PERSOONIA
Published by the Rijksherbarium, Leiden
Volume 11, Part 1, pp. 39–52 (1980)

COMPARISON OF ZYGOSPORE ORNAMENTATION IN INTRA- AND INTERSPECIFIC MATINGS IN SOME RELATED SPECIES OF MUCOR AND BACKUSELLA

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Zygospores resulting from intraspecific matings of *Mucor amphibiorum*, *M. inaequisporus*, *M. indicus*, *M. recurvus*, *M. variosporus*, *Backusella circina*, and *B. lamprospora* are compared with azygospores (zygospores) formed in matings of *Mucor amphibiorum* strain CBS 764.74 and strains of the other species by means of scanning electron microscopy. In general zygospores in interspecific matings cease to develop at an earlier stage than those of intraspecific matings. No proof could be obtained for our hypothesis that *M. amphibiorum* strain CBS 764.74 merely initiated a process of azygospore formation.

Sexuality in the Mucorales is based on the interaction of (+) and (−) thalli in heterothallic species, and on the interaction of (+) and (−) parts of the same thallus in homothallic species.

Sex specific substances are secreted by both (+) and (−), resulting in the production of sex hormones, trisporic acids, which initiate the formation of zygophores and probably also the mutual attraction prior to wall fusion and production of progametangia and gametangia. These first stages in the sexual process merely indicate that (+) and (−) interact hormonally; the reaction is mating type specific and can be induced by (+) and (−) strains of different species.

The fusion of (+) and (−) gametangia, the first step toward actual zygospore formation, is found only in matings of compatible strains of the same species. Both partners are involved in the formation of zygospores. Failure to fuse is caused not only by interspecific incompatibility, but also by physiological and environmental conditions. Partners isolated from the same source are often more prolific than partners isolated from different areas.

Occasionally a single gametangium gives riso to an azygospore. Two gametangia may be present, but in the absence of lysis of the fusion wall, an azygospore may develop from one of the gametangia. Azygospores are known in both hetero- and homothallic species, usually arising spontaneously in association with matings, rarely following induction by specifically distinct partners. Some heterothallic species have a pronounced tendency towards azygospore production in intraspecific matings, e.g. *Mucor indicus* Lendn. and *M. hiemalis* Wehmer f. *silvaticus* (Hagem) Schipper. Ling Young (*1930*) obtained an increased number of azygospores in intraspecific matings by growing one partner on a favourable medium and the other on an unfavourable one. Cutter (*1942*) studied a strain of *Zygorhynchus moelleri* Vuill. var. *agamus* Namyslowski which very infrequently produced zygospores, the majority of the zygospore-like bodies being azygospores.

Strains which produce only azygospores have been reported infrequently. *Absidia spinosa* Lendn. var. *azygospora* Boed. is an azygosporic variety of the homothallic zygosporic species

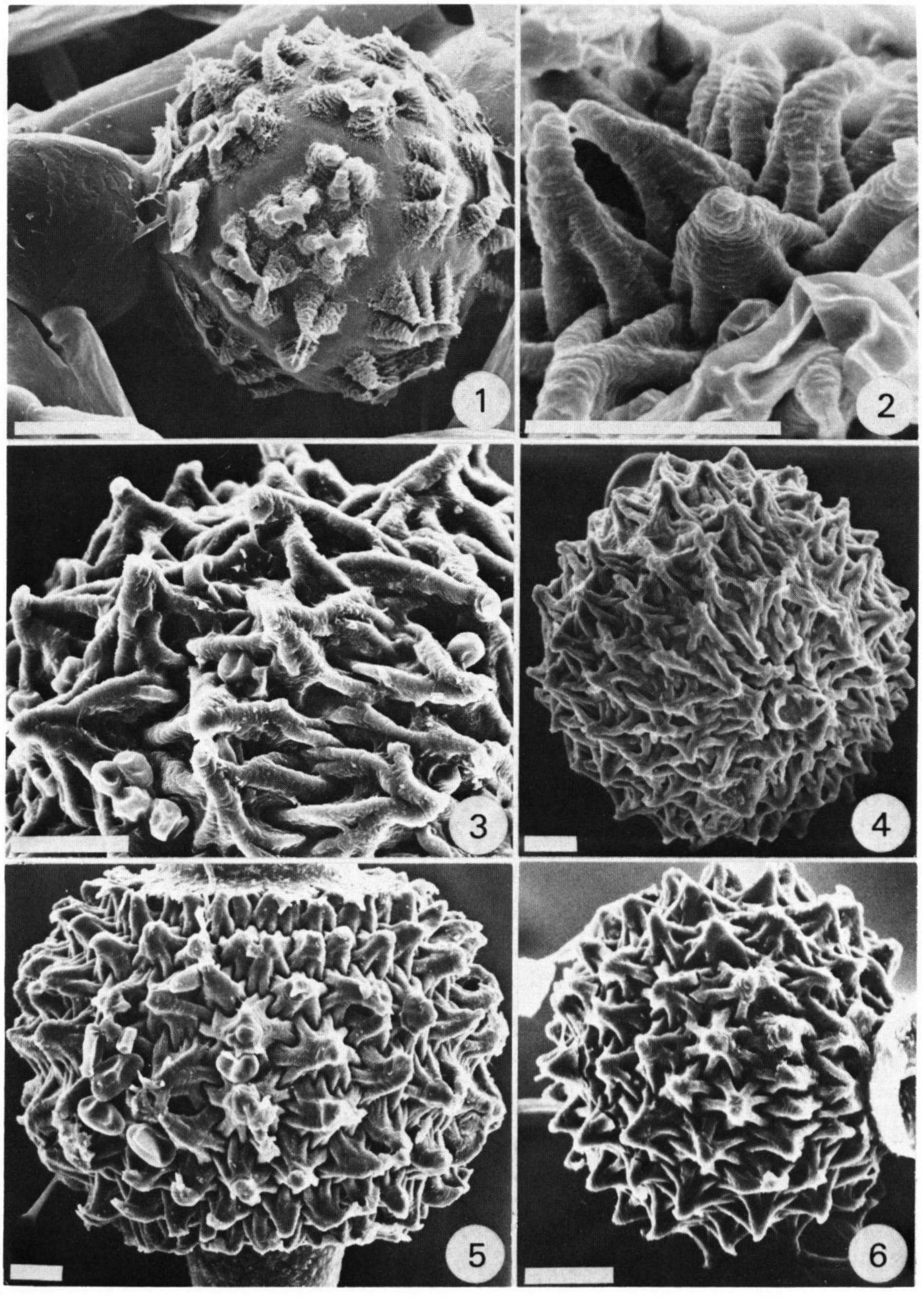
(Boedijn, *1958*). Certain heterothallic species have strains which produce only azygospores, e.g. *Mucor tenuis* Bainier (= *M. racemosus* Fres.) (Bainier, *1883*), *M. circinelloides* Tiegh. f. *azygosporus* Schipper, CBS 479.70 (Schipper, *1976*) and one strain of *Mucor indicus* Lendn., CBS 670.79, recently received for identification. '*M. globosus* Fischer, strain Naumov' was induced to produce azygospores when grown on meat peptone gelatin (Zach, *1935*). The latter strain was reported to have rough sporangiospores and is therefore probably a representative of *Mucor plumbeus* Bon. (with smooth columellae) or *M. fuscus* Bainier rather than of *M. globosus* (= *M. sphaerosporus* Hagem). Strain CBS 394.34 of *Rhizopus pseudochinensis* Yamazaki var. *thermosus* Takeda and CBS 344.29 of *R. pygmaeus* Naum. probably produced azygospores (as only one suspensor could be traced) in old slant cultures between the retracted agar and the glass. Azygospores could also be induced by inserting a slide at a sharp angle into the medium near the point of inoculation (cf. *Thermomucor* Subrahamanyam, Mehrotra & Thirum.; Schipper, *1979*). *Mucor bainieri* Mehrotra & Baijal, *in* Benjamin & Mehrotra, (*1963*), *M. azygospora* Benjamin, *in* Benjamin & Mehrotra (*1963*) and *M. ardhlaengiktus* Mehrotra & Mehrotra (*1979*) are obligate azygosporic species whose possible homo- or heterothallic counterparts are not known, although *M. ardhlaengiktus* is very similar to *M. variosporus* Schipper.

The occasional occurrence of azygospores in illegitimate contrasts was first observed by Blakeslee (*1915*). Schipper (*1978a*) found a great number of azygospores when testing interfertility among various species of *Mucor*. A few strains evoked reactions with strains of nearly all the species examined, resulting in the production of at least a few azygospores. These results motivated the present study.

The first question to be answered is which partner produces the azygospores. Is the common partner reacting to a stimulus received from the strain with opposite mating type, or are the azygospores produced by the various other strains, initiated by a stimulus from the common partner? Could both partners be reciprocally activated? When zygospores of the two partners in intraspecific matings are easily distinguished (e.g. roughened versus smooth walls, ridges versus warts) and the azygospores in interspecific contrasts show characters clearly pointing towards one of these types of zygospores, the question can be answered. Azygospores of undoubtful parentage have been induced in *Rhizomucor pusillus* (Lindt) Schipper (+) and (−) by both *Absidia corymbifera* (Cohn *in* Lichtheim) Sacc. & Trott. (Schipper, *1976*) and *A. blakesleeana* Lendn. (unpublished) of opposite mating type, in *Parasitella parasitica* (Bainier) Syd. (+) by *Mucor hiemalis* Wehmer f. *luteus* (Linnemann) Schipper (Schipper, *1978b*) and in *Gilbertella persicaria* (Eddy) Hesseltine (+) by *Rhizopus stolonifer* (Ehrenb. ex Fr.) Lind (−) (O'Donnell & *al.*, *1977*). When contrasting *Gilbertella persicaria* (−) and *Blakeslea trispora* Thaxt. (+), Hesseltine (*1960*) obtained zygospores similar to those of *Gilbertella* between parallel suspensors. If the *Gilbertella*-like zygospores were actually azygospores, then the parallel suspensors are quite extraordinary.

Unfortunately, zygospores of the *Mucor* species concerned are rather similar under the light microscope. The azygospores in the various contrasts display a wide variation in numbers and final degree of maturation. They may be rare, pale coloured and hardly ornamented when examined with the light microscope, but they can also be indistinguishable from mature

Figs. 1–4. *Mucor indicus* CBS 226.29 × CBS 422.71. — Figs. 5–6. *Mucor indicus* CBS 480.70 × CBS 423.71.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zygospores formed in intraspecific matings. Schipper *& al.* (*1975*) demonstrated that the ornamentation of zygospores from separate species of the genus *Mucor* shows a wide variation when examined with the scanning electron microscope (SEM).

The aim of the present study is to find out whether the ornamentation of the azygospores formed in interspecific contrasts of species of *Mucor* shows a distinct tendency toward the typical (species) ornamentation of one of the individual partners or whether the ornamentation is intermediate.

Zygospores and azygospores of the matings indicated in Table I were examined with the SEM (1) to gain an insight into the variation in ornamentation of the zygospore wall in each of the species concerned, and (2) to establish any partner resemblance of azygospores from interspecific contrasts.

Materials and methods

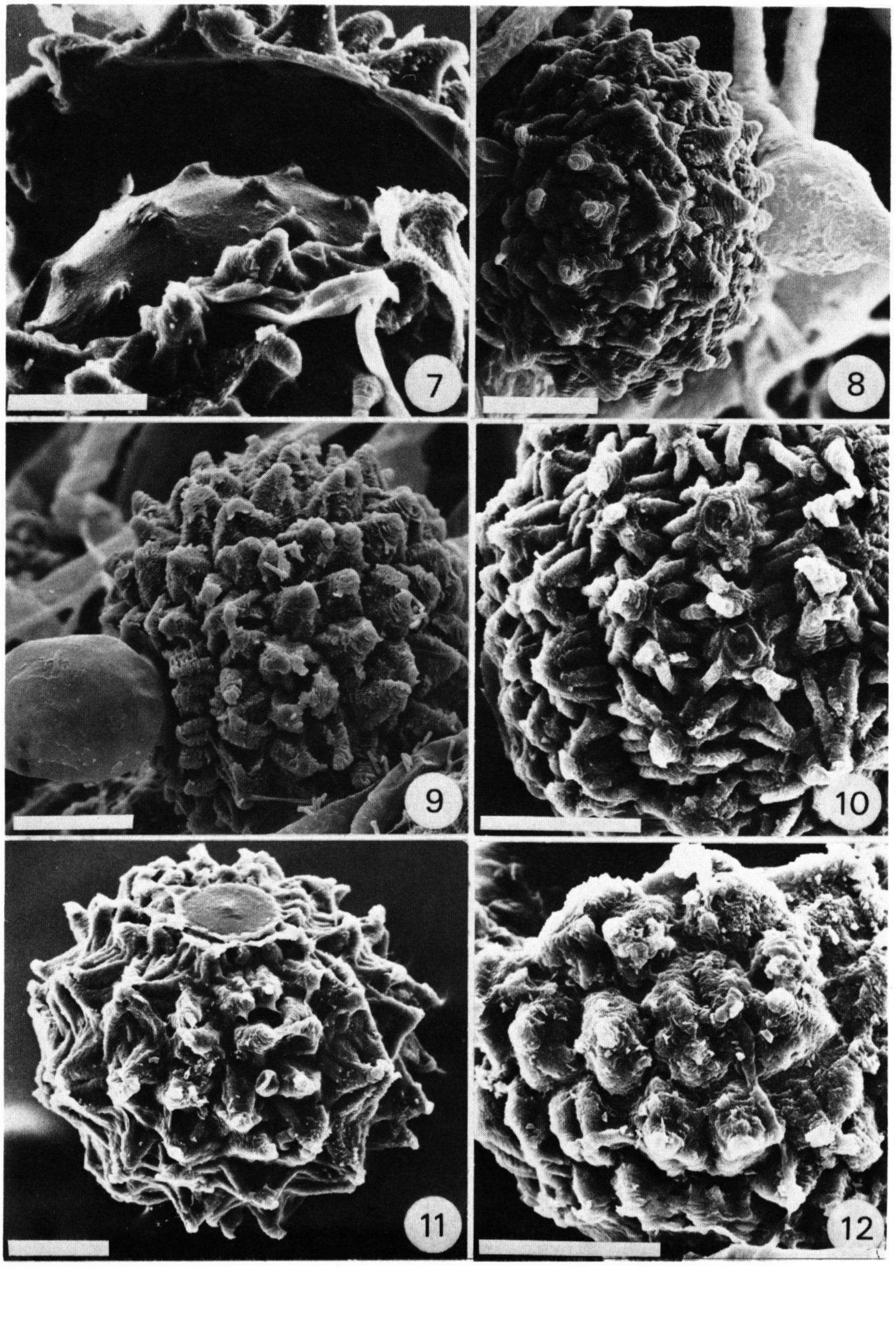
The following strains of fungi were used during this study.—
Mucor amphibiorum Schipper (+): CBS 763.74; CBS 764.74;
(−): CBS 185.77.
Mucor inaequisporus Dade (+): CBS 496.66;
(−): CBS 255.36; CBS 351.50; CBS 497.66.
Mucor indicus Lendner (+): CBS 120.08; CBS 226.29; CBS 480.70;
(−): CBS 422.71; CBS 423.71; CBS 424.71.
Mucor recurvus Butler (+): CBS 318.52; CBS 195.71;
(−): CBS 992.70; CBS 196.71; CBS 673.75.
Mucor variosporus Schipper (+): CBS 651.78; CBS 652.78;
(−): CBS 837.70; CBS 650.78; CBS 654.78.
Backusella circina Ellis & Hesseltine (+): CBS 323.69; CBS 128.70
(−): CBS 322.69; CBS 129.70
Backusella lamprospora (Lendn.) Benny & Benjamin (+): CBS 195.28;
(−): CBS 196.28; CBS 850.71.

Zygospores and azygospores, obtained by mating on beerwort agar at 24 °C, were transferred to specimen studs covered with double-sided adhesive tape and air-dried. The specimens were coated with gold in a sputter coater for 3.5 min. at 1.2 KV and examined with a Leitz AMR 100A scanning electron microscope.

Results

Seen under the light microscope, the zygospores of *Mucor amphibiorum*, *M. indicus*, *M. inaequisporus*, *M. variosporus*, *M. recurvus* and also of the related *Backusella circina* and *B. lamprospora* are rather similar. In each species the zygospores are black or brownish black,

Fig. 7. *Mucor indicus* CBS 120.08 × CBS 423.71. — Figs. 8–10. *Mucor amphibiorum* CBS 763.74 × CBS 185.77. — Fig. 11. *Mucor amphibiorum* CBS 764.74 × *Mucor indicus* CBS 423.71. — Fig. 12. *Mucor amphibiorum* CBS 764.74 × *Mucor indicus* CBS 422.71.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stellate and of about the same size (up to 80–100 µm in diam., except those of *M. recurvus*, which are up to 160 µm in diam). Under a light microscope the deeply darkened mature zygospore wall disguises any later stages of ornamentation. SEM observations revealed that breakage of the gametangial wall follows a general pattern in all species. The development of this pattern, however, may stop at any stage, usually depending on the species.

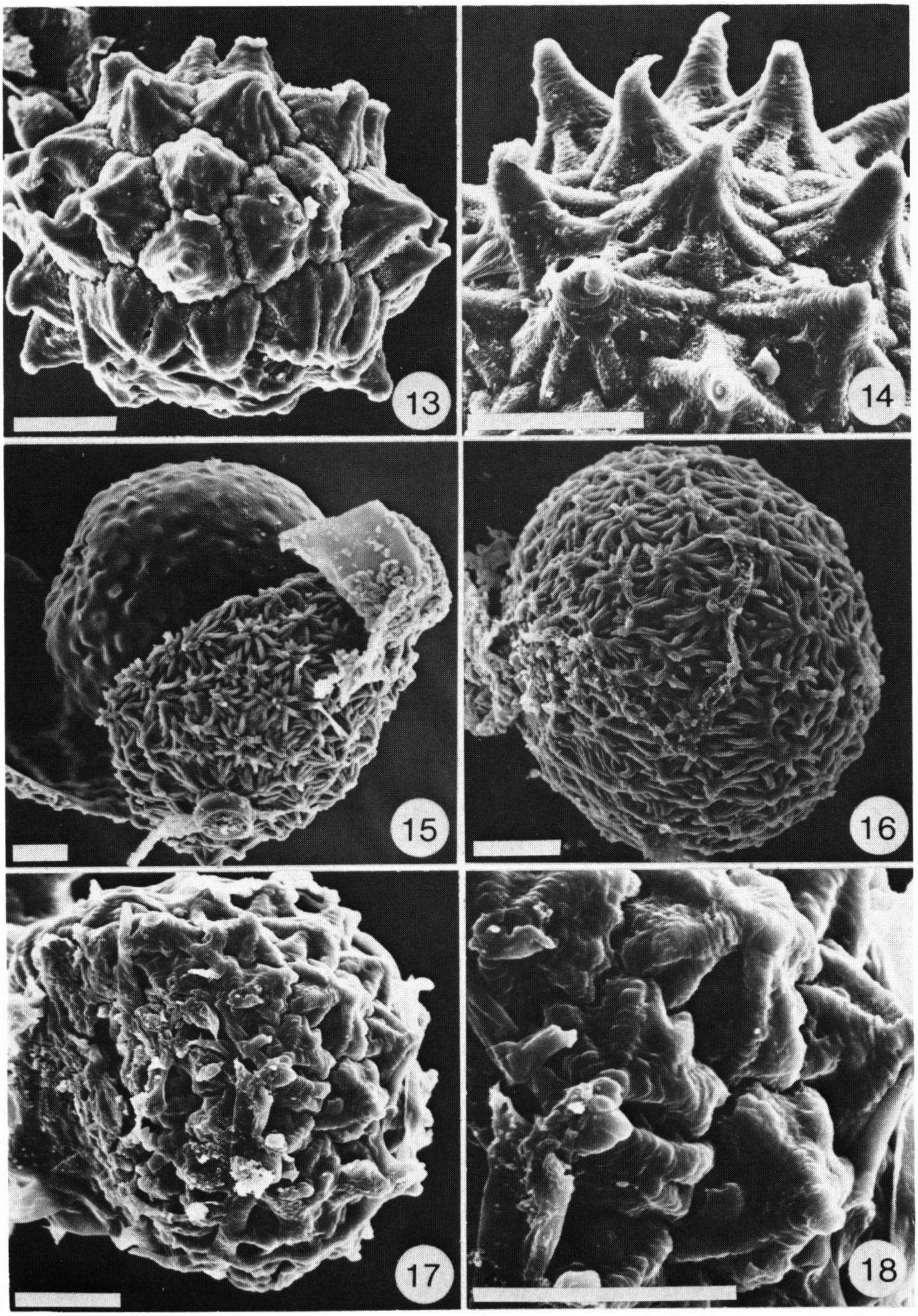
TABLE I

Survey of contrasts yielding sufficiently developed zygospores

+		M. amphibiorum 185.77	M. inaequisporus 255.36	351.50	497.66	M. indicus 422.71	423.71	424.71	M. recurvus 992.70	196.71	673.75	M. variosporus 837.70	650.78	654.78	B. circina 322.69	129.70	B. lamprospora 196.28	850.71
M. amphibiorum	763.74	O																
	764.74	O		X	X	X	X				X	X			X		X	
M. inaequisporus	496.66		O	O	O													
M. indicus	128.08					O	O	O										
	226.29					O	O											
	480.70					O	O	O										
M. recurvus	318.52								O									
	195.71								O	O	O							
M. variosporus	651.78											O	O	O				
	652.78											O						
B. circina	323.69															O		
	128.70														O	O		
B. lamprospora	195.28																	O

O intraspecific X interspecific

Figs. 13–16. *Mucor variosporus*. — Fig. 13. CBS 651.78 × CBS 654.78. — Fig. 14. CBS 651.78 × CBS 650.78. — Figs. 15–16. CBS 652.78 × CBS 837.70. — Figs. 17–18. *Mucor amphibiorum* CBS 764.74 × *M. variosporus* CBS 837.70.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After gametangial fusion a new zygospore wall is formed against the gametangial wall, subsequently breaking through at various places to form wart-like projections (Fig. 1). At first these warts may be more or less regularly grouped and often still connected by remnants of the old gametangial wall (Figs. 2, 8, 19), or they are isolated and regularly distributed from the very start (Figs. 30, 33), but they always become more or less regularly distributed. In this early stage the ornamentation is most spiny, i.e. here the distance from the apex to the base of the ornamentation is maximal. The warts (or spines) now start to split from the base toward the apex, resulting in 4–5 'arms', which interlock at the base (Figs. 3, 4). The division is never complete, always ending some distance from the top. Each arm may redivide, again from the base toward the top (Figs. 10, 29), though generally for a shorter distance and not through the whole width, being visible as a mere groove (Fig. 6). As the zygospore increases in size, the ornamentation becomes flattened (Figs. 16, 36), sometimes seeming nearly smooth when seen under the light microscope.

There is much variation in the final appearance of mature zygospores of *Mucor* and related genera, due to the fact that the development may stop at any stage. Moreover there is considerable variation in the degree of splitting, the length, and the length-width ratio of the arms. Within a single mating and generally within a species (exceptions will be discussed) the final stage is more or less similar, although this is not true for each individual zygospore; it is necessary to examine several zygospores of various ages. The appearance and variation of the various species is given below, followed by comparisons with interspecifically formed zygospores and azygospores of the species with *Mucor amphibiorum* CBS 764.74.

Contrasts which yielded insufficiently developed zygospores or azygospores have been omitted.

(1) *Mucor indicus* (Figs. 1–7).

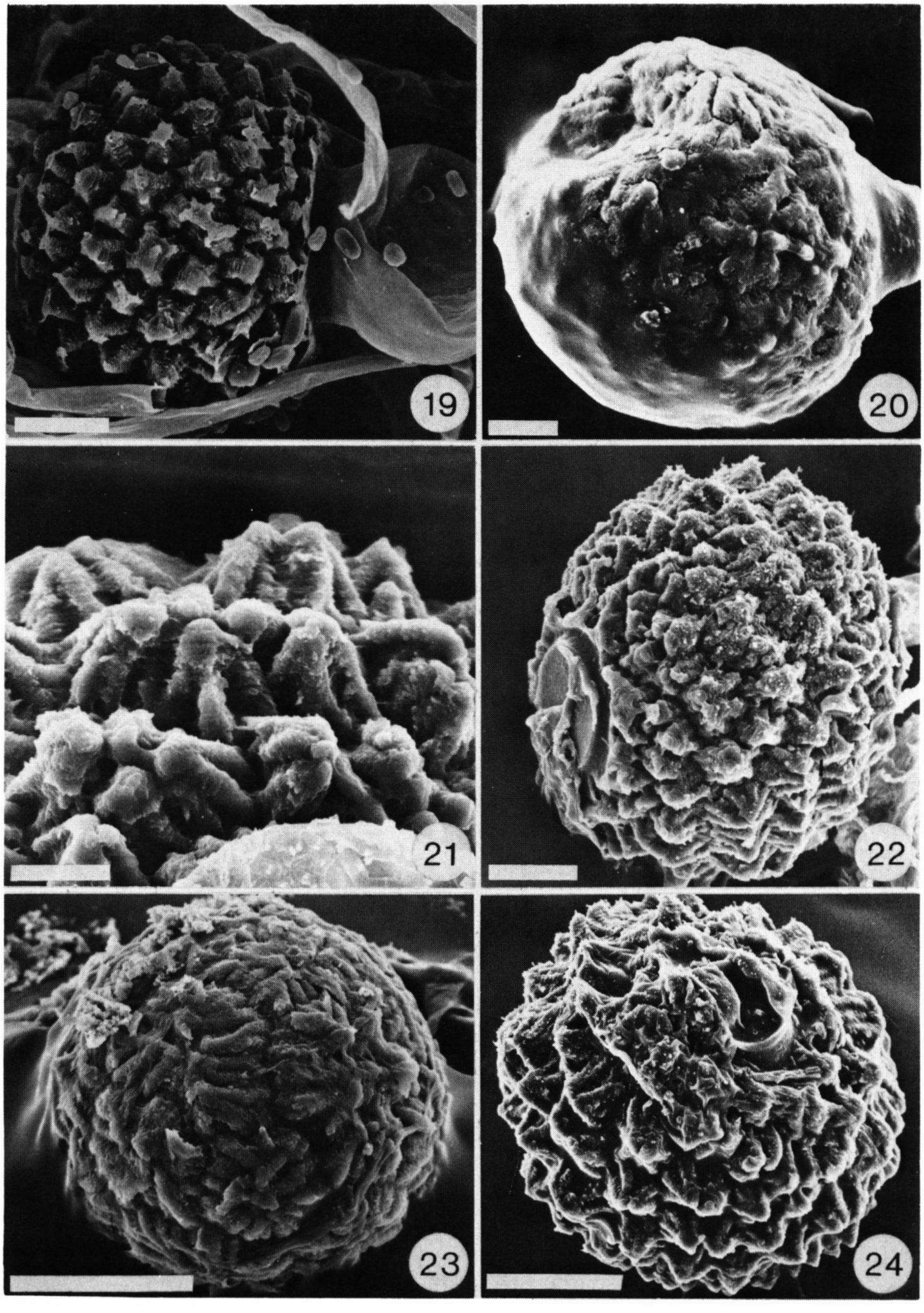
Young zygospores are stellate, warts (spines) 5–7 μm long. The ornamentation generally becomes gradually flattened, the individual arms reach up to 10 × 3 μm but are typically shorter. When well developed the zygospores ought to be classified in group A1 of Schipper *et al.* (*1975*) and not in group A2 as stated there (based on rather young zygospores). Nearly all matings examined finally showed this type of ornamentation, although the more spiny type sometimes remained dominant. Rarely flattened zygospores are found with the appearance of *Mucor recurvus* (or *Backusella lamprospora*) zygospores.

Matings with *M. amphibiorum* CBS 764.74 (Figs. 11–12) yield azygospores (zygospores) more closely resembling those of *M. indicus* than those of *M. amphibiorum*, with the restriction that the final stage with long, slender, separate arms is not found. The development stops at or before the more spiny phase. There are no warts with 8–10 arms.

(2) *Mucor amphibiorum* (Figs. 8–10).

Young zygospores are bluntly stellate, warts up to 4 μm long. Warts soon become more flattened and typically develop 8–10 arms of equal length (the primary and secondary splitting is often indistinguishable when the zygospore is mature). The arms remain rather compactly arranged and are up to about 4 × 1 μm. Sometimes warts are concrescent.

Figs. 19–22. *Mucor inaequisporus* CBS 496.66 × CBS 497.66. — Figs. 23–24. *Mucor amphibiorum* × *Mucor inaequisporus*. — Fig. 23. CBS 764.74 × CBS 351.50. — Fig. 24. CBS 764.74 × CBS 497.66.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There is some variation: matings with CBS 763.74 always show 8–10 more or less equal arms, while matings with CBS 764.74 may show dominant unequal splitting.

(3) *Mucor variosporus* (Figs. 13–16).

Young zygospores are stellate, warts up to 10 μm long, but sometimes considerably shorter (up to 6 μm). In all matings with CBS 651.78 this is the final stage; the warts may become somewhat flattened and blunt, but there are no deeply divided, slender arms. All other matings yielded zygospores of which the ornamentation became flattened and the arms long and slender (up to 5 × 1 μm).

Matings with *M. amphibiorum* CBS 764.74 (Figs. 17–18) resulted in the production of azygospores (zygospores) most like those of *M. variosporus*, and resembling the interspecifically formed azygospores (zygospores) of *M. indicus*.

(4) *Mucor inaequisporus* (Figs. 19–22).

The development of the zygospores stops at an early stage. The ornamentation is generally bluntly warted; warts up to 3 μm long, often without any sign of splitting, rarely stellate and basally split.

Mucor amphibiorum CBS 764.74 × *M. inaequisporus* CBS 351.50 (Figs. 23–24) yielded zygospores with blunt, very low warts with 8–10 arms. *Mucor amphibiorum* CBS 764.74 × *M. inaequisporus* CBS 497.66 produced azygospores (zygospores) with a very low and hardly differentiated ornamentation, though 8–10 arms could just be discerned.

(5) *Backusella circina* (Figs. 25–27).

Young zygospores have blunt, conical warts up to 6 μm long. These warts split into 4–5 arms, which usually redivide. There is normally little flattening, the arms often do not separate and are about 4–6 × 1.5 μm.

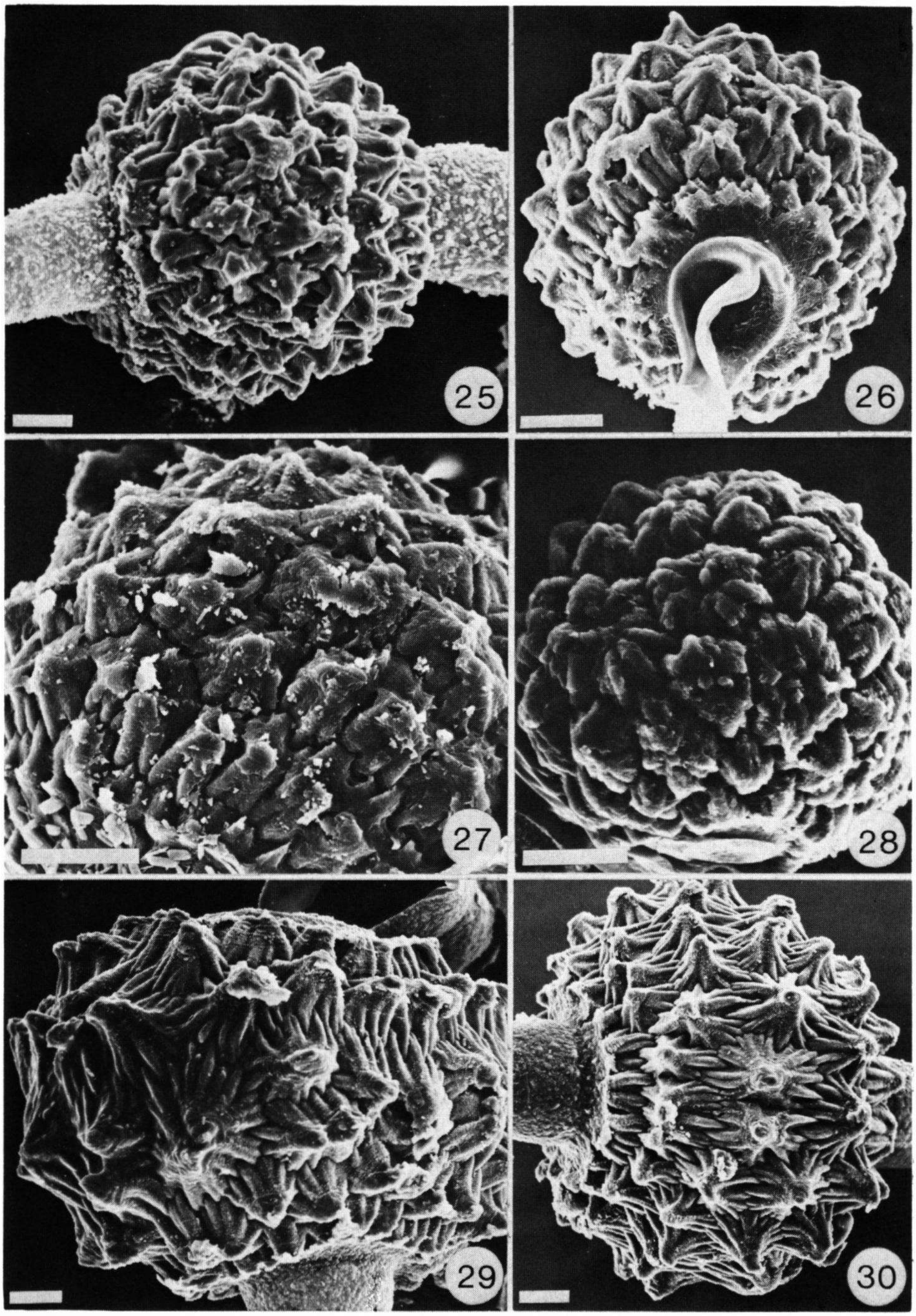
Azygospores (zygospores) produced in matings with *M. amphibiorum* CBS 764.74 (Fig. 28) develop warts with 4–5 arms, but warts with 8–10 arms are also found. The ornamentation remains low.

(6) *Backusella lamprospora* (Figs. 29–31).

The zygospore is slightly flattened between the broad suspensors. Warts are few and generally split to form 8–10(–12) about equal arms, measuring 6–10 × 1.5 μm (although zygospores with unequal arms are always found). Old zygospores may demonstrate a disrupted ornamentation with extremely long arms, caused by the increase in volume of the zygospore (and the scarcity of the warts).

Matings with *M. amphibiorum* CBS 764.74 (Fig. 32) yield azygospores (zygospores) with a low and sometimes indistinct ornamentation. The warts typically develop 4–5 arms, though warts with 8–10 arms are occasionally found.

Figs. 25–27. *Backusella circina*. — Fig. 25. CBS 128.70 × CBS 322.69. — Figs. 26–27. CBS 128.70 × CBS 129.70. — Fig. 28. *Mucor amphibiorum* CBS 764.74 × *Backusella circina* CBS 327.69. — Figs. 29–30. *Backusella lamprospora* CBS 195.28 × CBS 850.71.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(7) *Mucor recurvus* (Figs. 33–36).

The zygospores of *M. recurvus* are comparable to those of *B. lamprospora* in all respects. Azygospores (zygospores) produced in matings of *M. amphibiorum* CBS 764.74 with *M. recurvus* CBS 673.75 were rare and showed an indistinct ornamentation.

Discussion

Interspecific matings of *Mucor amphibiorum* CBS 764.74 with strains of the other species produced azygospores (zygospores) generally resembling zygospores formed in intraspecific matings of these species, but stopping at an earlier stage in the development. This probably indicates that CBS 764.74 merely initiated azygospore production, but did not actually make a genetic contribution.

Therefore, a few other attempts were made to identify the potential parent. These experiments which are briefly summarized below, remained negative.

(1) Strains separated by a cellophane membrane, a method used by Burgeff (*1924*), did not show any reactions.

(2) Single cultures of the species concerned were grown together with *Blakeslea trispora* (+) and (−). Abundant (smooth) zygospores were produced in *Blakeslea*, indicating the presence of trisporic acids in the medium. The only detectable effect on the third partner was an orange-yellow colouring of the contacting zone of the aerial mycelium.

(3) Matings of *Circinella umbellata* produce smooth-walled zygospores. Contrasts with the *Mucor* species concerned at best resulted in the production of an orange-yellow mycelium, except in that progametangia were produced in a contrast with *M. guilliermondii* Nodson & Phillippov.

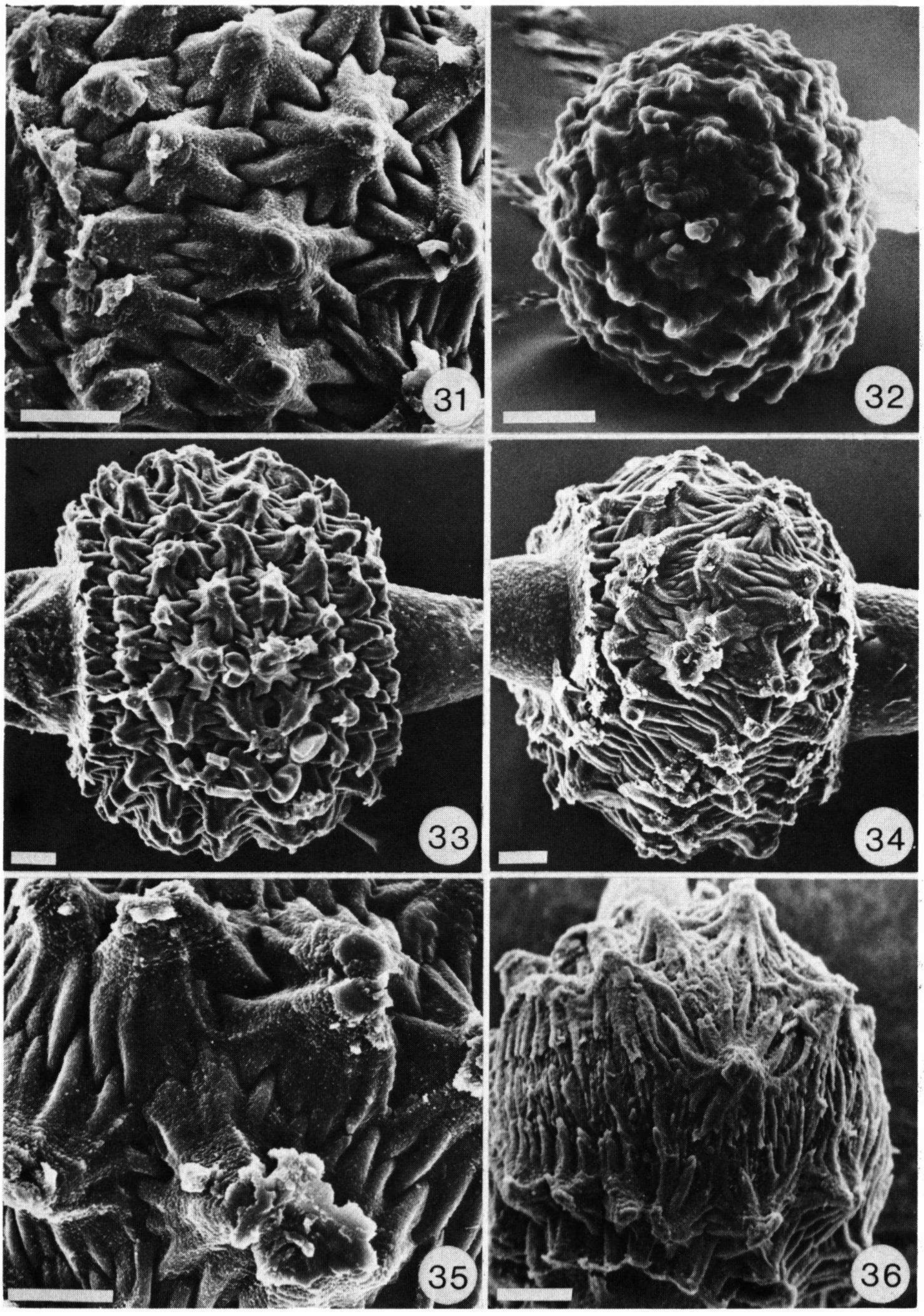
(4) *Mucor amphibiorum* CBS 764.74 (+) and *Backusella circina* CBS 322.69 (−), partners with a good mating potential, were used in a trial experiment, in which mating partners were roughly separated after two or three days.

To conclude, our results strongly suggest that *Mucor amphibiorum* CBS 764.74 induces azygospore formation in a number of species. This is an exceptional situation invoking many physiological questions, which cannot be answered without further study. Our results, combined with the occurrence of many other aberrations of patterns widely accepted as normal, tempted us to wider consideration (Schipper & Stalpers, *1980*), especially in the light of recent discoveries of mating type switching in yeasts.

Acknowledgement

The authors are grateful to Miss H. Pannebakker for printing the photographs.

Fig. 31. *Backusella lamprospora* CBS 195.28 × CBS 850.71. — Fig. 32. *Mucor amphibiorum* CBS 764.74 × *Backusella lamprospora* CBS 196.28. — Figs. 33–36. *Mucor recurvus*. — Fig. 33. CBS 195.71 × CBS 992.70. — Figs. 34–36. CBS 195.71 × CBS 196.71.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