

Relationships of *Amanita* to *Armillaria*

S. HELFER & R. WATLING

Royal Botanic Garden, Edinburgh, EH3 5LR, Scotland

HELFER, S. & R. WATLING (1989). Relationships of *Amanita* to *Armillaria*. — SYDOWIA 41: 144–149.

Photomicrographs using Nomarski interference contrast supported by transmission and scanning electron micrographs are offered to demonstrate that the ornamentation recorded for certain *Amanita* spp. is quite different to that found in the genus *Armillaria*.

Whilst examining the problems which surround the nomenclature, taxonomy and relationships of the genus *Armillaria* it was found necessary to explore at least some aspects of members of the genus *Amanita*.

Armillaria is now restricted to those agarics related to the "Honey fungus", *A. mellea* (VAHL: FR.) KUMMER, as outlined by WATLING, KILE & GREGORY (1982). However, *Armillaria* in the classical literature covered many quite different elements some of which are now distributed in *Floccularia*, *Oudemansiella* and *Tricholoma* (Tricholomataceae), *Limacella* (Amanitaceae) etc. This is undoubtedly the reason why there is still some nomenclatural confusion as to the correct delimitation of the genus. Members of the genus *Floccularia*, eg. *F. straminea* (KROMBH.) POUZAR, and *F. albolaripes* (ATK.) ABRAHAM & WATLING (in press) are macroscopically similar to members of the genus *Amanita*, and although the development of the basidiomata is different the hymenophoral trama is divergent and basidiospore walls are hyaline and amyloid. DENNIS, WAKEFIELD & BISBY (1954) discussed a scenario in which if careful consideration was not given to the nomenclature then even *Amanita* and *Armillaria*, through *Floccularia*, might in some instances, depending on delimitation and typification of genera, be synonymised.

This supposed closeness between *Amanita* and *Armillaria* could have been reflected in the findings of KUHNER (pers. comm.) which showed that some common European species of *Amanita* possessed ornamented basidiospores. BAS (1969) indicates that *Amanita princeps* CORNER & BAS from Singapore, placed in subgenus *Amanita* sect. *Vaginatae*, showed some differentiation in the structure of the basidiospores which lead Chr. WATLING to carry out a preliminary study of some common British species utilizing electron microscope techniques.

This work confirmed that structural differentiation could be documented but the wall was so much thinner than that found in *Armillaria* spp. (BENNEL & al., 1985). Thus rather more critical fixation techniques would be necessary to enhance the different structures. Whilst the senior author (S. H.) was refining the techniques two papers of interest appeared, *viz.* REID (1987) and KOTILOVÁ-KUBIČKOVÁ & POUZAR (1988). In the first REID reported his own observations on *A. umbrinolutea* (GILLET) BAT. and those of SPOONER's of a reticulate pattern in the basidiospores of *Amanita gemmata* (FR.) GILL. supported by light micrographs. The second report was more extensive and confirmed that further studies should be undertaken. The two Czech authors in addition to *A. umbrinolutea* also supplied figures of basidiospores of *A. caesarea* (SCOP.: FR.) GREV., *A. pantherina* (DC.: FR.) SECR., *A. vaginata* (BULL.: FR.) VITT. and *A. citrina* SCHAEFF.: S. F. GRAY. The present paper extends these observations using various light and electron microscopic techniques to address the problem.

Materials and methods

Specimens examined

Amanita submembranacea (BON) GRÖGER; (SH: 218). – *A. muscaria* (L.: FR.) HOOKER; (SH: 217). – *A. citrina* (SCHAEFF.) S. F. GRAY; (Wat.: 9642). – *A. fulva* (SCHAEFF.) SECR.; (Wat.: 9654). – *A. nivalis* GREV.; (Wat.: 20257).

Armillaria mellea (VAHL: FR.) KUMMER; (Wat.: 9660). – *A. nigritula* P. D. ORTON; (Wat.: 3387). – *A. bulbosa* (BARLA) KILE & WATLING (= *A. gallica* MARXMÜLLER & ROMAGNESI; (Wat.: 20833). – *A. cepistaepes* f. *pseudobulbosa* MARXMÜLLER & ROMAGNESI; (SH: 225). – *A. cepistaepes* f. *pseudobulbosa* MARXMÜLLER & ROMAGNESI; (SH: 226).

To avoid inconsistencies all the material used in this study was taken from dry herbarium specimens which are deposited in Edinburgh (E).

Sample preparation

a. *Light microscopy*. – Sections were cut from gills and suspended in 10% annonia for at least 2 hours on the microscope slide. Examination was carried out using bright field and Nomarski interference contrast on a Leitz Dialux 20 compound microscope. Micrographs were taken as optical sections at high magnification using Ilford FP4 film.

b. *Transmission electron microscopy (TEM)*. – Sections were cut from gills and rehydrated for two hours in 2.5% glutaraldehyde at pH 7.2 in 0.06 M phosphate buffer, prior to osmium tetroxide fixation (1% aqueous, two hours), dehydration in a graded ethanol series

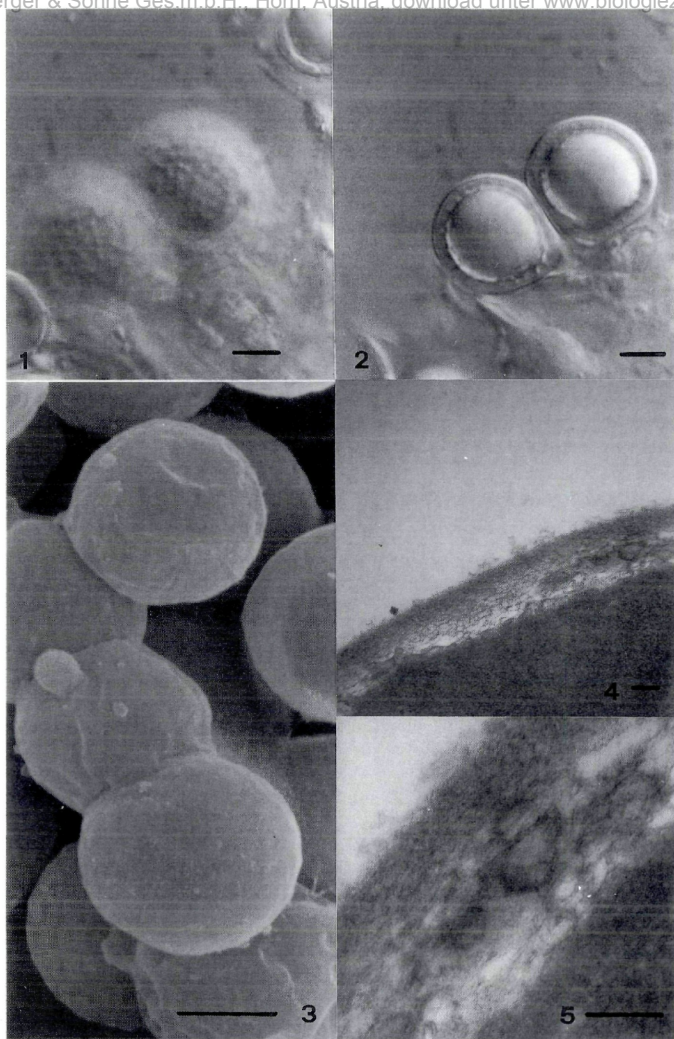


Plate 1: figs 1–5: *Amanita submembranacea*. 1. Basidiospores showing reticulate pattern; interference contrast on top section of spores. Scale bar represents 5 μm . – 2. Same spores as fig. 1; reticulation restricted to the inside of the spores; interference contrast through centre of spores. Scale bar represents 5 μm . – 3. Critical point dried spores showing \pm smooth surfaces with slight drying damage; SEM. Scale bar represents 5 μm . – 4. Basidiospore wall with internal ornamentation; TEM. Scale bar represents 0.1 μm . – 5. Detail from fig. 4; TEM. Scale bar represents 0.1 μm .

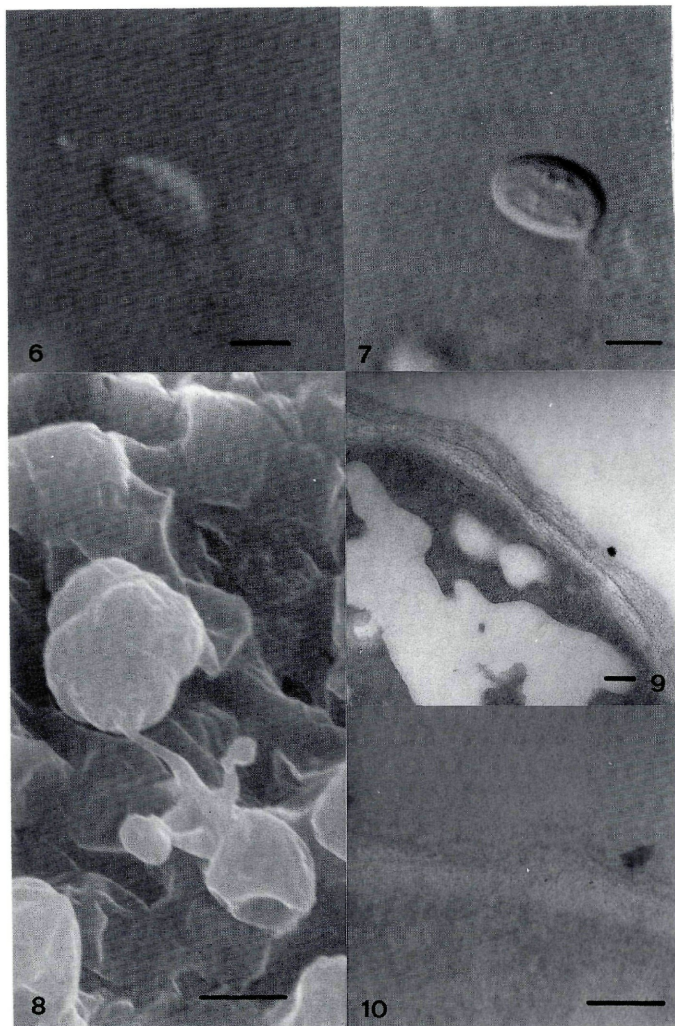


Plate 2: figs 6–10; *Armillaria mellea*. – 6. Basidiospore showing only slight ornamentation: interference contrast on top section of spore. Scale bar represents 5 μm . – 7. Same spore as fig. 6; only cytoplasmic structures visible; interference contrast through centre of spore. Scale bar represents 5 μm . – 8. Critical point dried basidium bearing one mature and three immature spores; coarse surface pattern; SEM. Scale bar represents 5 μm . – 9. Basidiospore wall showing external ornamentation; TEM. Scale bar represents 0.1 μm . – 10. Detail from fig. 9; TEM. Scale bar represents 0.1 μm .

(50%, 70%, 85%, 95%, 100%) and impregnation in acrylic resin (LR White). After polymerisation of the resin sections were taken using a Reichert Om U2 ultramicrotome, contrasted with lead citrate (REYNOLDS, 1963) for 7 min and examined in an AEI EM6M electron microscope at 80 kV accelerating voltage. Micrographs were taken on Agfa RA 710P film.

c. *Scanning electron microscopy (SEM)*. – Samples were rehydrated and dehydrated as for TEM and specimens were critical point dried in CO₂ after dehydration (Balzers CPD 010). Alternatively material was used directly from herbarium collections. Dried specimens were mounted onto aluminium stubs, coated with gold palladium for 2 mins at 20 mA and observed in a Jeol JSM 200T electron microscope at 25 kV accelerating voltage. Micrographs were taken on Ilford Pan F film.

Results and discussion

Although differences in spore shape and size could be recorded, the spore surface features of the different *Amanita* species were essentially identical. The same applied to the *Armillaria* species.

Photomicrographs, scanning and transmission electron micrographs are offered of basidiospores of *Amanita submembranacea* and *Armillaria mellea* which demonstrate that the ornamentation seen in a percentage of the basidiospores of the *Amanita* is composed of a reticulate pattern on the outer surface of the protoplast and is not formed as lenses of material within the ecto- and endospore as demonstrated in *Armillaria* (see also BENNELL & al., 1985). This cytoplasmic pattern is particularly demonstrable when Nomarski interference contrast is used (figs 1 & 2). Scanning electron micrographs show that the basidiospores of *Amanita* are in fact quite smooth and show little sign of collapse (fig. 3) compared with the rather coarse pattern revealed in *A. mellea* where collapse reflects the wall structure (figs. 6–8). Smooth basidiospores are observed in many European taxa of *Amanita* both with inamyloid and amyloid basidiospores. TEM micrographs reveal an internal ornamentation in the *Amanita* spores (figs 4 & 5) and varying wall thickness in those of the *Armillaria* species (figs 9 & 10). The comparison between dry herbarium material and critical point dried material showed that no artifacts had been introduced using the latter procedure or during the dehydration for TEM preparation.

Conclusions

On the evidence presented the structure of the basidiospore wall of *Amanita* is different to that of *Armillaria*. A link cannot be made between the genera on this character alone. Further studies are now

required on species of *Amanita* from SE Asia and *A. roanokensis* COKER from N. America which is reported as having basidiospores decorated with minute amyloid warts (BAS, 1969), something KOTILOVÁ-KUBIČKOVÁ & POUZAR (1988) noted in several European taxa.

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Zeitschrift/Journal: [Sydowia](#)

Jahr/Year: 1989

Band/Volume: [41](#)

Autor(en)/Author(s): Helper S., Watling Roy

Artikel/Article: [Relationships of Amanita to Armillaria. 144-149](#)