography, and isolate data for *Lentinus tigrinus* (Bull.:

TABLE 1. *Panus* isolates used in this study.

Species	Isolate number ^a	Country of origin
<i>P. conchatus</i>	VT-1502	USA
	TMI-1293	Japan
	TMI-1294	Japan
	TMI-1295	Japan
<i>P. rudis</i>	VT-915	USA
	VT-340	USA
	D-743	USA
	TMI-183	Japan
<i>P. fulvus</i>	FPL-4147	USA

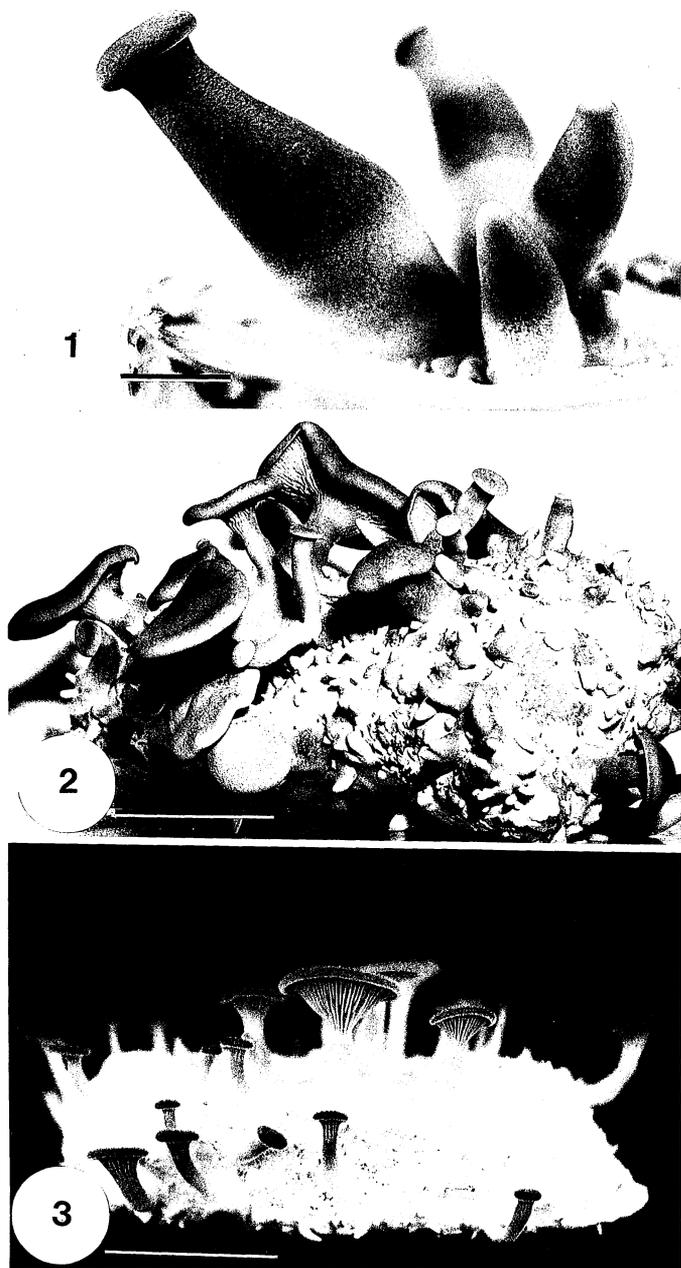
^a VT isolates were provided by Dr. Orson K. Miller, Jr., Virginia Polytechnic Institute, Blacksburg, Virginia. FPL isolate was provided by Dr. Harold H. Burdsall, Jr., USDA Forest Products Laboratory, Madison, Wisconsin. D isolate was provided by Dr. Rytas Vilgalys, Department of Botany, Duke University, Durham, North Carolina. TMI isolates are from culture collection of Tottori Mycological Institute. All isolates are deposited at TMI.

Fr.) Fr. and *L. crinitus* (Linn.: Fr.) Fr. were the same as those that we reported previously (Hibbett, Murakami, and Tsuneda, 1993).

RESULTS

***Panus conchatus*—Macroscopic observations**—Mycelium grew evenly through sawdust medium and fully colonized substrate in about 3 wk. Initially, the colonized spawn was white, with a firm consistency. After about 1 mo spawn blocks became soft and began to pull away slightly from the walls of their containers, possibly due to moisture loss. After 6–8 wk, numerous spherical, cespitose primordia were formed on the surface of the spawn blocks. After initiation, primordia elongated and became cylindrical (Fig. 1). From the outset, primordia had a purple-lavender color. Pileus initiation began 3–5 d after primordium initiation (Fig. 4), when the primordia were approximately 2–4 cm long and up to 1 cm wide near the base. Pilei were initially visible as mamillate protuberances at the apex of the primordium that broadened and flattened out as the young hymenophore became macroscopically visible under the incurved margin (Fig. 1). Pilei usually expanded asymmetrically so that mature sporocarps had excentric to lateral stipes, but some sporocarps had central stipes. Pilei were initially convex, then became depressed in the center, and finally became shallowly infundibuliform, with an incurved margin and deeply decurrent lamellae (Fig. 2). The mature hymenophore was composed of more or less parallel lamellae with scattered anastomoses, especially over the stipe apex, and numerous lamellulae. Pilei were glabrescent to finely velutinate. Stipe surfaces occasionally developed a loose tomentum near the base, but otherwise had a similar texture to that of the pilei. The largest sporocarps produced had pilei 9 cm broad, with stipes 7 cm long and up to 2 cm wide at the base. From primordium initiation to maturity took 5–9 d.

Morphology of cultured sporocarps agreed well with descriptions of field-collected material (e.g., Pegler, 1983). The primary difference between the cultured and natural sporocarps was that the cultured sporocarps tended to have slightly more elongate stipes, with a more central position than the natural materials. Mature cultured spo-



Figs. 1–3. Sporocarp ontogeny in *P. conchatus* and *P. rudis* (macroscopic aspects). 1. *Panus conchatus* D-613 primordia and sporocarps with young pilei. Bar = 1 cm. 2. *Panus conchatus* D-613 sporocarps approximately 7 d after primordium initiation. Bar = 5 cm. 3. *Panus rudis* D-743 sporocarps approximately 5 d after primordium initiation. Bar = 5 cm.

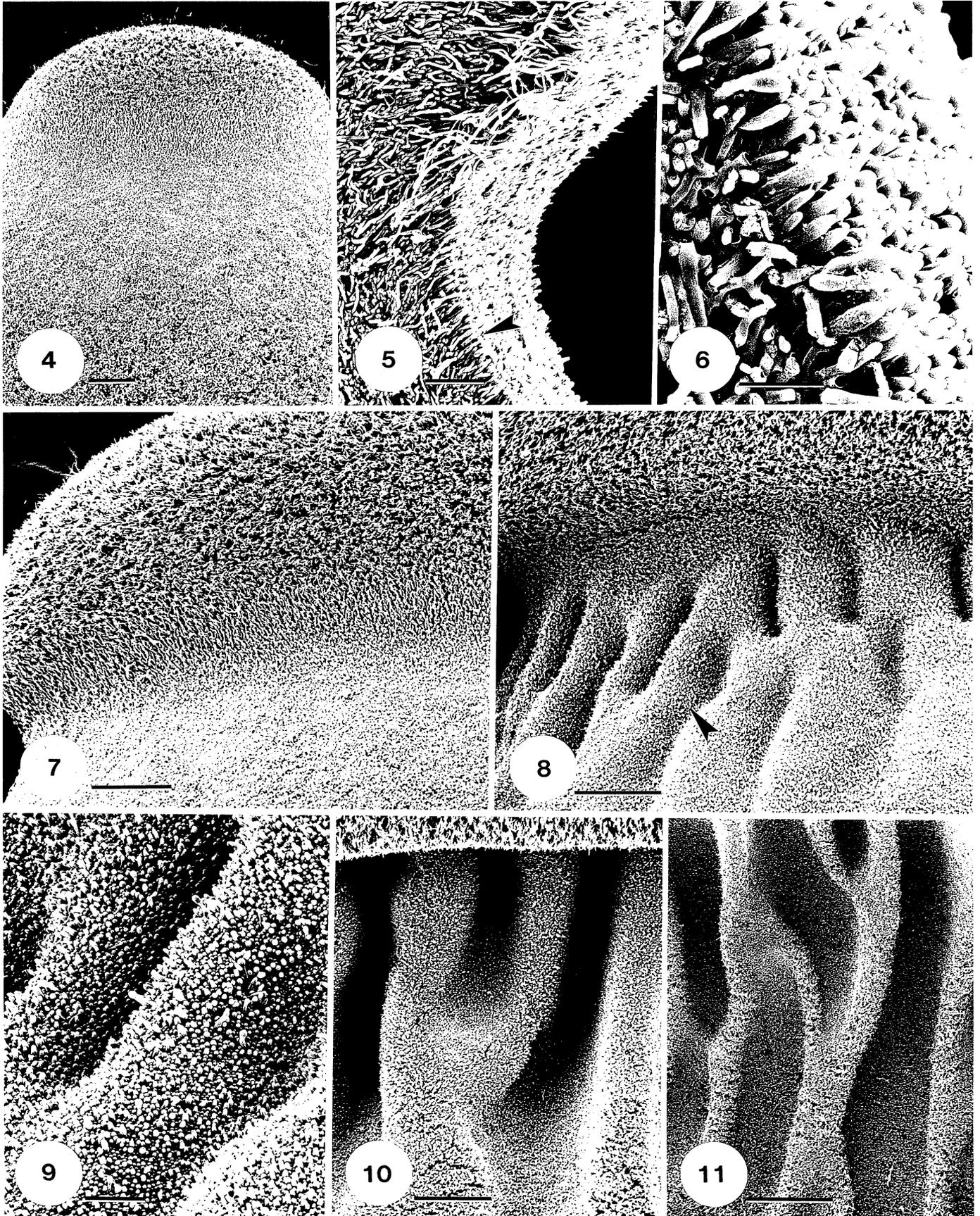
rocarps also retained the lilac tints, which are lost in mature natural sporocarps, possibly due to weathering.

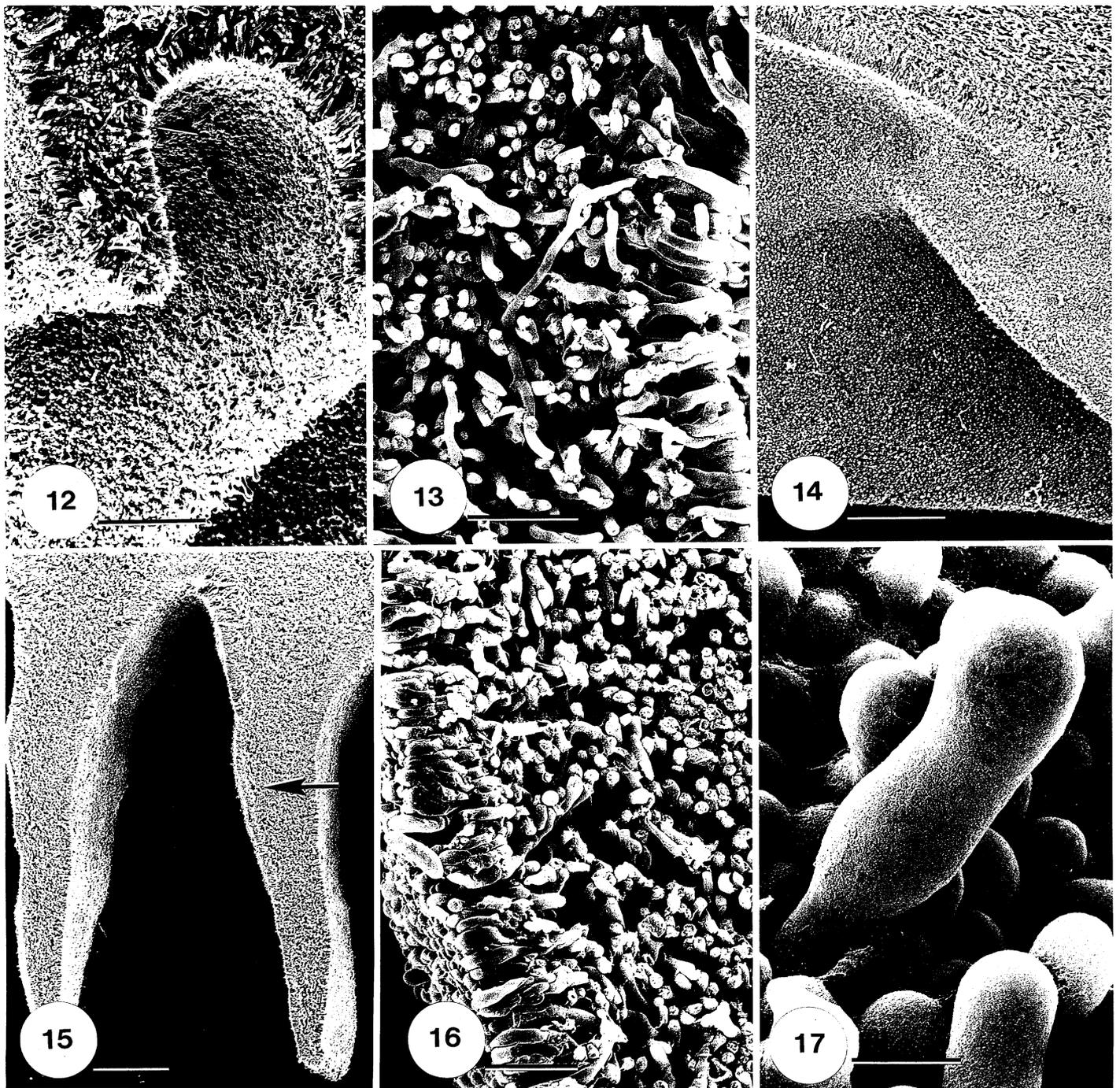
SEM observations—The surface of the primordium in the zone of hymenophore differentiation was composed of an erect palisade of basidioles and immature cystidia (Figs. 5, 6). Hymenophore differentiation began soon after pileus differentiation (Fig. 7). At the earliest point at which it was visible, the hymenophore surface was composed of broad, undulating ridges, covered by the palisade of basidioles and young cystidia (Figs. 8–10). The undulations in the hymenophore surface were caused by localized periclinal growth of hyphae in the primordium context directly below the structures of the hymenophore (Figs. 12, 13). This kind of early hymenophore morphology has been termed the cantharelloid hymenophoral type (Reijnders and Stalpers, 1992). With continued growth, the ridges of the hymenophore became taller and more numerous, with occasional anastomoses, leading to a venose or cantharelloid appearance (Figs. 10, 11).

Hymenophore development was accomplished by periclinal growth of the hymenophoral trama (Figs. 12, 13). At maturity, the hyphae of the hymenophoral trama were radially aligned, more or less parallel to the undersurface of the pileus (Figs. 14–16). The radial alignment was most clearly seen in tangential section, where numerous transversely cut ends of the tramal hyphae were visible (Fig. 16). This type of hymenophoral tramal anatomy has been termed radiate construction (Corner, 1981; Pegler, 1983; Pegler and Young, 1983). With light microscopy of hand-sectioned material it is usually described as producing an irregular trama (e.g., Largent, Johnson, and Watling, 1977). The mature hymenium was composed of basidia, basidioles, and thick-walled, clavate metuloidal cystidia projecting above the hymenial surface (Fig. 17).

***Panus rudis*—Macroscopic observations**—Mycelium fully colonized spawn in 2–3 wk. Fully colonized spawn blocks were white and had a firm texture. In contrast to *P. conchatus*, *P. rudis* spawn blocks did not soften appreciably prior to fruiting. Primordia were formed in clusters on the sides as well as the top of the spawn blocks. Sporocarp growth was macroscopically similar to that of *P. conchatus*, except that the stipe and pileus surfaces became strigose as the sporocarp enlarged. Pileus differentiation began 1–2 d after primordium initiation, when the primordia were 1–3 cm long. Mature sporocarps had depressed to infundibuliform pilei up to 4 cm wide, with central to lateral stipes up to 4 cm long by 1 cm broad at the base (Fig. 3). Again, there was a slight elongation of stipes and a greater tendency toward central attachment relative to natural material. Purple-lavender colors in *P.*

Figs. 4–11. *Panus conchatus* D-613 early hymenophore ontogeny (SEM). 4. Primordium prior to pileus differentiation. Bar = 200 μ m. 5. Early pileus differentiation, longitudinal section. Arrowhead indicates enlargement in Fig. 6. Bar = 50 μ m. 6. Detail from Fig. 5 showing closed palisade of immature hymenial elements in zone of hymenophore differentiation. Bar = 20 μ m. 7. Surface view of differentiating pileus, approximately equivalent stage to Figs. 5, 6. Bar = 200 μ m. 8. Early hymenophore differentiation at stipe apex below expanding pileal margin. Note broad undulations in zone of hymenophore differentiation. Arrowhead indicates location of enlargement in Fig. 9. Bar = 200 μ m. 9. Detail from Fig. 8. Bar = 50 μ m. 10. Later hymenophore differentiation. Undulations have become taller and more distinctly lamellate, with some anastomoses at the stipe apex. At this stage, the hymenophore is macroscopically visible. Bar = 200 μ m. 11. Later hymenophore differentiation. Anastomoses of lamellae are pronounced and give hymenophore a cantharelloid aspect. Bar = 400 μ m.





Figs. 12–17. *Panus conchatus* D-613 later stages of hymenophore differentiation (SEM). 12. Tangential section through immature hymenophore. Arrowhead shows location of enlargement in Fig. 13. Bar = 100 μm . 13. Detail from Fig. 12 showing predominance of transversely cut hyphal ends. This illustrates periclinal growth of tramal hyphae, leading to a radiate type of tramal anatomy. Bar = 25 μm . 14. Radial section through mature hymenophore showing a lamellar anastomosis. Pileus margin is to the left of the frame. Bar = 200 μm . 15. Tangential section through mature hymenophore. Arrow shows comparable location of enlargement in Fig. 16. Bar = 200 μm . 16. Tangential section through mature hymenophore showing transversely cut hyphal ends, which indicate radiate type of tramal anatomy. Bar = 20 μm . 17. Metuloidal cystidium in immature hymenium. Bar = 5 μm .

rudis were also retained longer in the cultured sporocarps than in field-collected materials, which are often tan to brown at maturity. Development from primordia to mature sporocarps took 4–6 d.

SEM observations—Development of the hymenophore was similar to that of *P. conchatus* (Figs. 20–22). However, the primordium (Fig. 18) was covered by a loose weft of hyphae that formed an evanescent veil that was not ob-

served in *P. conchatus* (Figs. 19–22). The veil was stretched and ruptured as the pileus expanded and remained as scattered remnants on the shoulder of the stipe apex (Figs. 19–22). The veil was completely disrupted by the time the hymenophore had developed distinct cantharelloid undulations, and by maturity no remnants were visible. Anatomy of the hymenophoral trama was similar to that of *P. conchatus* (Figs. 24–26). The mature hymenium contained basidia, basidioles, and metuloidal cystidia which were more numerous and prominent than those of *P. conchatus*, especially at the margins of the lamellae (Figs. 23, 24).

***Panus fulvus*—Macroscopic observations**—Mycelium colonized sawdust medium completely in 3–4 wk. After about 1 mo, the spawn block had a firm consistency and an even white color. Later, the blocks became softer and began to pull away from the walls of the container slightly. Occasionally a dark wine-purple tomentum developed on the tops of the spawn blocks and on the sides where the spawn block had pulled away from the container. At this stage the spawn blocks developed a distinctive aroma that was reminiscent of wet rotting leaves.

After 3–4 mo, pseudosclerotia were dug out of the surrounding spawn. One or (rarely) two irregularly shaped pseudosclerotia were formed inside of each culture container (Fig. 27). The pseudosclerotia formed inside 300-ml bottles were 2–4 cm wide × 2–6 cm long, and weighed 20–35 g fresh. Pseudosclerotia were composed of a matrix of sawdust medium bound together and impregnated by white mycelium. Pseudosclerotia had a hard texture and did not exude any liquid. The medium remaining around the pseudosclerotia had a very soft, pulpy consistency and was completely saturated.

A thin covering of aerial white hyphae began to grow out over the surface of the pseudosclerotia within a day of being placed in the incubator. From 3 to 5 d the hyphae darkened to a purple-lavender color and thickened to become a dense tomentum (Fig. 28). The tomentum was absorbent and became densely matted when the pseudosclerotia were misted. All pseudosclerotia developed a tomentum within several days of being placed in the incubator. One or two primordia appeared on most pseudosclerotia 2–3 wk after they were placed in the incubator (Fig. 28). On one occasion, primordia were produced on the surface of a spawn block inside an unopened culture container. The bases of these primordia were connected to a pseudosclerotium buried inside the spawn.

Initially, primordia were smooth, conical, approximately 75 mm wide, and unpigmented (Fig. 28). Primordia were produced on the sides or near the bottom of the pseudosclerotia and initially grew out at roughly horizontal angles. Within 3 d, the apices of the primordia started to turn upward. As the primordia elongated, they developed a lilac-purple pigment from the base, and later developed a dark, velutinate tomentum, but the primordium apices remained glabrescent and unpigmented (Figs. 28–30). Apical growth of the primordium continued for approximately 2 wk. Pileus initiation began 10–15 d after primordium initiation, when the primordia were 8–12 cm tall. Pileus initiation was macroscopically similar to that in *P. conchatus* and *P. rudis* (Fig. 29). As the pilei expanded, they developed a fine purple-brown tomentum

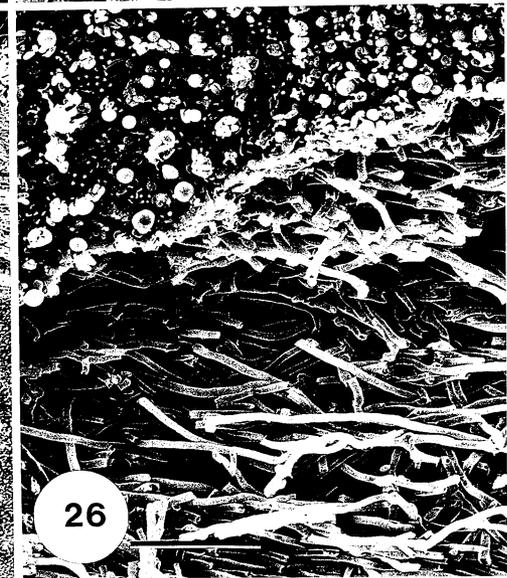
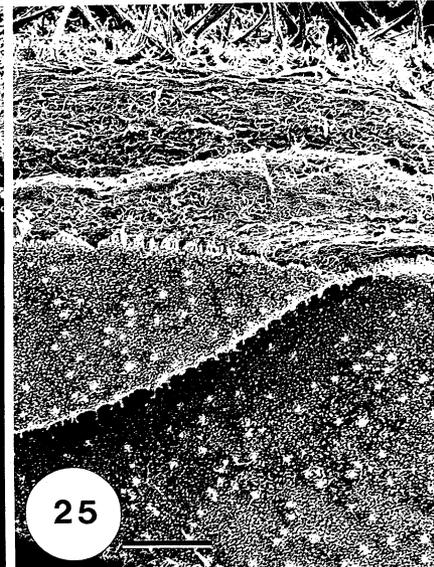
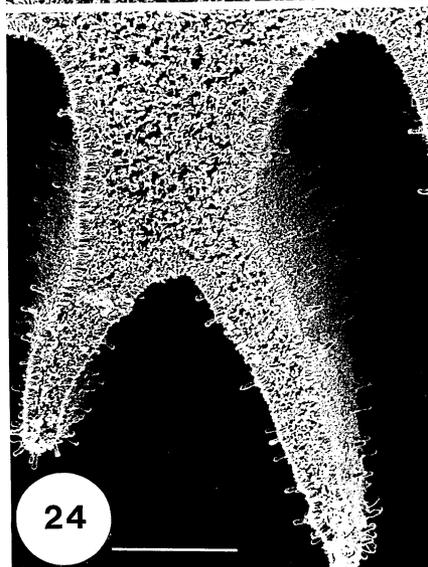
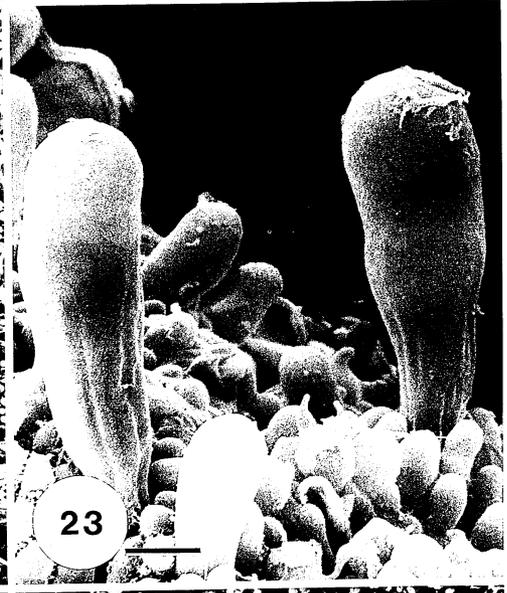
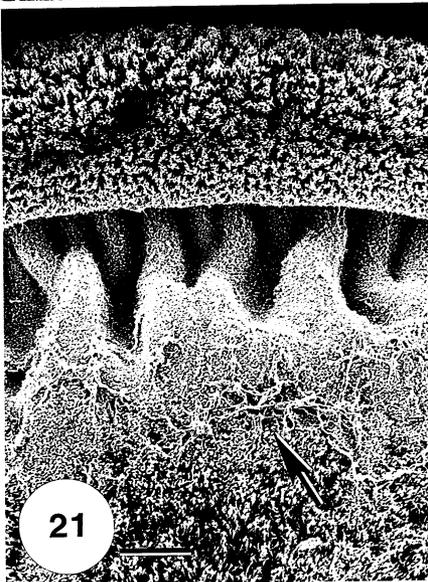
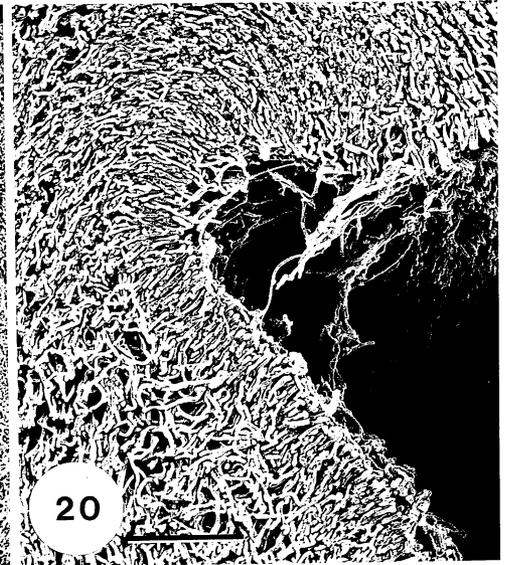
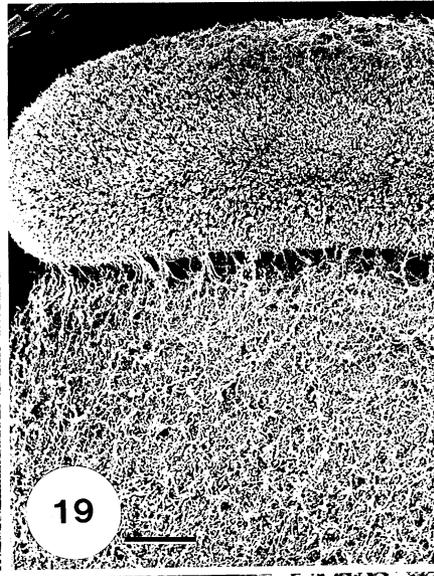
like that covering the stipe. Pilei were convex at first, and later became deeply infundibuliform, with an inrolled to inflexed margin and central stipe attachment (Figs. 29, 31). The mature hymenophore was composed of decurrent, moderately crowded, narrow lamellae. The lamellae were light brown, with few anastomoses. Stipe elongation continued after pileus differentiation. At maturity, sporocarps were up to 24 cm tall, 1 cm broad at the base, with pilei up to 4 cm broad.

Panus fulvus demonstrated a capacity for dedifferentiation and regeneration (Fig. 29). Primordium apices were removed for SEM at various points after pileus differentiation had begun. After sectioning, new primordial apices developed from the cut stipe surfaces. Freshly cut stipes developed a flared swelling at the point of the cut within 1 d. Within 2 d, a mound of white mycelium appeared above the cut end of the stipe. A new primordium apex developed from the mound of mycelium above the cut surface after 2–3 d. The regenerated primordia grew normally, and after approximately 5 d had typical pigmentation and began to differentiate pilei. We never observed regeneration of cut primordium apices in *P. conchatus* or *P. rudis*.

SEM observations—Hymenophore differentiation was similar to that in *P. conchatus* and *P. rudis*, except that the ridges composing the early hymenophore were not as rounded as they were in the other species, and had fewer anastomoses (Figs. 32–35). This resulted in a less venose, more regular appearance in the early hymenophore of *P. fulvus* than in *P. conchatus* and *P. rudis*. Hymenophore differentiation was achieved by cantharelloid growth of the tramal hyphae which led to a strongly radiate tramal anatomy, as in the other *Panus* species (Figs. 36–39). In addition to basidia and basidioles, the mature hymenium contained metuloidal cystidia and scattered emergent skeletal hyphae (Fig. 40). The cystidia in *P. fulvus* did not project as far above the surface of the hymenium as those in *P. conchatus* and *P. rudis*. *Panus fulvus* cystidia had a slight ventricose shape, often with subapical constrictions that were lacking in the clavate cystidia of *P. conchatus* and *P. rudis* (Fig. 40). No veils or other protective structures were observed.

DISCUSSION

Taxonomic significance—The ontogeny and early morphology of the hymenophore in *Panus* is qualitatively different from that of *Lentinus tigrinus* and *L. crinitus* (Figs. 41, 42; Hibbett, Murakami, and Tsuneda, 1993). The initial structures of the *Lentinus* hymenophore are formed by outward, apical growth of hyphae on the primordial surface which gives rise to irregularly crested ridges of hyphae (Figs. 41, 42). Hymenium differentiation involves a developmental switch from indeterminate apical growth of hyphae to determinate growth with specialization of hyphal ends as basidia or sterile hymenial elements. In *L. tigrinus* and *L. crinitus*, hymenium differentiation occurs in a zone behind the margin of the developing lamellae. Hyphae at the margin of the lamellae maintain apical growth, and consequently the margins of the lamellae are often lacerate to denticulate at maturity,



with emergent, undifferentiated tramal hyphae (Corner, 1981; Pegler, 1983; Reijnders and Stalpers, 1992).

In *Panus*, the structures of the hymenophore develop primarily from localized, periclinal multiplication of sub-surface hyphae, resulting in cantharelloid folds and ridges (Figs. 7–10, 21, 22, 33–35). The hyphal ends on the surface of the primordium in the developing hymenophore in *Panus* form a regular, closed palisade of basidioles and cystidia. This palisade covers the primordium from the base up to and including the margins of the lamellae and remains intact at all stages of development. Thus, in the *Panus* hymenophore there is no apparent distinction between zones of hymenium differentiation and hymenophore expansion.

Similarity in ontogeny is a component of the similarity test of taxic homology (Patterson, 1982; but see Roth, 1988). The differences between the early morphologies of the *Panus* and *Lentinus* hymenophores support the view that they are not homologous, which is consistent with the conclusions of our previous molecular systematic studies. This suggests that *Panus* and *Lentinus* should be maintained as separate genera, but their limits are still not clear. Early morphology of the hymenophore might provide useful characters for refining the limits of *Panus* and *Lentinus*. However, we do not propose that the cantharelloid type of hymenophore ontogeny is a synapomorphy for *Panus*. Indeed, Reijnders and Stalpers (1992) have reported that in diverse basidiomycetes the cantharelloid type of early development can give rise to hymenophores that are composed of pores, veins, lamellae, or spines.

Pigments may also provide useful characters for segregating *Panus* and *Lentinus*. Lilac-purple pigments have previously been noted in *Panus*, particularly in young sporocarps (Miller, 1967; Corner, 1981; Pegler, 1983). In *Panus*, purple pigments are brightest in young sporocarps and fade with age, especially in field-collected material. Pigment production is therefore another potentially informative character that is best observed in immature sporocarps.

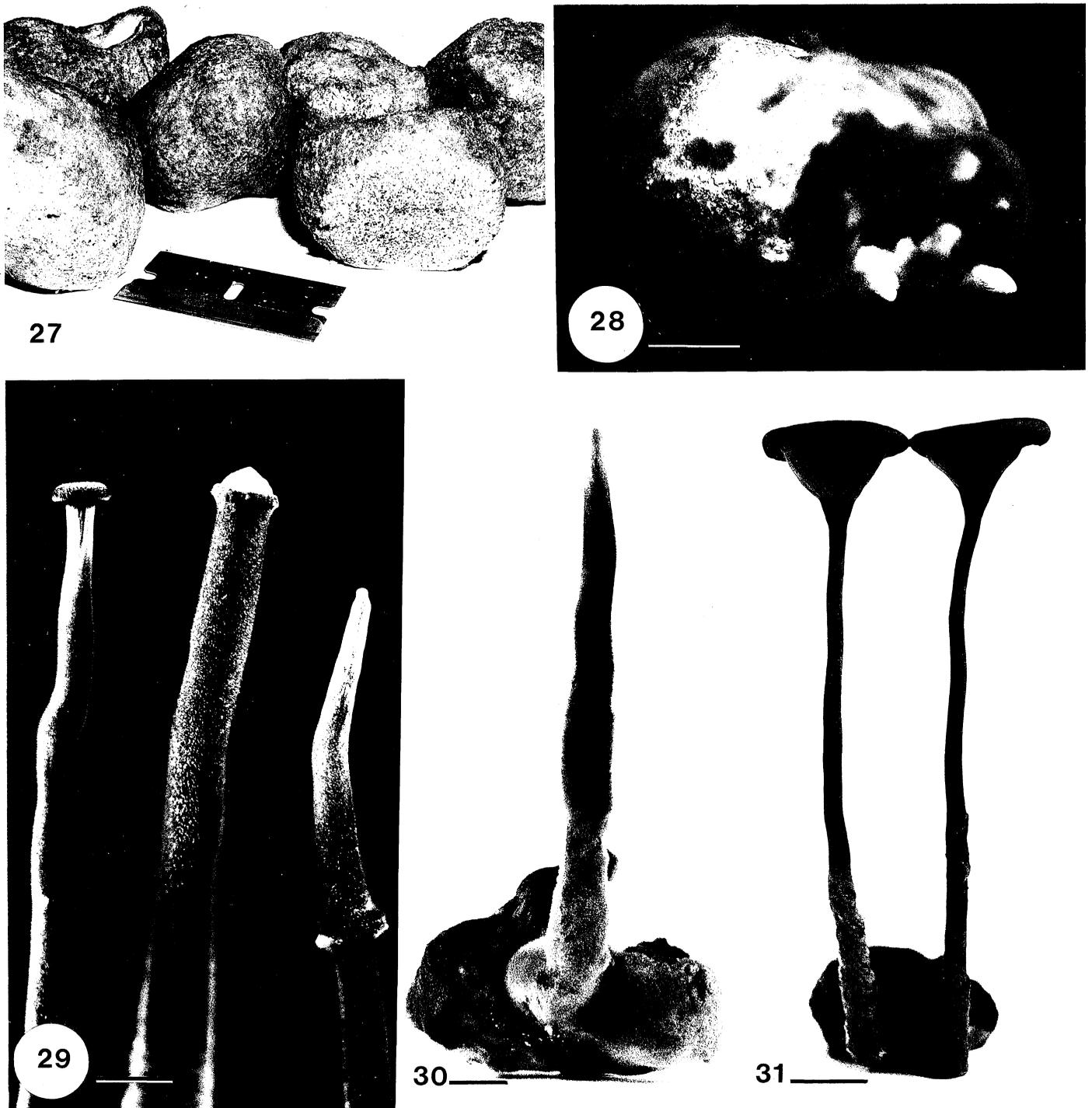
The *Panus* species that we examined have a strongly radiate construction of the hymenophoral trama. *Lentinus* has species with radiate, descending, or intermediate trama types (Corner, 1981; Pegler, 1983; Pegler and Young, 1983). We previously suggested, based on outgroup comparison to *Polyporus* (Hibbett, Murakami, and Tsuneda, 1993), that descending tramal construction is plesiomorphic within *Lentinus*. If this is correct, then the presence of the radiate construction of the trama in *Panus* and certain members of *Lentinus* is the result of convergent evolution.

Panus rudis and *Lentinus squarrosulus* (Hibbett, Murakami, and Tsuneda, 1993) both produce an ephemeral veil, but they are morphologically and developmentally distinct. The veil of *L. squarrosulus* is thicker and persists longer than that of *P. rudis*. In *L. squarrosulus*, hymenophore and hymenium differentiation begins before the veil has been ruptured, and the young lamellae develop inside longitudinal cavities formed by contiguous veil and hymenophore tissues. Fragments of the veil remain scattered on the surface of the lamellae and stipe apex in mature *L. squarrosulus* sporocarps (Hibbett, Murakami, and Tsuneda, 1993). In *P. rudis*, the veil is ephemeral, and there is no hyphal continuity between the veil and the developing hymenophore. No veil remnants could be found in mature *P. rudis* sporocarps. These differences suggest that veils of *P. rudis* and *L. squarrosulus* are not homologous and support the commonly held view (e.g., Reijnders, 1991) that presence or absence of veils has little phylogenetic informativeness at high taxonomic levels. Veil characters might be informative in a phylogenetic study of species within *Panus*. Our observations in *P. rudis* suggest that veils in other *Panus* species might be apparent only in very young sporocarps.

Our results suggest that early developmental observations could provide useful characters for reassessing the limits of *Panus* and *Lentinus*. So far, we have been able to study only a handful of *Panus* and *Lentinus* species, and so we caution against overinterpretation of our results. We would be particularly interested in observing the ontogeny of those species of *Lentinus* that have a radiate anatomy of the hymenophoral trama (e.g., *L. sajor-caju* Fr.). Furthermore, we do not wish to imply that characters arising early in ontogeny should be weighted more heavily than other characters. We therefore recommend that ontogenetic data be obtained from more representatives of *Panus* and *Lentinus*, especially tropical species, and that these data be evaluated in terms of their congruence with other independent phylogenetic characters.

Developmental evolution—Comparison of developmental patterns within *Panus* may provide insights into general modes of developmental and morphological evolution in basidiomycetes. We could discern four variable aspects of ontogeny in *Panus*: 1) duration of the primordial phase (short in *P. conchatus* and *P. rudis*, long in *P. fulvus*); 2) formation of the pseudosclerotium (present in *P. fulvus* only); 3) presence or absence of dedifferentiation and regeneration potential of cut primordium apices (present in *P. fulvus* only); and 4) formation of an ephemeral veil (present in *P. rudis* only). To understand the evolution of developmental programs in *Panus* it is nec-

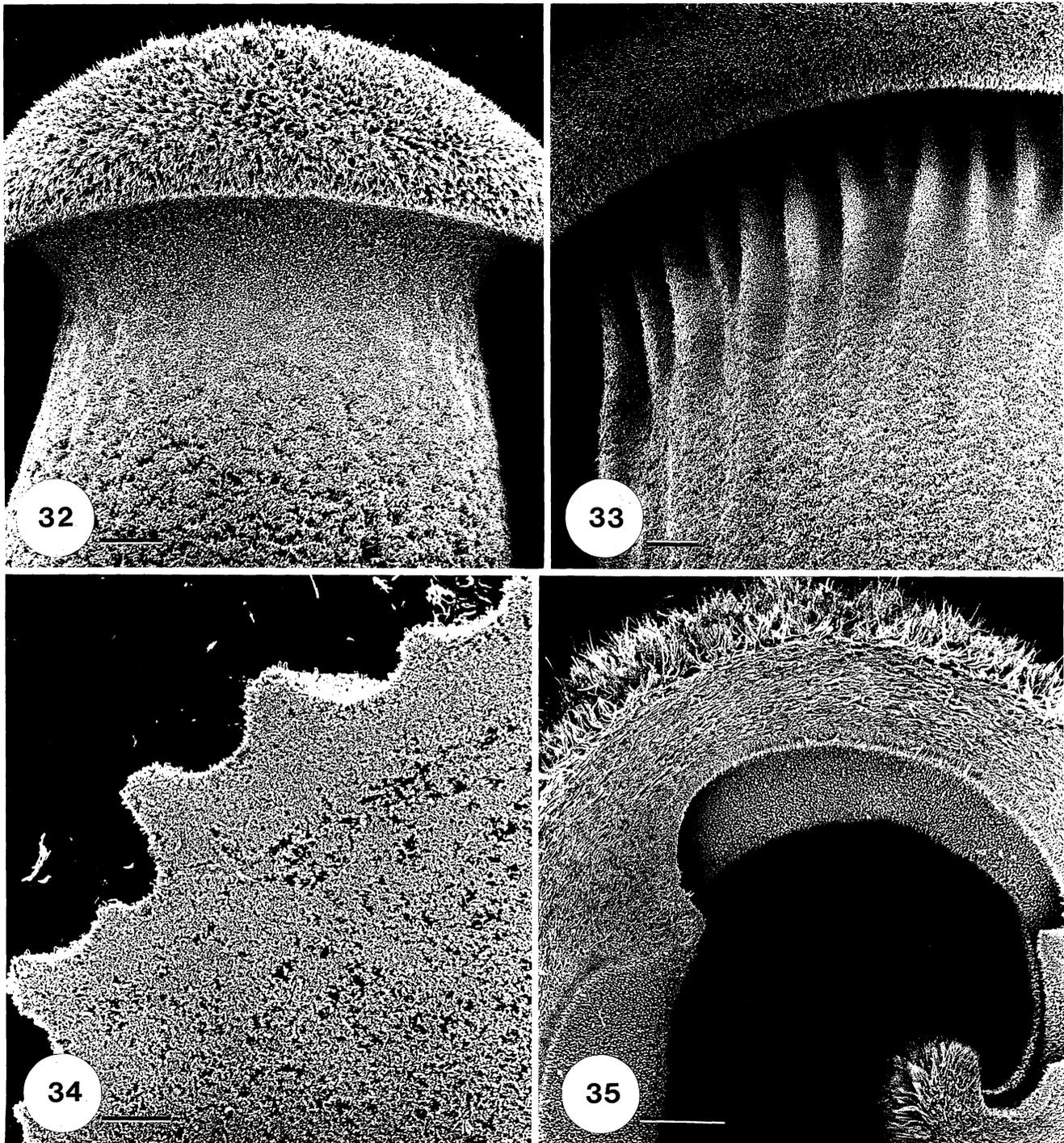
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Figs. 18–26. Sporocarp ontogeny in *P. rudis* TMI-183 (Fig. 18 only) and D-743 (SEM). 18. Primordium. Bar = 200 μ m. 19. Early pileus differentiation. Note strands of hyphae forming ephemeral veil stretched between margin of pileus and shoulder of stipe. Bar = 200 μ m. 20. Longitudinal section through developing pileus at approximately same stage as Fig. 19. Note veil tissue stretched between pileus and stipe, and closed palisade of hymenial elements on stipe shoulder in zone of hymenophore differentiation. Bar = 75 μ m. 21. Early hymenophore differentiation showing cantharelloid aspect. Arrow indicates location of enlargement in Fig. 22. Bar = 200 μ m. 22. Detail of Fig. 21 showing veil remnants on stipe apex. Bar = 50 μ m. 23. Metuloidal cheilocystidia on margin of immature lamella. Bar = 5 μ m. 24. Tangential section through mature hymenophore showing lamellar anastomosis. Note cystidia on faces and margins of lamellae. Bar = 200 μ m. 25. Radial section through mature hymenophore showing primary lamella and lamellula. Margin of pileus is to the left of the frame. Bar = 150 μ m. 26. Radial section through mature hymenophore with part of hymenium chipped away to reveal radiate hymenophoral trama. Orientation is same as in Fig. 25. Bar = 50 μ m.



Figs. 27–31. *Panus fulvus* ontogeny (macroscopic aspects). 27. Fresh pseudosclerotia. Note cut surface of pseudosclerotium showing sawdust medium impregnated with mycelium. Razor blade = 39 mm. 28. Pseudosclerotium with tomentum and two conical primordia. Bar = 1 cm. 29. Regeneration series. Left: unsectioned sporocarp with immature pileus with inrolled margin. Center: sporocarp approximately 1 d after sectioning. A mound of white mycelium has formed above the surface of the cut. Right: Approximately 5 d after sectioning. A new primordial apex has regenerated and elongated. The base of the regenerated apex has become pigmented and velutinate. Pileus initiation is barely visible as a mamillate protuberance at the regenerated apex. Each is from a different pseudosclerotium. Bar = 1 cm. 30. Elongate primordium approximately 10 d after initiation. Bar = 1 cm. 31. Mature sporocarps approximately 3 wk old. Bar = 2 cm.

essary to infer the evolutionary polarity of these developmental characters. The ontogenetic polarity criterion, controversial under any circumstance, is clearly inappropriate here because it would introduce an unacceptable

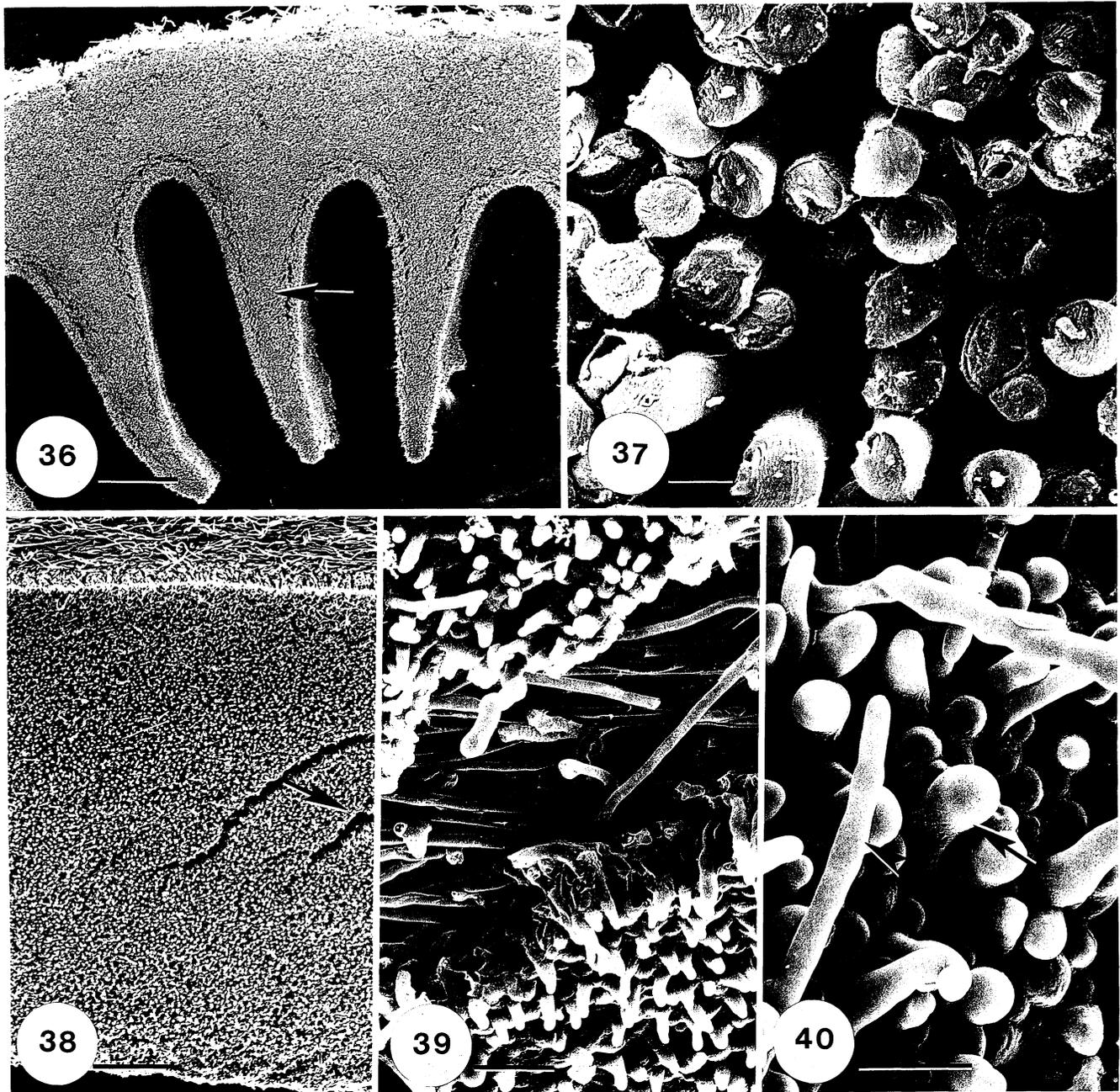
element of circularity. Outgroup comparison is a preferable method for inferring evolutionary polarity of character states (Stevens, 1980; Maddison, Donoghue, and Maddison, 1984; Brooks and Wiley, 1985). Our previous



Figs. 32–35. *Panus fulvus* hymenophore ontogeny (SEM). 32. Early pileus differentiation. Structures of hymenophore are not yet visible. Bar = 200 μm . 33. Early hymenophore differentiation showing deep decurrent cantharelloid ridges. Bar = 200 μm . 34. Transverse section through developing hymenophore. Approximately same stage as Fig. 33. Bar = 100 μm . 35. Later hymenophore differentiation. Radial section. Bar = 200 μm .

cladistic analysis of rDNA sequence data and morphological characters (Hibbett and Vilgalys, 1993, fig. 4) suggested that *Panus* is derived from within a clade that contains *Lentinellus* Karst., *Pleurotus* (Fr.) Quél., *Collybia* Kummer, *Mycena* (Pers.: Fr.) S. F. Gray, and other agaricoid genera. However, the position of the *Panus* clade was not strongly supported by bootstrapping or decay

indices. Our outgroup hypothesis, and consequently our assessment of polarity, must therefore be regarded as provisional. The potential outgroup genera all grow by rapid, “mushroom” growth, lack pseudosclerotia of the *P. fulvus* type, and lack the ability to regenerate cut primordium apices. Therefore, we propose that the prolonged primordial phase, presence of a pseudosclerotium, and abil-

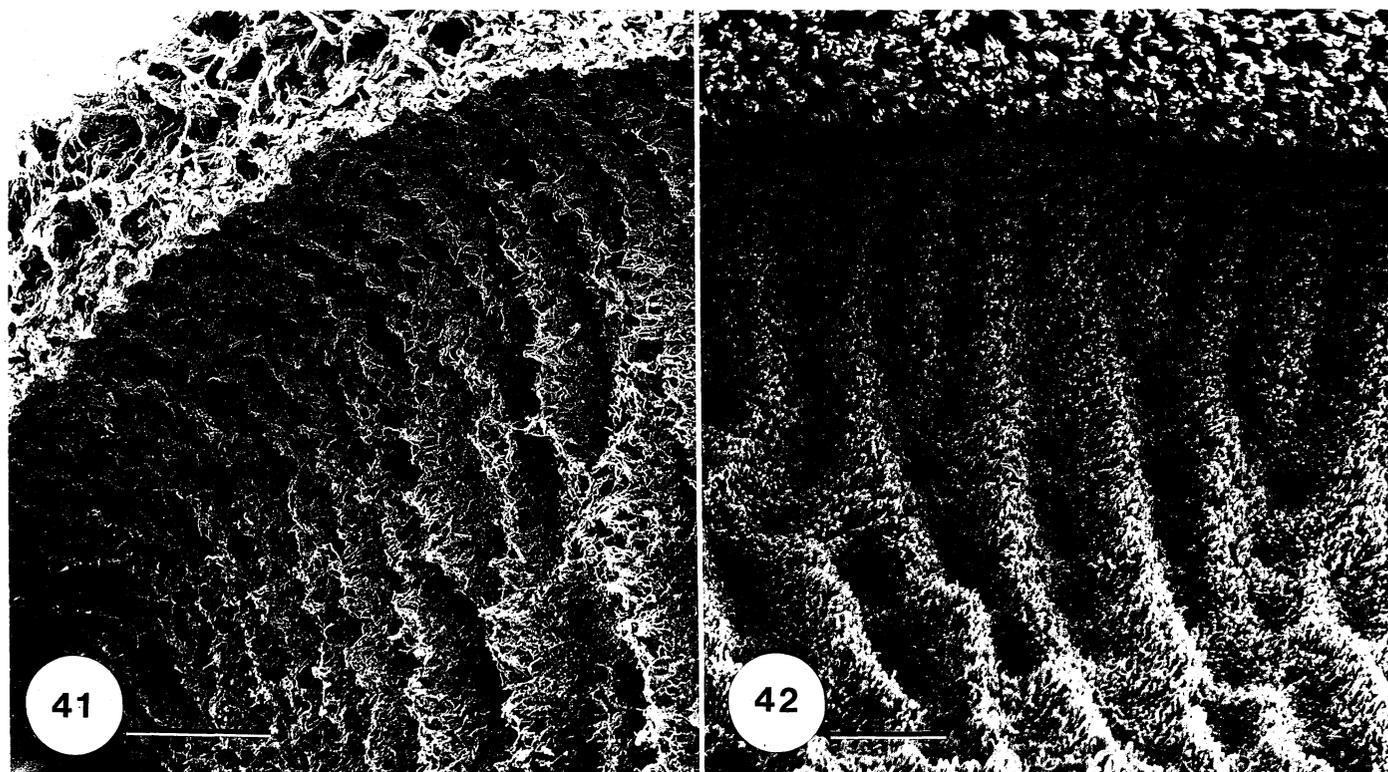


Figs. 36–40. *Panus fulvus* hymenophoral anatomy (SEM). 36. Tangential section through mature hymenophore. Arrow shows location of enlargement in Fig. 37. Bar = 200 μm . 37. Detail from Fig. 36 showing transversely cut thick-walled skeletal hyphae in radiate hymenophoral trama. Bar = 2 μm . 38. Radial section through mature hymenophore. Arrow shows location of enlargement in Fig. 39. Bar = 100 μm . 39. Detail from Fig. 38. Hymenium has cracked slightly, revealing strongly radiate trama. Bar = 15 μm . 40. Hymenium in immature sporocarp showing cystidium (arrow) and emergent skeletal hyphae (arrowhead). Bar = 10 μm .

ity for dedifferentiation and regeneration are all derived within *Panus*. Presence or absence of veils is highly variable among the potential outgroups to *Panus* and so we cannot infer the polarity of this character. Presence of veils could be either plesiomorphic, which would imply a loss within *Panus*, or apomorphic, which would imply a gain.

Ontogenetic order of appearance of characters has been advocated as a criterion for inferring evolutionary polarity of character states (Nelson, 1973, 1978; Kraus, 1988). In

general, our results do not support the use of ontogeny as a criterion for assessing evolutionary polarity of sporocarp morphological characters. Two of the inferred modifications to development—evolution of the pseudosclerotium in *P. fulvus* and evolution of the veil in *P. rudis*—involve nonterminal additions or deletions to developmental programs. Neither is compatible with ontogenetic polarity assessment, which assumes that evolution proceeds by terminal addition to development, resulting in recapitulation. Nonterminal additions and deletions to



Figs. 41, 42. Early hymenophore morphology in *Lentinus* (SEM). Note narrow, crested ridges of hyphae forming hymenophore. Compare to Figs. 8–10, 21, 33, and 34. **41.** *Lentinus crinitus*. Bar = 200 μm . **42.** *Lentinus tigrinus*. Bar = 100 μm .

developmental programs disrupt parallels between ontogeny and phylogeny and invalidate the ontogenetic polarity criterion (Gould, 1977; Brooks and Wiley, 1985; Kluge, 1985; Mabee, 1989).

Dedifferentiation and regeneration, which we observed in *P. fulvus*, are also incompatible with the ontogenetic polarity criterion. The ontogenetic criterion is based on the biogenetic law sensu Nelson (1978, p. 327), which states that "given an ontogenetic character transformation, from a character observed to be more general to a character observed to be less general, the more general character is primitive and the less general advanced." As noted by Nelson (1978) and others (e.g., Kluge, 1985; Mishler, 1988), dedifferentiation clearly violates the biogenetic law.

Prolongation of the primordial phase involves changes in the relative timing of developmental events and is therefore an example of heterochrony (Gould, 1977). Specifically, this is an example of hypermorphosis (Gould, 1977); there is a delay in the offset of primordial growth in the *P. fulvus* type ontogeny relative to that of the *P. conchatus*-*P. rudis* type ontogeny (we cannot rule out that changes in rate of growth have also occurred). Hypermorphosis results in recapitulation because derived ontogenies proceed past their ancestral points of termination (Gould, 1977). Therefore, we feel that this developmental modification might be correctly polarized by the ontogenetic criterion. It should be noted that this is a local hypermorphosis (Raff and Wray, 1989; McKinney and McNamara, 1991) that affects only growth of the primordium and stipe. *Panus fulvus* sporocarps do not re-

capitulate the mature morphology of *P. conchatus* or *P. rudis* sporocarps, but *P. fulvus* primordia do pass through a stage that resembles the terminal developmental stage of *P. conchatus* and *P. rudis* primordia.

Ontogeny and phylogeny have been jointly studied in too few groups of fungi to permit broad generalizations about the frequency of various modes of fungal developmental evolution. Our observations in *Panus* suggest that basidiomycete sporocarp ontogeny is evolutionarily flexible, and that any stage of development can be subject to modification. This suggests that direct observation of ontogeny should not be relied upon as a method for inferring evolutionary polarity of fungal morphological character states. Mycology has a rich tradition of developmental studies (cf. Reijnders, 1991), but rigorous, detailed phylogenetic hypotheses for fungi have only recently become routinely accessible, via molecular techniques (Bruns, White, and Taylor, 1991; Hibbett, 1992; Kohn, 1992). Because they can be fruited in culture, saprophytic fungi like *Panus* offer exciting possibilities for empirical studies of the relationship between development and evolution in fungi.

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