

SPORE ORNAMENTATION AND SPECIES CONCEPT IN SYNCEPHALASTRUM

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Morphological studies and mating experiments indicate the synonymy of *Syncephalastrum verruculosum* and *S. racemosum*. The ornamentation of the sporangiospores is due to folds of the merosporangial wall. It is highly variable and cannot be used for species delimitation.

In his revision of the merosporangiferous Mucorales Benjamin (1959) considered all the species (6) and varieties (1) described in the genus *Syncephalastrum* J. Schroet. as synonyms of the only accepted species, *S. racemosum* Cohn ex J. Schroet. He studied more than thirty isolates and concluded that variation in vesicle diameter, number of spores per sporangium and size and shape of the spores (globose to ovoid) had no taxonomic value at species level. Since 1959 two additional species have been described, viz. *S. alma-ataense* Novobr. (1972) and *S. verruculosum* Misra (1975).

Benjamin (1959) described the sporangiospores as smooth, but in later reports they have been repeatedly described as roughened or verruculose (Young, 1968; Matsushima, 1975; Watanabe, 1975). Misra used the ornamentation of the sporangiospores as a main character to distinguish *S. verruculosum*. O'Donnell (1979) published SEM photographs of nearly smooth sporangiospores, while those published by Cole and Samson (1979) were definitely warty. In both cases the strain had been grown on malt extract agar (MEA). In 1981 a strain on MEA (Merck) with smooth spores was sent for identification; after transfer on CBS beerwort agar it produced warty sporangiospores. This phenomenon and the different interpretation of the taxonomic value of the spore ornamentation prompted us to a reconsideration of this character.

MATERIAL AND METHODS

Strains examined. — CBS 348.35 (-), originating from Blakeslee's collection. — CBS 440.59 (+), CBS 442.59 (+), CBS 443.59 (+), CBS 444.59 (-), all isolated from soil, California, U.S.A., sent by R.K. Benjamin — CBS 441.59, isolated from coyote dung, California, U.S.A., sent by R.K. Benjamin. — CBS 213.78 (-), type strain of *S. verruculosum*, from air, Gorakhpur, India, sent by P.C. Misra. — CBS 556.81 (-), CBS 557.81 (+), from foodstuff, the Netherlands.

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All strains were grown at 25°C on PDA, YpSs, YEA (yeast + malt extract agar), malt extract no. 5398 (Merck, pH = 5.6), malt extract CM59 (Oxoid), CBS beerwort agar, containing 4% sugar, at pH = 4, 5, 6 and 7. To establish an eventual influence of the temperature, strains were also grown at 20, 30 and 36°C. Slides were mounted in water, 0.1 N NaOH, 0.1 N HCl, Congo Red in 10% NH₄OH and Cotton Blue in lactic acid. Mating experiments were performed on YE agar at 25°C in the dark. SEM preparations were made according to Samson & al. (1979); after critical point drying some spores were gently squashed between object glasses in order to get more information on the nature of the ornamentation. Capitalized colour names refer to Ridgway (1912); colour codes refer to Kornerup & Wanscher (1978).

RESULTS

On beerwort agar all strains resembled each other in general morphology. The variation encountered did not exceed the usual range in species of Mucorales. All strains contained ornamented spores, albeit that smooth spores also occurred and that the ratio smooth versus ornamented spores and the degree of ornamentation (slightly roughened versus distinctly verrucose) varied. On all other media similar results were obtained. CBS 556.81 which was received with smooth sporangiospores, later also produced warty spores on Merck malt agar, the medium on which it was originally received. Various temperatures (20, 25, 30, 36°C) did not affect the ornamentation.

When mounted in 1 N HCl the ornamentation disappeared almost completely (Fig. 4); in 1 N NaOH and in Congo Red the ornamentation seemed to be less distinct, but did not disappear. In Cotton Blue the ornamentation was similar to that seen in water.

SEM photographs revealed that the ornamentation is formed by the merosporangial wall. When this wall is damaged (Fig. 5–6) or dissolved (Fig. 4), the smooth or nearly smooth spore wall is clearly visible. The type of ornamentation (Fig. 1–3) varied from wart-like to wrinkled or plate-like, often within the same strain. In addition the density of the warts varied considerably.

The mating experiments also indicate that only one species is involved (Table 2).

DISCUSSION

The merosporangial wall, which remains in close contact with the spore wall (Cole & Samson, 1979), is responsible for the ornamentation. TEM photographs of Fletcher (1972) and also our own observations suggest that the warts and ridges are mere folds of the sporangial wall. The ornamentation is thus due to a deformation of the sporangial wall during maturation. This may be caused by a shrinkage of the sporangiospores during the process of maturation, which indeed seems to occur. This explanation, however, is only partly satisfactory; it may account for the long and low irregular ridges as seen in the lower spores of Fig. 1, but hardly for the rather high and abrupt plate-like structures as seen in Fig. 6. In additional explanation may be found in the residual contents which remain in the sporangium after the spores have been separated and fill some space

	<i>S. racemosum</i> after Benjamin (1959)	<i>S. verruculosum</i> after Misra (1975)	<i>S. racemosum</i> CBS 441.59 (Benjamin)	<i>S. verruculosum</i> CBS 213.78 (Type strain)
Conditions	Yp5s or PDA, 21 °C	SMA or PDA, 25 °C	CBS beerwort agar, 25 °C	CBS beerwort agar, 25 °C
Colony	nearly white or Drab (7D3) to Deep Neutral Gray (18F3) 0.5–1.5 cm high	greenish grey to light brown 1 mm high	Deep Olive Gray (28D2) 1.5 cm high	Deep Olive Gray (28D2) to Dark Olive Gray (29E2) low, aerial mycelium collapsed
Sporangio- phores	erect, ascending or recumb- ent, 10–25 µm diam. branching racemose or cymose	erect or ascending 7–13 µm diam. cymosely branched	up to 15 µm diam. series of short, racemose late- ral branches and a few cymose branches	up to 7.5(–15) µm diam. series of racemose lateral branches
Sporangio- vesicles	lateral branches straight or recurved	lateral branches straight or recurved	lateral branches straight or recurved	lateral branches mostly straight, some recurved
Terminal vesicles	globose or ovoid 30–80 µm diam. pale or brownish usually subtended by septa	globose 25–40 µm diam. light brown usually subtended by septa	globose, rarely ovoid up to 40 µm diam. brownish often subtended by septa	globose or ovoid up to 30(–40) µm diam. brownish usually subtended by septa
Sporangio- spores	pale to dark grey or brownish 40–150 µm diam.	dark grey 45–90 µm diam.	pale to dark grey or brownish up to 80 µm diam.	pale to dark grey or brownish up to 80 µm diam.
Mero- sporangia	rod-like (3–)5–10(–18) spores	clavate, 13–23 × 4–7 µm (2–)3–5(–6) spores	clavate, av. 25 × 5 µm av. 7 spores	clavate, av. 25 × 5 µm av. 6 spores
Sporangio- spores	globose to ovoid, rarely oblong to cylindrical about 3–5 µm diam. smooth	globose to subglobose, rarely obovoid or oblong 4–7 µm diam. verruculose	globose to subglobose 3–5 µm diam. slightly verruculose	subglobose 4–6(–7) µm diam. verruculose

Table 1. Comparison of literature data and observations of *S. racemosum* and *S. verruculosum*.

	+	440.59	442.59	443.59	199.81	557.81
348.35	+			+	+	+
441.59	+		+			+
444.59	+		+	+	+	+
213.78	+			+	+	+
556.81	+		+	+	+	+

Table. 2. Results of matings in *Syncephalastrum* (+ = zygospores produced).

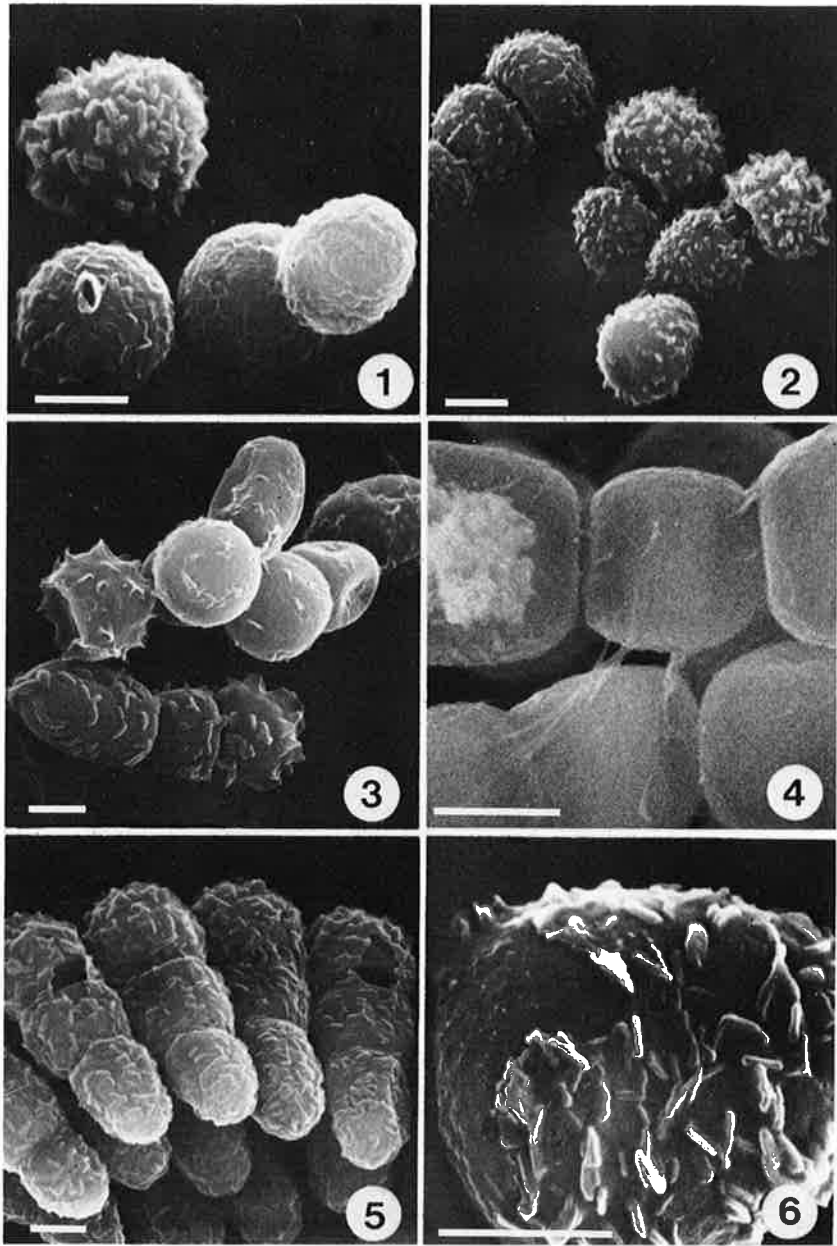
between the spores and the sporangial wall. This wall is partly broken down from the inside (Fletcher, 1972). The liquid then either evaporates or is absorbed by the spores and consequently the meiosporangial wall collapses.

The above results clearly demonstrate that the ornamentation of the sporangiospores has no taxonomic value at species level. Other characters used by Misra (1975) to separate *S. verruculosum* from *S. racemosum* are summarized in Table 1 and compared with the description of Benjamin (1959) and our own observations on an original strain of Benjamin (CBS 441.59) and the type strain of *S. verruculosum*. These data clearly indicate, that *S. verruculosum* falls within the morphological variability of *S. racemosum*. The results of the mating experiments (Table 2) support this statement.

No material was available for a study of *S. alma-ataense*. This is probably another synonym of *S. racemosum* as the only distinguishing character would be the slow growth on Czapek agar (Novobranova, 1972).

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Figs. 1–6. — Sporangiospores of *Syncephalastrum racemosum*. — 1. *CBS 440.59* on CBS beerwort agar. — 2. *CBS 348.35* on CBS beerwort agar. — 3. *CBS 213.78* on CBS beerwort agar. — 4. *CBS 213.78* after treatment with 0.1 N HCl. — 5. Intact merosporangia of *CBS 199.81* on Merck malt agar. — 6. A spore of *CBS 213.78* on Merck malt agar. (The bar represents 2 μm .)