BASIDIOME DEVELOPMENT OF XEROMPHALINA CAMPANELLA (TRICHOLOMATALES, BASIDIOMYCETES)

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The agaricoid Hymenomycete *Xeromphalina campanella* is exocarpic, apertopileate and amphiblemate. Metablemas develop separately on the pileus and on the stipe, but they do not form any kind of veil. The pileoblema becomes a gelatinous pileipellis, and the cauloblema forms a hairy coating on the lower part of the stipe of the mature basidiomes. The hymenophoral trama is bidirectional in the gill rudiments, but becomes more physalo-irregular at maturity and contains many narrow hyphae with smooth or incrusted walls. The context of the stipe resembles a sarcodimitic structure, but the thin-walled inflated cells are rarely fusiform, although they are frequently gradually narrowed at one end. Between the physalohyphae, narrow, incrusted hyphae and ramified connective hyphae occur in the stipe and in the pileus context. The hyphae of the pileus of a young basidiome contain granular deposits of glycogen.

The only note on the basidiome development of *Xeromphalina campanella* published so far consists of a few lines and a single photograph at the end of a taxonomic paper by Hintikka (1957). Since no trace of any kind of veil is visible in the photograph, Hintikka cautiously concluded that the development is probably gymnocarpic. Singer (1965) was more confident and stated that his *X. austroandina* is gymnocarpic, based on the "same observations as indicated by Hintikka (1957) for *X. campanella*", without giving any further details. Later, in his fundamental work on the genera of gill fungi, Singer (1986: 425) only mentioned the gymnocarpic development of *X. campanella*, giving credit to Hintikka, but he did not mention his own observations on *X. austroandina* any more. Since the reported observations are rather scanty and no other species of this genus was studied, Watling & Turnbull (1998: 147) cautiously wrote that the development is "not known".

In March 2002 a large population of X. campanella basidiomes with primordia in all developmental stages was found near Lausanne, Switzerland, which enabled a study of the carpogenesis of this species.

MATERIAL AND METHODS

Xeromphalina campanella (Batsch) Maire, 'Les Liaises', north of Lausanne, Switzerland, 800 m above sea level; basidiomes densely gregarious on the black remains of a stereoid fungus still attached to the fallen conifer trunk it was growing on. Leg. H. Clémençon, 18.III.2002, coll. *HC 02/009* (LAU).

Patches of the black, fibrous substrate were inspected with a dissecting microscope. Excised substrate fragments bearing primordia were fixed for 25 h at $20-22^{\circ}$ C in a solution of 0.9% formaldehyde and 1.25% glutaraldehyde in tap water (63 mg/L Ca++).

No vacuum was applied. The fixed material was transferred to cold methyl cellosolve and carried over ethanol and propanol into butanol (Feder & O'Brien, 1968). It was embedded in methacrylate, and the microtome sections were stained with aluminiumzirconium-haematoxylin (Clémençon, 2000), basic fuchsine or with the tannin-iron reaction for polysaccharides followed by haematoxylin as follows (all manipulations done at room temperature of 20-22°C): a) Immerse the sections in a 3% tannin (tannic acid) solution in distilled water for 30-40 minutes. Do not use gallic acid; it does not work. b) Rinse in 3 or 4 charges of distilled water, about 5 minutes each. c) Immerse in 5% iron(III)chloride in distilled water for 20-40 minutes. The sections stain dark gray to black, methacrylates remain unstained. d) Rinse in 2 or 3 charges of distilled water, about 5 minutes each. e) Stain in 0.1% ripened hematoxylin in distilled water for 15-20 minutes. f) Rinse in 2 or 3 charges of distilled water, about 5 minutes each. g) Stabilise the stain in calcium-rich tap water. If no hard tap water is available, use a 0.2% sodium bicarbonate solution. h) Briefly rinse in distilled water, dry the sections on a hot bench or in an oven, apply a synthetic resin (Entellan or a similar product) and a cover glass. As a result the cell walls and gelatinous substances colour dark gray to black; some cell contents, mainly protein crystals, are sometimes stained. Selected sections were photographed with an Olympus DP11 digital camera mounted on a Leitz Orthoplan microscope. The photographs were prepared for printing using Adobe Photoshop with a Macintosh G4 computer.

RESULTS

A summary of the early development of X. campanella is shown in Figs. 1 and 2. Already the smallest nodulus is hairy (Figs. 1a, 2a). The Figs. 1c-e and 2c-e show the first developmental stages of the pileus, the growth of the metablema, and the beginning of hyphal inflation. The smallest nodulus studied was 160 μ m wide and 150 μ m high and had a stalk 65 μ m long and 35 μ m thick, sunken in the substrate (Fig. 3). The parallel hyphae of the stalk deviate and ramify at the base of the nodulus and become subregularly arranged, still growing upward. The context in the centre of the nodulus is slightly more irregular than in the peripheral parts. The hyphae of the surface of the nodulus have a slightly thickened, light brown wall, staining deeper in the permanent



Fig. 1. Selected stages of early primordium development. Living specimens photographed on the substrate, illustrating the hirsute metablemas and the initiation of the pileus.

mounts. The nodulus is moderately hairy from free hyphal ends. The small groove on the top has no relation with the funnel-shape of the mature basidiome, as in other noduli and young primordia studied it is lacking (e.g. Fig. 2a).

In the next stage (Figs. 1b, 2b) a primordial shaft has begun its upward growth from the top of the nodulus, changing the nodulus into a cone-shaped body with a rounded base and a narrow, flat top (Fig. 4). The hyphae in the rudiment of the primordial shaft are subregularly arranged in a general vertical orientation. The bulbous nodulus bears many slightly thick-walled peripheral hyphae in an obliquely upward and outward orientation, giving this part a hirsute appearance. These hairs constitute a noduloblema. The cauloblema on the young primordial shaft is less developed, so this part is less hirsute than the nodulus. The cauloblema and the noduloblema intergrade smoothly, forming a continuous hairy coating. Most hyphal cells are binucleate (Figs. 5a, b).

The pileus initial becomes visible as a short horizontal rim on top of the primordial shaft (Figs. 1c, 2c, 6-8). It consists of more or less horizontal or obliquely downward oriented hyphae. No prehymenial palisade is present at this stage. The pileoblema is strongly developed and consists of greatly inflated hyphae. The top of the primordial shaft is usually slightly convex because of the form of the pileoblema, but in many primordia the future funnel-shaped pileus is already perceptible by the general orientation of the thin generative hyphae of the context beneath the pileoblema (Fig. 8a). Under the pileus initial the primordial shaft is composed of subregular generative hyphae with a general vertical orientation, growing upward. This part is the stipe initial. In most primordia it is covered with a cauloblema consisting of hyphal ends growing in an oblique outward-upward direction. At the base, the hyphae of the nodulus started to inflate (Figs. 6, 8b).

The pileus margin grows outward and curves downward, and the underside of the pileus initial becomes lined with a prehymenial palisade (Fig. 9). The pileoblema and the cauloblema grow considerably and become a loose layer of erect hyphae, but the prehymenial palisade remains naked. The stipe base consists of the nodulus with a characteristic secondary structure composed of irregularly arranged and irregularly inflated hyphae. Hyphal inflation is minimal in the stipe just below the pileus, and strong again in the top part of the pileus, contributing here to the expansion of the cap.

During further development the pileus margin grows outward and curves downward, but it never reaches the stipe surface (Figs. 2e, 10, 14). The pileoblema is most voluminous in the centre of the cap, where it forms a flat, inverted cone filling the funnelshaped pileus rudiment. Laterally the pileoblema forms a thin periclinal layer on the pileus context. Although the transition from pileus context to pileoblema is gradual, the funnel-shaped, denser pileus context is perceptible in Fig. 10. The prehymenial palisade begins to produce meiotic basidia protruding considerably beyond the level of the palisade cells (Figs. 10, 11). In the stipe context many hyphae swell and elongate enormously and become multinucleate (Figs. 12, 13). Some hyphae end bluntly within the stipe context (Fig. 13), but the end cells do not become acrophysalides.

With the growth of the pileus, the prehymenial palisade becomes arched, and individual hyphae of the cauloblema may grow into the space between the pileus margin and the stipe, but no 'partial veil' is formed. The pileoblema grows down laterally on the pileus margin, its hyphae taking a periclinal orientation, but it does not join the cauloblema (Fig. 14). It is not yet gelatinous. In this developmental stage the gill initials are still lacking (Fig. 15).

When the pileus reaches a diameter of about 1 mm, gills begin to grow down from its lower surface. The first gill trama is bidirectional with the vertical hyphae slightly diverging (Fig. 16). Maturing basidia protrude considerably beyond the general level of the hymenium. When the pileus reaches a diameter of about 3 mm, more pronounced plectological differentiations take place. In the gill trama the hyphae of the mediostratum begin to inflate (Figs. 17a, b), and the pileoblema changes its architecture. Its hyphae become radially adherent to the pileus surface, and a gelatinous substance is formed, so that the result is a radial ixocutis. Some hyphae of the pileoblema remain more or less erect, and their often inflated end cells are sometimes called pileocystidia (Miller, 1968; Klán, 1984; Redhead, 1988). In the pileus context many cells now contain granular glycogen staining brown in iodine solutions and black with the tannin-iron reaction (Fig. 18). In the hymenium well-differentiated pleurocystidia and cheilocystidia are present, but the latter are by far the more numerous. The surface of the pleurocystidia seems to be sticky enough to capture spores liberated by the basidia (Fig. 19). The time of the first appearance of the hymenial cystidia is difficult to establish, since big, prominent cells protruding beyond the level of the prehymenial palisade may develop either into basidia or cystidia.

Basidiomes with a pileus 4-5 mm wide are fully differentiated. The mature gill trama consists of irregular to bidirectional physalohyphae, and thin, brown-walled, irregularly arranged hyphae with smooth or incrusted walls and almost no stainable cell content (Figs. 20 a–d). A few very thin and extremely thin-walled hyphae rich in cytoplasm occur in the gill trama and in the pileus context (Figs. 21a, b). The pileus is covered by an ixocutis of radial hyphae bearing some erect hyphae and isolated inflated cells called pileocystidia (Fig. 22). The ixocutis and the pileocystidia are the product of the pileoblema. The stipe of the mature basidiome is composed of three hyphal types (Figs. 23a–c). Most prominent are the greatly inflated, multinucleate and highly vacuolated physalohyphae. Their cells are sometimes cylindrical, sometimes elongate-conical at one septum and cylindrical-blunt at the other (Figs. 23a, b), only rarely the cells are fusiform. Because of the conical structures Redhead (1987) called this context sarcodimitic. Besides the physalohyphae many thin, approximately cylindrical hyphae with brown incrusted walls, and thin, highly ramified, colourless connecting hyphae are present in the stipe context (Fig. 23c). The context of the pileus has the same structure.

DISCUSSION

Using the traditional terminology, the development of *X. campanella* should be called gymnocarpic, as did Hintikka (1957) and Singer (1986). The primordia lack any kind of veil, universal or partial, the pileipellis does not extend over the pileus margin toward the stipe, and the pileus margin itself never touches the stipe surface. Thus no closed gill cavity ever exists. But this simple term does not take into account the massive development of metablemas on the stipe and on the pileus.



Fig. 2. Selected stages of early primordium development. Longitudinal sections showing the development of the metablemas, the stipe rudiment and the pileus initial.



Fig. 3. A nodulus rooting in the substrate, the dead remains of a stereoid fungus. The hyphae in the centre are slightly more irregularly arranged than in the peripheral parts. The erect hyphae on the surface can be termed a noduloblema. The slight indent on top is accidental; it is not related to the funnel shape of the mature pileus.



Fig. 4. A young primordium composed of a rooted nodulus and a conical primordial shaft bearing a developing cauloblema.



Figs. 5a, b. Top and base from the same primordium shown in Fig. 4, different sections. The hyphae of the top are subparallel and growing upward; in the base they are more irregularly arranged and more inflated. Nuclear pairs indicated by the circles.



Fig. 6. A young primordium detached from its substrate and its root broken off. The pileus starts to form, and the pileoblema begins to grow out. A prehymenial palisade is still lacking.



Fig. 7. Detailed view of the section shown in Fig. 6. The voluminous cells on the top belong to the pileoblema, the narrower, club-shaped, downward oriented surface cells are the precursors of the pileus margin and the very narrow, more or less horizontal surface cells are the precursors of the palisade.



Figs. 8a, b. Top and base of a slightly more developed primordium showing a more pronounced pileus margin, a more developed pileoblema and already some pigmented cells in the primordial shaft. The funnel shape of the future pileus is already perceptible. Under the left pileus margin, the very young palisade initial composed of clavate, horizontal hyphal end cells is visible. In the base, the hyphae are more inflated than in the shaft, and in the rooting base they are subparallel.



Fig. 9. Although in this primordium the cauloblema and the pileoblema are well developed, no partial veil is formed. The underside of the pileus bears a palisade of narrowly clavate cells, but no basidia are present yet. The stipe rudiment consists of densely packed, subparallel hyphae. The nodulus in the base of the stipe has developed the secondary structure characterised by inflated, irregular hyphae. The rooting base is broken off.



Fig. 10. Pileus of an older primordium with well-developed prehymenial palisade from which numerous young basidia are projecting. The pileus is funnel-shaped, but the funnel is filled with the large-celled pileoblema.



Fig. 11. Prehymenial palisade with a young basidium projecting far beyond the general level of the palisade. Even at this early primordial stage, meiosis is already completed in this basidium, and three of the four nuclei are visible.



Fig. 12. The stipe of the primordium shown in Fig. 11 contains many inflated hyphae, but no sarcodimitic structure is visible yet.



Fig. 13. An enlarged view of the stipe context of the primordium shown in Figs. 11 and 12 reveals many free hyphal end cells, but they are not swollen into acrophysalides. The physalohyphae are multinucleate.



Figs. 14a, b. Well-developed metablemas cover the pileus and the stipe, but they do not meet and do not form a 'partial veil'.



Fig. 15. The prehymenial palisade of the same primordium shown in Fig. 14 is smooth, no gill initials are present. The projecting cell is either a young basidium or a young cystidium.



Fig. 16. A primordial gill in perradial section shows the divergent-bidirectional arrangement of the trama hyphae. Some basidia already bear sterigmata and spores.



Figs. 17a, b. Plectological differentiation in an older primordium is initiated by turgescent inflation of some hyphae in the gill trama.



Fig. 18. At the time of gill trama differentiation glycogen granules are present in the hyphae of the pileus trama. Some cells containing glycogen are indicated by arrows. Tannin-iron reaction followed by haematoxylin.



Fig. 19. A young basidiome with a cap diameter of 3.1 mm has a fully developed hymenium with sporulating basidia and pleurocystidia. The latter are sticky and trap released spores. The gill trama is physalo-irregular, and the subhymenium is slightly gelatinous. Tannin-iron reaction followed by haematoxylin.



Figs. 20a-d. Architecture of the mature gill trama in perradial (a, b), parahymenial (parallel to the hymenium, c) and paracial (parallel to the gill edge, d) sections. The wide physalohyphae are more or less bidirectionally oriented, with some hyphae running down from the gill base to the gill edge, others running more or less parallel to the gill edge. Between the physalohyphae occur many narrow, cylindrical, more irregularly arranged hyphae.



Figs. 21a, b. Very thin, thin-walled hyphae with strongly stainable content are present in the gill trama (a) and in the pileus trama (b).



Fig. 22. The pileoblema is now a gelatinous cutis with radial, periclinal hyphae. Some erect hyphae and some erect inflated cells, called pileocystidia, can still be found on the cap of the mature basidiome.



Figs. 23a-c. Architecture of the mature stipe. The context is composed of strongly inflated physalohyphae reminiscent of the sarcodimitic structure (a), but very often without fusiform shape (b). Narrow, cylindrical and strongly incrusted hyphae and some tortuous, frequently ramified, smooth connective hyphae occur between the physalohyphae (c).

Cauloblemas and pileoblemas often produce a partial veil, e.g. in *Panus tigrinus* (Kühner, 1925) and *Boletinus cavipes* (Kühner, 1926), and the difference with *X. campanella* concerns only the degree of the growth of the metablemas: a slightly more extended growth of either metablema would produce a partial veil, and, as a consequence, the developmental type of *X. campanella* would change from gymnocarpy to secondary angiocarpy, creating the illusion of a fundamentally different carpogenesis.

In the terminology proposed by Clémençon (1997), X. campanella is exocarpic since the primordium does not develop within a primordial shaft, amphiblemate since a pileoblema and a cauloblema are produced independently and never form a partial veil, and apertopileate since the pileus margin never touches the stipe.

Xeromphalina campanella is a good example to illustrate the fact that 'metablema' is not synonymous with 'emanated veil', since in this fungus the pileoblema and the cauloblema independently develop into a pileipellis and into a hirsute stipe covering, respectively.

The structure of the hymenophoral trama in the genus *Xeromphalina* is controversially described in the literature. According to Singer (1986: 424) the gill trama of mature basidiomes "is regular, its hyphae close, rather voluminous, subparallel and axiallarly arranged ..."; where 'axial' means "... the hyphae run ... from the plane of attachment to the pileus down to the edges of the hymenophore ..." (Singer, 1986: 56). Klán (1984: 215) confusingly writes "Hymenophoral trama regular, composed of irregular to loosely interwoven thin-walled hyphae ...", and Miller (1968: 159) describes the gill trama

"of loosely interwoven to loosely parallel, thin- to thick-walled hyphae". According to Redhead (1988) the "lamellar trama hyphae [are] bidirectionally arranged", using the concept of the bidirectional gill trama introduced by Clémençon (1982). In primordia of *X. campanella* the gill trama is bidirectional at first but becomes irregular at maturity, a final condition best observed in parahymenial sections. It is conceivable that some species conserve the bidirectional arrangement of the hyphae longer than the species studied here, explaining the wording used by Redhead (1988). Horak (1979) and Watling & Turnbull (1998) remain silent about the structure of the hymenophoral trama.

Intracellular glycogen has been observed in several agaricoid fungi, e.g. in *Coprinus cinereus* (Madelin, 1960; Matthews & Niederpruem, 1972), *C. trisporus* (Clémençon, 1997), and *Lepista flaccida* (Clémençon, unpublished observation), so the presence of this polysaccharide in *X. campanella* is not surprising.

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