PERSOONIA
Published by the Rijksherbarium, Leiden
Volume 7, Part 2, pp. 289–292 (1973)

SPORE ORNAMENTATION IN RAMARIA AS DEPICTED BY SCANNING ELECTRON MICROGRAPHS[1]

RONALD H. PETERSEN and RONALD W. PEARMAN

Department of Botany, University of Tennessee, Knoxville, Tennessee 37916, *U.S.A.*

(With Plates 24–26)

Scanning electron micrographs of *Ramaria* spores show three distinct ornamentation patterns: (1) spiny ornamentation; (2) raised patches or warts; and (3) longitudinally or obliquely oriented ridges. These observations fully support none of the provious taxonomic schemes at the subgeneric level, and some nomenclatural adjustments are recommended.

Although a number of papers have illustrated basidiospores with scanning electron micrographs, only one has shown a spore of *Ramaria* (Perreau & Heim, 1969). Bigelow & Rowley (1968) and Grand & Moore (1970) offered photos of spores of *Gomphus floccosus*, which recent authors have placed in the Gomphaceae with *Ramaria*, and Pegler & Young (1971) have shown spores of *Gymnopilus* and *Hebeloma*, which Petersen (1968) conjectured as perhaps related to the Gomphaceae.

Corner (1970) described three subgenera within *Ramaria*. Subgenus *Echinoramaria* was based on spiny spore ornamentation, while subgenera *Lentoramaria* and *Ramaria* were characterized by a combination of habit, hyphal construction of the fruit body, and spore ornamentation. Although for any student of the genus these designations were obvious and clear, such groupings were really based on experience, with the characters hardly definitive or empirically measurable. Similarly, Petersen (1967) suggested four subgeneric complexes, also based on character combinations, and that scheme was of dubious usefulness. In order to clarify some emerging concepts of subgeneric classification, spores of representative species of *Ramaria* were examined under the scanning electron microscope to corroborate data already gathered in observations with bright field and phase contrast microscopy with the aid of cotton blue staining (Petersen, 1967, 1971a, b, c).

Spores for examination were obtained from spore prints from specimens in herb. TENN. Spore prints on glass or wax-impregnated paper were found preferable to those on paper. Spores were transferred directly to double-faced cellophane tape which had been mounted on standard 3/4 inch aluminum studs, and then coated with vaporized carbon and gold in vacuo using a Denton vacuum coater equipped with a random rotating head. The spores were viewed on an AMR model 900

[1] This project was supported in part by National Science Foundation grant GB 32104, and represents contribution no. 396 from the Botanical Laboratory, University of Tennessee.

scanning electron microscope and photographed with a Polaroid Land camera.

Using these methods, three clearly discernable subgeneric groups may be identified on spore ornamentation characters as follows:

COMPLEX 1.—*Ramaria grandis* (Pk.) Corner was chosen to represent the echinulate-spored group because spore ornamentation is gross in this species and good spore prints were readily available. Line drawings of such spores [Coker, 1923; Corner, 1950 under *R. zippelii* (Lév.) Corner] had already shown the spiny ornamentation but subsequently Petersen (1967) illustrated spores stained in cotton blue, in which the ornamentational spines stained much more strongly than the interspine areas.

Scanning electron micrographs of such spores (Pl. 24 Figs. 1–3) completely corroborate the data gathered with cotton blue and bright field microscopy. Not only are some spines longer than others, but some are partially joined by low, raised isthmuses. The spines (Fig. 3) are seen as extensions of the spore surface, rather than protrusions through an outer layer as suggested for *Clavulinopsis helvola* (Pers. per Fr.) Corner. The apiculus, marked in the figures, seems accompanied by a collar-like neck, but this feature is obscure, as it also is with the light microscope. Likewise the type of apiculus as categorized by Pegler & Young (1971) remains unknown.

Although such spores are obviously different from those described below, additional representatives of this group must be observed, especially those with smaller, more delicate spore ornamentation, for instance *R. ochraceo-virens* (Jungh.) Donk.

COMPLEX 2.—A spore of *Ramaria subbotrytis* (Coker) Corner is illustrated (Pl. 25 Figs. 4, 5). Spore ornamentation consists of raised patches or warts on the spore surface, either discrete or somewhat meandering. In cotton blue, it is these raised patches which stain most strongly, as evidenced by comparison of the photo with line drawings of stained spores with similar ornamentationl patterns (see Petersen, 1967, 1971a). Based on such comparisons, it would appear that high magnification (2500 × or higher) and cotton blue staining reveal all of the ornamentation seen under higher magnification by scanning electron microscope examination.

Perreau & Heim (1969) illustrated a spore of *R. stricta* (Pers. per Fr.) Quél. as seen through the SEM, and the ornamentational pattern was very similar to that of *R. subbotrytis*. This is supported by observations with the light microscope. Such similarities detract from definitive or diagnostic differences between Corner's 1970) subgenera *Lentoramaria* and *Ramaria*, but also mitigate against resurrection of the name *Clavariella* Karsten, based on *C. apiculata* (Fr.) Karsten ast ype, as suggested by Petersen (1971b).

Only one confusing variation of this ornamentational pattern has been observed. In some species [i.e. *R. xanthosperma* (Pk.) Corner] the raised pattern takes the form of irregular ridges often oriented longitudinally or obliquely on the spore. Such patterns superficially resemble the striate appearance described below. Care in observations, however, readily reveals the true ornamentation type.

COMPLEX 3.—As the type species of *Ramaria*, *R. botrytis* (Pers. per Fr.) Bourd.

Pl. 26 (Figs. 6–8) was chosen as representative of the "striate spored" complex. Line drawings and descriptions (Coker, 1923; Corner, 1950) had suggested such ornamentation, but later drawings (Petersen, 1967; Marr, 1968) showed the pattern more clearly under cotton blue staining. All drawings, even with stain, had indicated that the stained areas (not nearly as cyanophilous as in the preceding complexes) were raised, perhaps as folds of a thin outer spore wall. When such drawings are compared with SEM images, however, it is clear that the areas which stain with cotton blue are not raised, but are the narrower, attenuate, sometimes reticulate sunken areas between the longitudinally or obliquely arranged raised striae. This is in contrast to the situation described in complex 2 above, where the raised ornamentational patches stain more strongly than the lower spore surfaces. Although additional spores must be examined, such a discrepancy indicates that a wider separation between complexes 2 and 3 may be warranted. In such an event, complex 2, hitherto grouped under subgenus *Ramaria* by Corner (1970), would require a new name. Thus far, the only name available is "Laeticolores Marr" (1968), but this name has not been validly published.

Figure 8 shows the abaxial spore surface in *R. botrytis*, on which the obliquely oriented ridges diverge downward adaxially. Such a divergent pattern is also observable under the light microscope with cotton blue stain.

Such "ground truth" gained under the SEM thus far substantially corroborates the observations already made with the light microscope. Several tentative conclusions seem more evident: (1) subgenus *Echinoramaria* seems well founded, and with further investigations may require generic rank (in which case the unfortunate name *Phaeoclavulina* Brinkmann is available); (2) little distinction may be made between subgenus *Lentoramaria* and subgenus *Ramaria* sensu Corner, and further attempts to ease the taxonomy of this interface must be made; and (3) separation of subgenus *Ramaria* sensu Petersen (striate-spored species) from "Laeticolores Marr" nom. inval. seems obvious on spore ornamentation and staining patterns.

LITERATURE CITED

BIGELOW, H. E. & J. R. ROWLEY (1968). Surface replicas of the spores of fleshy fungi. *In* Mycologia **60**: 869–887.

COKER, W. C. (1923). The clavarias of the United States and Canada. Chapel Hill.

CORNER, E. J. H. (1950). A monograph of *Clavaria* and allied genera. *In* Ann. Bot. Mem **1**.

—— (1970). Supplement to "A monograph of *Clavaria* and allied genera." *In* Beih. Nova Hedwigia **33**.

GRAND, L. F. & R. T. MOORE (1970). Ultracytotaxonomy of Basidiomycetes. I. Scanning electron microscopy of spores. *In* J. Elisha Mitchell scient. Soc. **86**: 106–117.

MARR, C. D. (1968). The genus *Ramaria* in western Washington. Ph. D. Dissertation, ined. Univ. Washington, Seattle.

PEGLER, D. N. & T. W. K. YOUNG (1971). Basidiospore morphology in the Agaricales. *In* Beih. Nova Hedwigia **35**.

PERREAU, J. & R. HEIM (1969). L'ornementation des basidiospores au microscope électronique à balayage. *In* Revue Mycol. **33**: 329–340.

PETERSEN, R. H. (1967). Evidence on the interrelationships of the families of clavarioid fungi. *In* Trans. Br. mycol. Soc. **50**: 641–648.
—— (1968). Notes on cantharelloid fungi. I. *Gomphus* S. F. Gray and some clues to the origin of ramarioid fungi. *In* J. Elisha Mitchell scient. Soc. **84**: 373–381.
—— (1971a). Familial interrelationships in the clavarioid and cantharelloid fungi. *In* R. H. Petersen (Ed.), Evolution in the Higher Basidiomycetes 345–374. Knoxville.
—— (1971b). The genera *Gomphus* and *Gloeocantharellus* in North America. *In* Nova Hedwigia **22**: 1–112.
—— (1971c). Type studies in clavarioid fungi. IV. Specimens from Herbarium Fries at Uppsala, with notes on cantharelloid species. *In* Friesia **9**: 369–388.

EXPANATION OF PLATES 24-26

PLATE 24

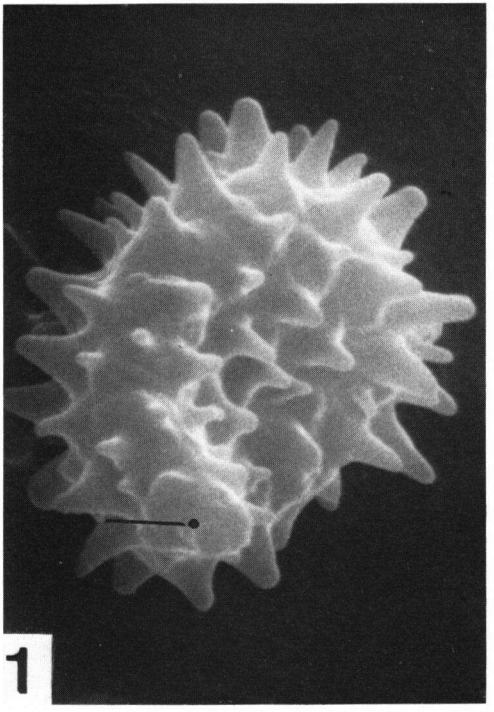
Figs. 1–3. *Ramaria grandis* (Pk.) Corner, TENN 36164. figs. 1, 2: × 5000; 3: × 10,000.

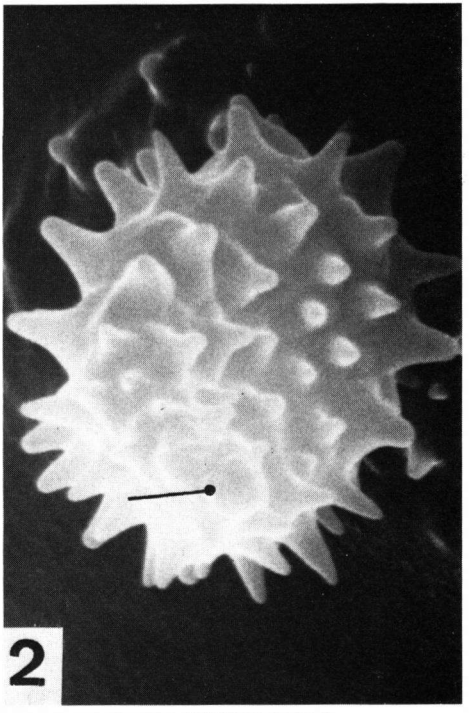
PLATE 25

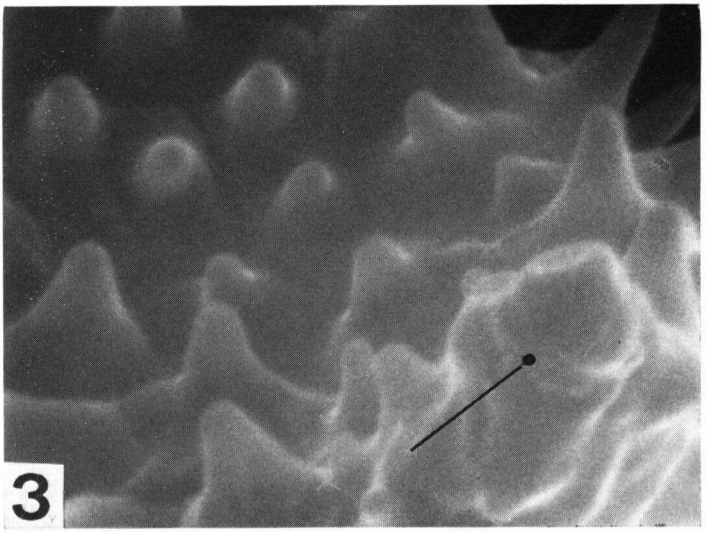
Figs. 4, 5. *Ramaria subbotrytis* (Coker) Corner, TENN 31279. Fig. 4: × 5000; 5: × 10,000.

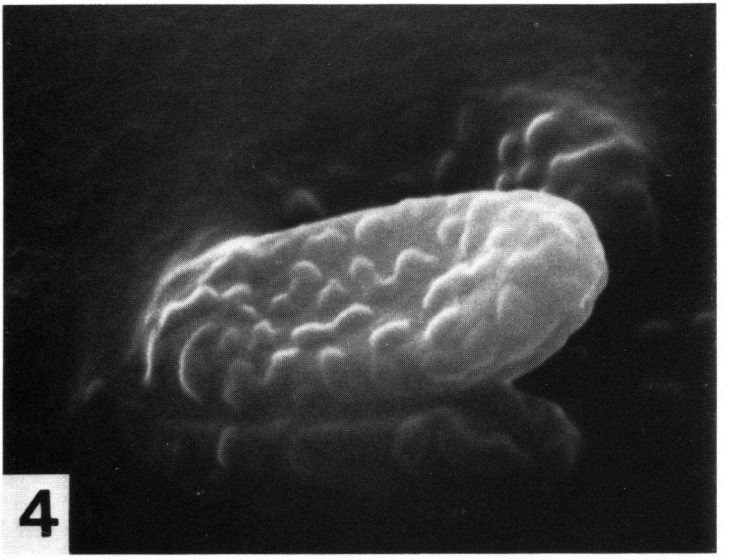
PLATE 26

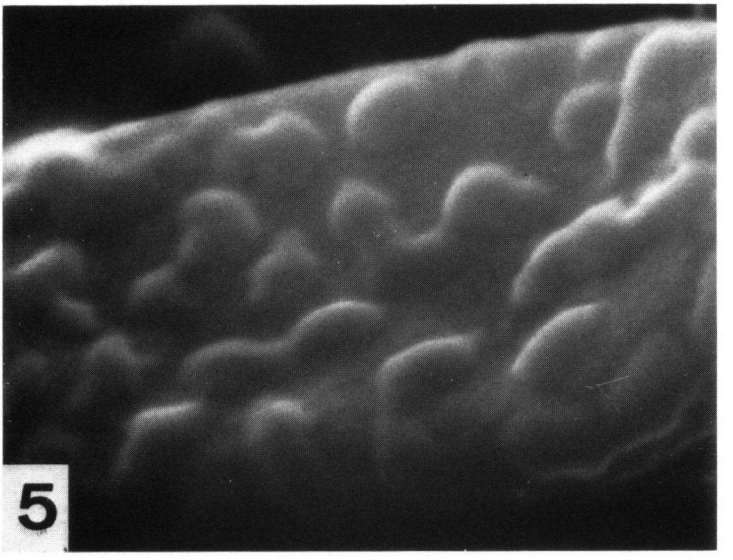
Figs. 6–8. *Ramaria botrytis* (Pers. per Fr.) Bourd., TENN 31138. Figs. 6, 8: × 5000; 7: × 10,000.

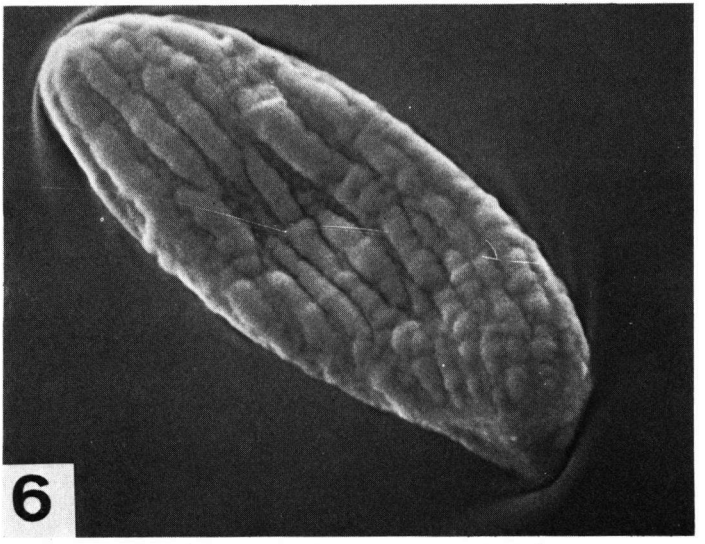

1

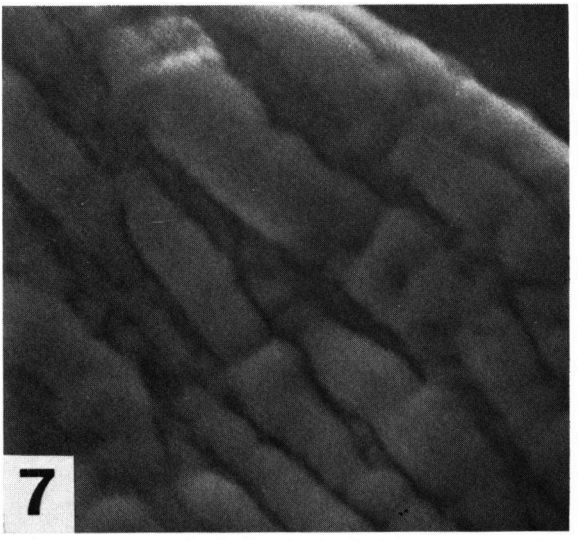

2

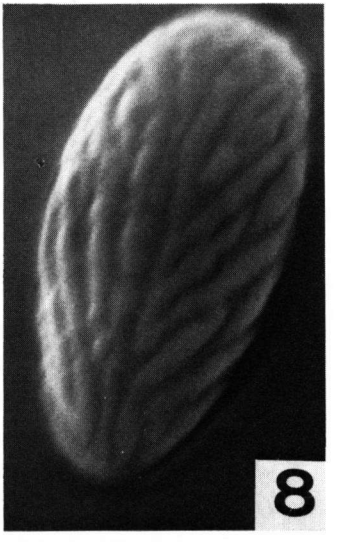

3


4


5


6


7


8