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# BASIDIOME DEVELOPMENT OF BAEOSPORA MYOSURA (TRICHOLOMATALES, BASIDIOMYCETES)

## H. CLÉMENÇON

Institut d'Ecologie, Université de Lausanne, CH-1015 Lausanne, Switzerland. E-mail: Heinz.Clemencon@bluewin.ch

For the first time, the primordial development of a species of *Baeospora* is described and found to be metablematic, but the spurious partial veil vanishes in mature specimens. The pubescence of the mature stipe is a cauloblema. Although the pileus is described as dry in fully grown fruit-bodies, some young basidiomes have a locally slightly gelatinous pileipellis.

The species of the small agaricoid genus *Baeospora* Singer "are completely known except for the development of the carpophores" (Singer, 1986), a statement confirmed by Watling & Turnbull (1998) and Vellinga (1999), and still true in December 2001 when I came across a plentiful crop of young basidiomes of *Baeospora myosura* (Fr.: Fr.) Singer in all phases of development, from the young nodulus to the mature carpophore, growing on a dead cone of *Picea excelsa*. The cone was inspected under a stereomicroscope, and fragments of the cone with attached primordia were fixed, dehydrated, embedded and sectioned for the study of fruit-body development.

# MATERIAL AND METHODS

Excised cone fragments bearing the fungus were fixed by immersion in a solution of 0.9% formaldehyde and 1.25% glutaraldehyde in a 0.05 M sodium cacodylate buffer of pH 6.9. No vacuum was applied, and the material was fixed for 24–48 hours at a room temperature of about 20°C. Some material floated on the solution, but this did not reduce the quality of the fixation, as the aldehyde gasses penetrated into the fungi.

The fixed material was transferred directly to ice-cold 2-methoxy-ethanol (methyl cellosolve) without intermediate steps, and carried over ethanol and propanol into butanol, according to the procedure outlined by Feder & O'Brien (1968). For embedding, a mixture of 7 g glycol methacrylate and 3 g butyl methacrylate, complemented with 3 drops of terpineol per ml of methacrylate mixture, activated with 0.3% benzoyl peroxide and promoted with 0.04% N, N-dimethyl aniline was used. After polymerisation at room temperature for 12–16 hours, the blocks were cured at 60–65°C for another 10–12 hours.

The microtome sections, usually  $4-6 \mu m$  thick, were stretched on a drop of terpineolsaturated distilled water at room temperature and affixed to the glass slide by drying the preparations on a hot bench of about 70°C. The dry sections had to be baked onto the glass for at least 2 hours at 115–120°C to prevent formation of blisters during staining. Most sections were stained with aluminium zirconium haematoxylin at 38–40°C, resulting in blue cytoplasm and red-mauve walls (Clémençon, 2000). Some sections were stained with 0.01% basic fuchsine in distilled water for a few minutes at room temperature.

Selected sections were photographed with an Olympus DP11 digital camera mounted on an Orthoplan Leitz microscope. The photographs were optimised for printing using Adobe Photoshop with a Macintosh G4 computer.

Voucher specimens of *B. myosura* (Fr.: Fr.) Singer have been deposited in LAU under the accession number HC 01/082.

### RESULTS

The smallest stage observed is a nodulus about 125  $\mu$ m high and 85  $\mu$ m in diameter (Fig. 1). It consists entirely of irregularly interwoven hyphae. The middle part is slightly less dense than the base and the top. At this stage no metablema (Clémençon, 1997) is present.

The next stage has the form of an elongate ellipsoid about 115  $\mu$ m wide and 305  $\mu$ m high (Fig. 2). The basal part is a tightly woven nodulus supporting a primordial shaft consisting of upward growing slightly interwoven hyphae. In the nodulus the hyphae are irregularly arranged and about 2.5-4.5  $\mu$ m wide; in the centre of the shaft they are subregular and 1.5-4  $\mu$ m wide; and in the top they are again irregularly arranged, but they measure only about 1.5-2.5  $\mu$ m. At the sides, a few hyphae grow away from the main body announcing the future metablema. As the primordia develop on the inner side of a scale of the *Picea* cone, the primordial shaft is not geotropically upright, but it grows into the space between two scales.

Fig. 3 shows a slightly larger developmental stage covered with a loose metablema and topped by an early pileus initial. The diameter of the hyphae diminishes from 2–5.5  $\mu$ m in the nodulus at the base of the primordium to about 1.5–2.5  $\mu$ m in the young pileus. There is no trace of a prehymenial palisade.

As the pileus rudiment enlarges, a small, irregular prehymenial palisade develops between the pileus edge and the stipe (Fig. 4c, d), but it does not always circle the whole circumference of the primordium. The expansion of the pileus separates the metablema into two parts that can be called pileoblema and cauloblema (Clémençon, 1997). The prehymenial palisade initial is not covered by a metablema, but some hyphae of the pileoblema may locally grow below the pileus edge (Fig. 4c). In the young pileus the hyphae are irregularly and tightly interwoven and are only about  $1-3.5 \mu m$  thick, but in the basal part of the primordium they now measure up to  $6 \mu m$  in diameter (Fig. 4e). The middle part of the primordium is the very short stipe initial. Its hyphae form a more or less irregular context with only a slight longitudinal tendency.

Fig. 5 shows the upper part of a primordium about 450  $\mu$ m high. In the lower part of the stipe, a few hyphae inflate conspicuously, announcing the formation of the spindle-shaped physalohyphae characteristic of the sarcodimitic stipe context of the mature basidiome (Vellinga, 1999). As the pileus grows and expands laterally, the prehymenial palisade becomes more conspicuous. The cauloblema now grows upwards towards the pileus edge and may locally bridge the gap between the pileus and the stipe, thus forming a very scant partial veil. This veil remains very thin and shows many gaps where the prehymenial palisade remains naked. The Figs. 6a-d show two 'cauloblematic bridges' from the same primordium.

The scant 'cauloblematic bridges' persist for some time, but they never form a complete partial veil. In another primordium of about the same size, the metablema does not bridge the gap between stipe and pileus margin at any point, although the pileoblema and the cauloblema are well-developed (Fig. 7). In both primordia the prehymenial palisades are well-formed but still smooth, since no gills are yet formed (Fig. 9).

Gills are formed by downward growth of generative hyphae (Fig. 8a, d). The primordial gill trama is subregular, slightly divergent and weakly bidirectional by the presence of a few hyphae roughly parallel to the gill edge. Surprisingly, the pileipellis is gelatinous at this stage (Fig. 8b, c).

The conclusion is that *B. myosura* is exocarpic metablematic with a spurious amphicleistoblema that does not leave any trace in mature fruit-bodies.



Figs. 1–3. First developmental stages of *Baeospora myosura*. – 1. This nodulus is composed entirely of intertwined hyphae, but shows already a slight plectological differentiation; the centre is less dense than the bottom and the top part. No metablema is present yet. 2. The primordial shaft consists of upward growing, subregular hyphae. In the nodulus at the base the hyphae are wider and irregularly arranged. A few hyphae grow out laterally from the shaft; this is the metablema rudiment. 3. The pileus initial is formed, but there is no prehymenial palisade yet. The metablema is well-developed.



Figs. 4a-e. Anatomy of a young primordium with well-developed metablemas. The pileoblema is growing out from the pileus surface (b), pileus margin is forming and the first rudiment of the prehymenial palisade is visible (c, d). The pileoblema grows downward over the pileus margin in the direction of the stipe (c). In the base of the stipe, the hyphae of the nodulus are strongly inflated (e).



Fig. 5. A slightly older primordium in a lateral longitudinal section, showing the well-developed cauloblema that locally bridges the gap between the pileus margin and the stipe, thus forming a spurious and incomplete partial veil. In the stipe a few hyphae are enormously enlarged; they are the forerunners of the spindle-shaped physalohyphae of the future sarcodimitic context.



Fig. 6a-d. In the next developmental stage the spurious partial veil formed by the cauloblema and the pileoblema becomes more evident. The prehymenial palisade and the pileus margin are well-developed.



Fig. 7. Whole view of a primordium with well-developed pileus margin, prehymenial palisade and metablemas. There is no trace of a partial veil in this specimen.



Fig. 8a-d. Tangential section through a young basidiome with developing gills (d). The pileipellis is locally gelatinous (b in tangential section, from a; c in median section), but is otherwise dry (a, left side).



Fig. 9. Tangential section through a primordium with well-developed yet spurious partial veil. The prehymenial palisade is still smooth.

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