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ZYGOSPORE ORNAMENTATION IN THE GENERA MUCOR AND ZYGORHYNCHUS

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(With Plates 51–57, one Table, and one Text-figure)

Zygospores of species of the genera *Mucor* and *Zygorhynchus* were studied by means of scanning electron microscopy. The types of ornamentation are described and compared with those of some species of other genera of the Mucorales. The possibility of grouping the species according to zygospore characters is discussed.

INTRODUCTION

Zygospores are an important criterium in the taxonomy of the Mucorales. If mating partners are available and the suitable conditions are known, zygospores of most *Mucor* species can be obtained in 4–7 days on one of the ordinary media. The size, shape, colour and a rough estimate of the ornamentation of the wall of the zygospores can be easily determined by light microscopy (LM) under low magnification. A detailed examination of the ornamentation by means of LM, however, is difficult because of the heavy pigmentation and thickness of the zygospore wall.

Hawker & Gooday (1968) and Hawker & Beckett (1971) studied the zygospore wall structure of *Rhizopus sexualis* by means of transmission electron microscopy (TEM). The zygospore wall is formed by deposition of material inside the original gametangium wall, which remains as torn fragments on the outside of the mature zygospores. There are two thick layers: an outer electron dense warty coating and an inner electron transparent layer, separated by a thin 'smoothing' layer. Hawker & Gooday (1968) also provided some micrographs showing the ornamentation of *Rhizopus sexualis* as seen by means of scanning electron microscopy (SEM), but no further research has been done on this subject.

The purpose of this paper is to compare the wall ornamentation of mature zygospores of all available *Mucor* species known to produce them. The zygospores of *M. plasmaticus*, recently reported by Pidoplichko & Milko (1971), were not available for study. Zygospores of the genus *Zygorhynchus* have also been included because of the close relationship between *Zygorhynchus* and *Mucor*. In addition, zygospores of some representatives of other, more or less related members of the Mucorales are compared. In some cases azygospores were also examined.

The SEM micrographs, the descriptions of zygospore ornamentation, the grouping of the species after this character, and the comparison with other genera were done by Samson and Stalpers, while Schipper provided the zygospores and gave the taxonomical comment on the grouping of the *Mucor* and *Zygorhynchus* species.

MATERIALS AND METHODS

Zygospores were obtained in matings of the tester pairs of the species as indicated by Schipper (1973, 1975) in petridishes under conditions favourable for zygospore production in the species.

STRAINS EXAMINED:
Mucor alpinus CBS 105.10
Mucor aromaticus CBS 195.71 (+) × 196.71 (—)
Mucor azygosporus CBS 292.61
Mucor bacilliformis CBS 251.53
Mucor bainieri CBS 293.63
Mucor circinelloides CBS 192.68 (+) × 394.68 (—); strain Hadlok 84 (+) × Hadlok 46 (—); CBS 192.68 (+) × 222.28 (—); CBS 479.70 (azygosporous strain)
Mucor flavus CBS 235.35 (+) × 234.35 (—)
Mucor genevensis CBS 144.08
Mucor griseocyanus f. *griseocyanus* CBS 223.56 (+) × 907.69 (—)
Mucor griseocyanus f. *jansenii* CBS 205.68 (+) × 204.68 (—)
Mucor griseocyanus f. *griseocyanus* CBS 907.69 (—) × f. *jansenii* CBS 205.68 (+)
Mucor hiemalis f. *hiemalis* CBS 200.28 (+) × 201.65 (—); CBS 972.68 (+) × 971.68 (—); CBS 978.68 (+) × 978.68A (—); CBS 975.68 (+) × 975.68 A (—)
Mucor hiemalis f. *luteus* CBS 244.35 (+) × 243.35 (—)
Mucor hiemalis f. *silvaticus* CBS 250.35 (+) × 249.35 (—)
Mucor hiemalis f. *corticolus* CBS 366.68 (+) × 365.68 (—)
Mucor inaequisporus CBS 496.66 (+) × 497.66 (—)
Mucor 'indicae-seudaticae' CBS 104.75
Mucor lamprosporus CBS 195.28 (+) × 196.28 (—)
Mucor lusitanicus CBS 277.49 (+) × 276.49 (—)
Mucor miehei CBS 282.68; CBS 182.67
Mucor mucedo CBS 144.24 (+) × 109.16 (—)
Mucor piriformis CBS 225.29 (+) × 169.25 (—)
Mucor pusillus CBS 253.53 (+) × 354.68 (—); CBS 245.58 (homothallic strain)
Mucor plumbeus CBS 213.75 (+) × 848.72 B (—); 210.75 (+) × 246.58 (—)
Mucor racemosus f. *racemosus* CBS 124.23 (+) × 260.68 (—)
Mucor racemosus f. *sphaerosporus* CBS 115.08 (+) × 238.35 (—)
Mucor racemosus f. *racemosus* CBS 260.68 (+) × f. *sphaerosporus* CBS 115.08 (—)
Mucor saturninus CBS 974.68 (+) × CBS 137.40 (—); CBS 637.65 (+) × 521.64 (—)
Mucor rouxii CBS 226.29 (+) × CBS 422.71 (—)
Zygorhynchus californiensis CBS 402.58
Zygorhynchus exponens var. *exponens* CBS 403.58
Zygorhynchus exponens var. *smithii* CBS 404.58
Zygorhynchus heterogamus CBS 338.70
Zygorhynchus japonicus CBS 254.69
Zygorhynchus macrocarpus CBS 215.57
Zygorhynchus moelleri CBS 406.58, CBS 501.66
Zygorhynchus psychrophilus CBS 336.68

Absidia spinosa CBS 106.08
Backusella circina CBS 128.70 (+) × 129.70 (—)
Gongronella butleri CBS 227.36 (+) × 226.36 (—)
Parasitella simplex CBS 412.66 (+) × 208.28 (—)
Phycomyces blakesleeanus CBS 283.35 (+) × 284.35 (—)
Pirella circinans CBS 306.29 (+) × 367.59 (—)
Rhizopus sexualis CBS 336.62
Syzygites megalocarpus CBS 372.29.

Zygospores, usually from 7 days old cultures, were transferred to squares of double-sided adhesive tape, attached to specimen stubs for the SEM and air-dried for 24 hours. The specimens were coated with gold in a sputter coater for 2 minutes at 1.2 mV. Preparations were examined with a Cambridge Stereoscan microscope at an accelerating voltage of 10 kV.

RESULTS AND DISCUSSION

TEM studies by Hawker & Gooday (1968) and Hawker & Beckett (1971) showed, that *de novo* wall formation in zygospores takes place along the inside wall of the cell after gametangial fusion combined with an increase in volume. From the present SEM studies it was concluded, that the new wall pierces the gametangial wall at various places simultaneously. Fragments of the gametangial wall can be seen at the most extended points of the ornamentation and around the suspensors (Pl. 55 fig. 27). The thick warty coating appears to be easily separable from the zygospore; gentle squashing or manipulation with a pair of needles is sometimes sufficient to remove this layer, e.g. *Mucor lusitanicus* (pl. 51 fig. 1). In contrast with *Rhizopus sexualis* (Hawker & Gooday 1968; Hawker & Beckett, 1971), the then exposed layer is nearly smooth and does not follow the shape of the warty layer.

The warts can remain rather flat and irregular in shape or in other species they enlarge to become more or less conical (Fig. 1, diagram). These conical warts have

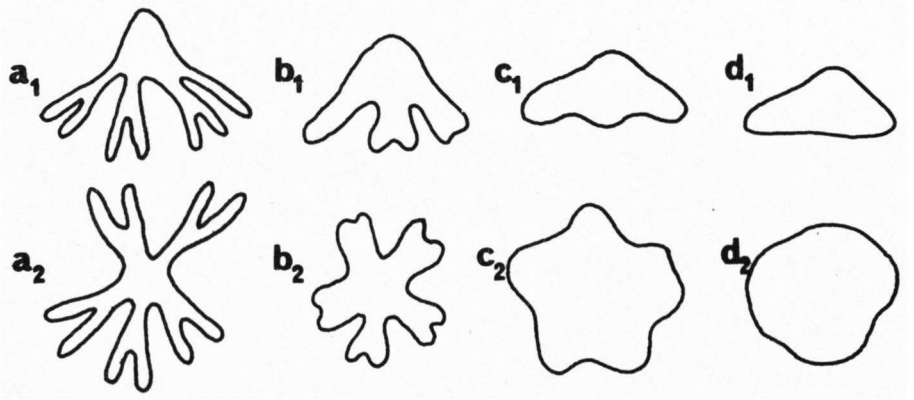

Fig. 1. Diagram of the ornamentation types in the different groups: a. Group A1; b. Group A2; c. Group B; d. Group C. (a_1–d_1 = sideview, a_2–d_2 = surface view).

the appearance of a starfish (indicated as stellate in LM descriptions); they consist of a raised central region and a number of arms, usually 4 to 5, but sometimes up to 8 or 10. The arms may interlock with those of the neighbouring warts and they may be bifurcately branched at their terminal ends. In some cases the arms tend to be free at the central region, giving the zygospore a spiny appearance (Pl. 52 fig. 10, 12), whilst in other cases arms are not actually formed, but just indicated by ridges (Pl. 53 fig. 13, 16). Length/width ratio of the arms and their number are more or less uniform within a species, but highly variable in the different species. In ageing zygospores the conical starfish-like warts may become flattened because of the increase in volume of the zygospore (Pl. 51 fig. 2–3). This feature is particularly developed in group A1.

In the species with starfish-like ornamentation the type of ornamentation is fairly constant, but in some species with irregular warts there is sometimes a tendency towards starfish-like ornamentation. This, for instance, is the case in *Mucor saturninus* (Pl. 54 fig. 24).

Three main types can be recognized in *Mucor* and *Zygorhynchus*; the delimitations, however, are not sharp and some species may eventually be placed in two groups.

GROUP A.—Zygospores covered with distinct starfish-like ornamentation which may become spiny.

1. Arms of ornamentation long and narrow, length/width=2.5–6. Warts never completely split up (Pl. 51 figs. 2–6; Pl. 52 figs. 7, 10).

Mucor aromaticus, M. bainieri, M. circinelloides, M. griseocyanus, M. lusitanicus.

2. Arms of ornamentation rather plump, length/width=1–2.5. Zygospores sometimes spiny (Pl. 52 figs. 8, 9, 11, 12; Pl. 53 figs. 13–18; Pl. 54 figs. 19–23).

Mucor azygosporus, M. bacilliformis, M. inaequisporus, M. rouxii, M. lamprosporus, M. plumbeus, M. racemosus, Zygorhynchus californiensis, Z. exponens, Z. heterogamus, Z. japonicus, Z. macrocarpus, Z. moelleri, Z. psychrophilus.

GROUP B.—Zygospores completely or at most places covered with an irregular, warty ornamentation; rarely with a tendency towards a starfishlike ornamentation.

1. Zygospores large, up to 180–290 μm in diameter. (Pl. 54 fig. 24; Pl. 55 figs. 25–27).

Mucor flavus, M. mucedo, M. piriformis, M. saturninus.

2. Zygospores smaller, up to 110 μm in diameter (Pl. 55 figs. 28–30; Pl. 56 figs. 31, 32).

Mucor genevensis, M. hiemalis f. *corticolus, M. hiemalis* f. *hiemalis, M. hiemalis* f. *luteus, M. hiemalis* f. *silvaticus.*

GROUP C.—Zygospores covered with distinct and separated warts. (Pl. 56 figs. 33, 34).

Mucor miehei, M. pusillus.

COMPARISON OF THE GROUPING ACCORDING TO THE ZYGOSPORE SCULPTURE WITH THAT BASED ON OTHER MORPHOLOGICAL AND INTERFERTILITY CRITERIA

GROUP A1—*Mucor circinelloides*, *M. griseocyanus*, *M. lusitanicus*, and *M. bainieri* form a natural group in view of general morphology. Interspecific mating of the heterothallic species may result in the production of zygospores. *Mucor aromaticus* differs from these species by the large dimensions of its sporogenous structures and by the colour and size of the zygospores which are produced high in the aerial mycelium. In some matings between strains of *M. griseocyanus* zygospores were observed resembling those of group A2.

GROUP A2—Besides a number of *Mucor* species this group includes all species of *Zygorhynchus*. *Mucor inaequisporus*, *M. rouxii*, and *M. lamprosporus* are rather closely related in view of the results of interspecific matings. *Mucor plumbeus* and *M. racemosus* form another group of related species. *Mucor bacilliformis* is homothallic. *Zygorhynchus exponens* and *Z. heterogamus* show a similar ornamentation to that of *M. azygosporus*, which differs from the other species of this group by the formation of azygospores. The zygospores of *Z. moelleri* resemble those of *M. rouxii* in their spiny appearance, while those of *Z. japonicus* are reminiscent of *M. bacilliformis*. The ornamentation of the zygospores of *M. bacilliformis*, *M. inaequisporus*, and *M. lamprosporus* is sometimes variable and approaches that of group B.

GROUP B1—The species classified in this group have large sporangiophores and show optimum development at lower temperatures. *Mucor mucedo* and *M. piriformis* are closely related, whilst *M. saturninus* and *M. flavus* are also related but to a lesser extend. In view of their ornamentation *M. flavus* and *M. saturninus* also have affinities with members of group B2. The taxonomy of the species of this group has been discussed in detail by Schipper (1975).

GROUP B2—The species are closely related and have been revised by Schipper (1973). A similar type of ornamentation is observed in CBS 109.10, deposited as *M. alpinus* Hansen.

GROUP C.—*Mucor pusillus* and *M. miehei* form a natural group and can be refered to as *Rhizomucor* sensu Hesseltine & Ellis (1973), a name published by Lucet & Costantin (1900) as a section of *Mucor*. The zygospores are small, up to 75 μm in diameter and only produced at temperatures between 30 and 40 °C. The ornamentation of the zygospores of *M. miehei* somewhat resembles that of *M. hiemalis*.

COMPARISON WITH OTHER GENERA

For reasons of comparison representatives of several other genera of Mucorales have been examined on zygospore ornamentation. The zygospores of *Absidia spinosa* Lendner, *Phycomyces blakesleeanus* Burgeff (Pl. 56 fig. 35) and *Syzygites megalocarpus* Ehrenb. ex Fr. (Pl. 56 fig. 36) have an ornamentation not comparable to that

Table 1. Zygospore characters of *Mucor* and *Zygorhynchus* species (mainly after Schipper, 1969, 1973, 1975, and Hesseltine & *al.*, 1959).

Name	Sexual reproduction	Max. diam.	Pigmentation	Group	Fig.
M. aromaticus Povah	het.	160 µm	black	A1	8
M. azygosporus R. K. Benjamin	azy.	180 µm	black	A2	15
M. bacilliformis Hesseltine	hom.	72 µm	black	A2	13
M. bainieri Mehrotra & Baijal	azy.	120 µm	rbr.–dbr.	A1	6
M. circinelloides Tieghem	het., azy	100 µm	rbr.–dbr.	A1	5
M. flavus Bain.	het.	180 µm	black	B1	27
M. genevensis Lendner	hom.	100 µm	brown–dbr.	B2	31
M. griseocyanus Hagem	het.	80 µm	rbr.	A1	7
M. hiemalis Wehmer	het.	100 µm	br.–black	B2	28–30, 32
M. inaequisporus Dade	het.	100 µm	black	A2	18
M. lamprosporus Lendner	het.	75 µm	brown	A2	17
M. lusitanicus Bruderlein	het.	115 µm	rbr.–dbr.	A1	1–4
M. miehei Cooney & Emerson	hom.	50 µm	rbr.–dbr.	C	34
M. mucedo L. ex Fres.	het.	250 µm	black	B1	25
M. plumbeus Bon.	het.	75 µm	dbr.	A2	14
M. piriformis Fischer	het.	240 µm	black	B1	26
M. pusillus Lindt	het.,hom.	70 µm	dbr.	C	33
M. racemosus Fres.	het.	110 µm	rbr.	A2	16
M. rouxii (Calmette) Wehmer	het.	100 µm	black	A2	12
M. saturninus Hagem	het.	180 µm	black	B1	24
Z. californiensis Hesseltine & *al.*	hom.	47 µm	brown–dbr.	A2	11
Z. exponens Burgeff	hom.	80 µm	rbr.–dbr.	A2	21
Z. exponens var. *smithii* Hesseltine & *al.*	hom.	62 µm	red–brbl.	A2	20
Z. heterogamus (Vuill.) Vuill.	hom.	70 µm	dbr.–black	A2	9
Z. japonicus Kominami	hom.	80 µm	brown–black	A2	22
Z. macrocarpus Ling	hom.	100 µm	brown–black	A2	19
Z. moelleri Vuill.	hom.	56 µm	brown–dbr.	A2	10
Z. psychrophilus Schipper & Hintikka	hom.	100 µm	black	A2	23

ABBREVIATIONS: hom. = homothallic; het. = heterothallic; azy. = azygosporous; brbl. = brownish black; dbr. = dark brown; rbr. = red-brown.

of *Mucor*; in addition the zygospores are enveloped by appendages arising from the suspensors (*Absidia, Phycomyces*) or by repeated ramification of zygophore branches (*Syzygites*). *Backusella circina* Ellis & Hesseltine (= *Mucor pseudolamprosporus* Naganashi) (Pl. 57 fig. 37) and *Rhizopus sexualis* (G. Smith) Callen (Pl. 57 fig. 38) have zygospores of the type of group A2. The zygospores of *Pirella circinans Bain.* (Pl. 57 fig. 39) closely resemble those of *Mucor mucedo* (group B1), except for the fact that they are much smaller (maximum diameter 140 µm). When well developed the ornamentation of the zygospores of *Parasitella simplex* Bain. (Pl. 57 fig. 40) is comparable with that of the species placed in group A2, but most zygospores have the type of ornamentation found in group B2. *Gongronella butleri* (Lendner) Peyronel & Dal Vesco (Pl. 57 fig. 41)

has regularly spiny zygospores which can not adequately be placed in one of the afore mentioned groups.

An undescribed thermophilic strain, sent to the CBS by Dr. M. J. Thirumalachar and tentatively named *Mucor indicae-seudaticae*, produced smooth-walled, brown zygospores (Pl. 57 fig. 42). Since zygospores and other morphological characters are not *Mucor*-like the classification of the species in a separate genus is advisable.

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EXPLANATION OF PLATES 51–57

Scale as indicated represents 10 μm, except in Figs. 35 and 36, 100 μm.

PLATE 51

Figs. 1–4. *Mucor lusitanicus*, CBS 277.49 ×276.49; Fig. 1. zygospore with partly removed outer wall; Figs. 2–4. different stages; Fig. 5. *M. circinelloides*, CBS 192.68 ×394.68; Fig. 6. *M. bainieri*, CBS 293.63.

PLATE 52

Fig. 7. *Mucor griseocyanus*, CBS 205.68 ×907.69; Fig. 8. *M. aromaticus*, CBS 196.71 ×196.71; Fig. 9. *Zygorhynchus heterogamus*, CBS 338. 74; Fig. 10. Z. *moelleri*, CBS 501.66; Fig. 11. *Z. californiensis*, CBS 402.58; Fig. 12. *M. rouxii*, CBS 226.29 ×422.71.

PLATE 53

Fig. 13. *Mucor bacilliformis*, CBS 251.52; Fig. 14. *M. plumbeus*, CBS 210.75 ×246.58; Fig. 15. *M. azygosporus*, CBS 292.61; Fig. 16. *M. racemosus*, CBS 260.68 ×115.08; Fig. 17. *M. lamprosporus*, CBS 195.28 ×196.28; Fig. 18. *M. inaequisporus*, CBS 496.66 ×497.66.

PLATE 54

Fig. 19. *Zygorhynchus macrocarpus*, CBS 215.57; Fig. 20. *Z. exponens* var. *smithii*, CBS 404.58; Fig. 21. *Z. exponens*, CBS 403.58; Fig. 22. *Z. japonicus*, CBS 254.69; Fig. 23. *Z. psychrophilus*, CBS 336.68; Fig. 24. *Mucor saturninus*, CBS 974.68 ×137.40.

PLATE 55

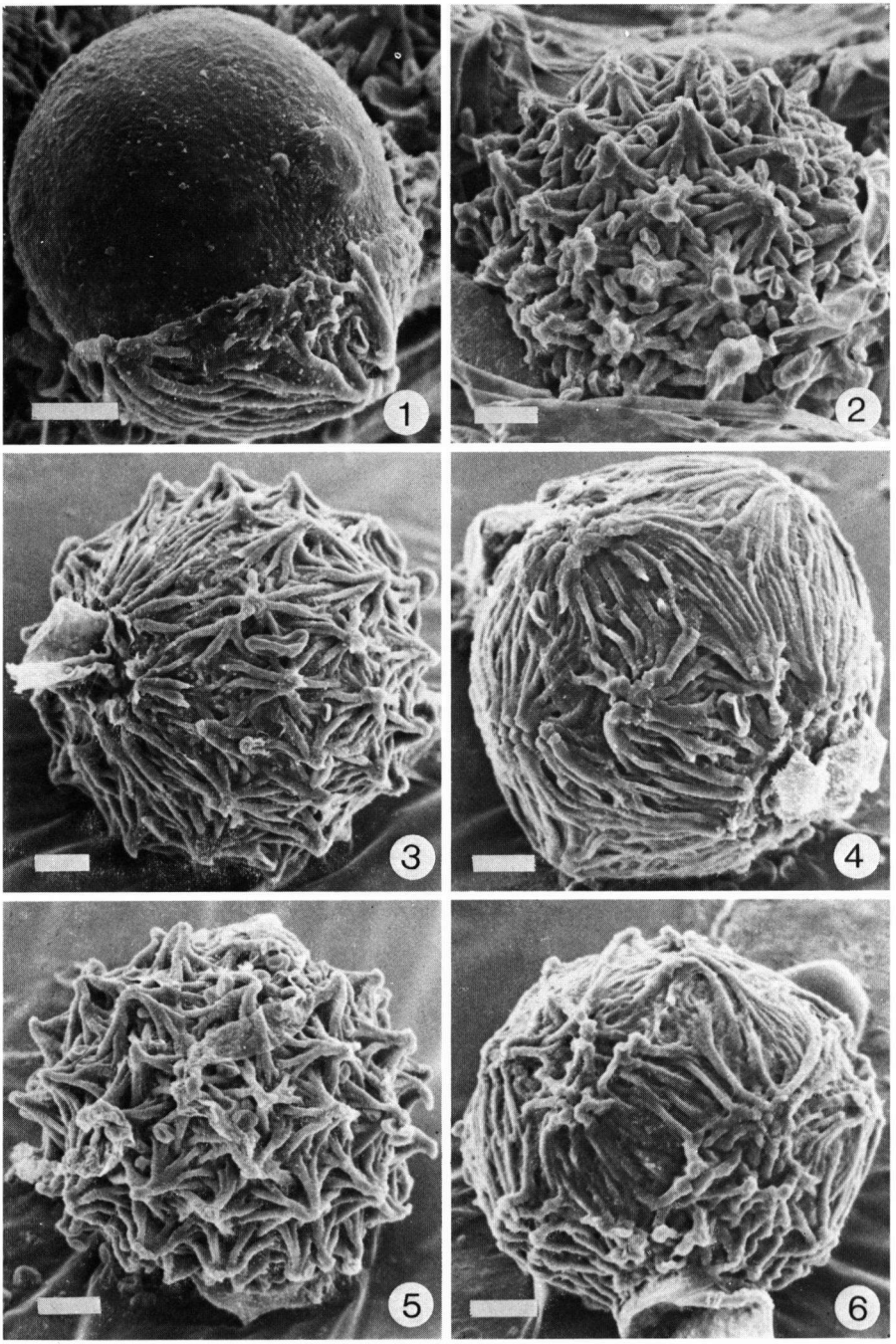
Fig. 25. *Mucor mucedo*, CBS 144.24 ×109.16; Fig. 26. *M. piriformis*, CBS 225.29 ×169.25; Fig. 27. *M. flavus*, CBS 235.35 ×234.35; Fig. 28. *M. hiemalis* f. *corticolus*, CBS 366.68 ×365.68; Fig. 29. *M. hiemalis* f. *silvaticus*, CBS 250.35 ×249.35; Fig. 30. M. *hiemalis* f. *hiemalis*, CBS 200.28 ×201.65.

PLATE 56

Fig. 31. *Mucor genevensis*, CBS 144.08; Fig. 32. *M. hiemalis* f. *luteus*, CBS 244.35 ×243.35; Fig. 33. *M. pusillus*, CBS 245.58; Fig. 34. *M. miehei*, CBS 182.67; Fig. 35. *Phycomyces blakesleeanus*, CBS 283.35 ×284.35; Fig. 36 *Syzygites megalocarpus*, CBS 372.29.

PLATE 57

Fig. 37. *Backusella circina*, CBS 128.70 ×129.70; Fig. 38. *Rhizopus sexualis*, CBS 336.62; Fig. 39. *Pirella circinans*, CBS 306.29 ×367.59; Fig. 40. *Parasitella simplex*, CBS 412.66 ×208.28; Fig. 41. *Gongronella butleri*, CBS 227.36 ×226.36; Fig. 42. *Mucor* '*indicae-seudaticae*', CBS 104.75.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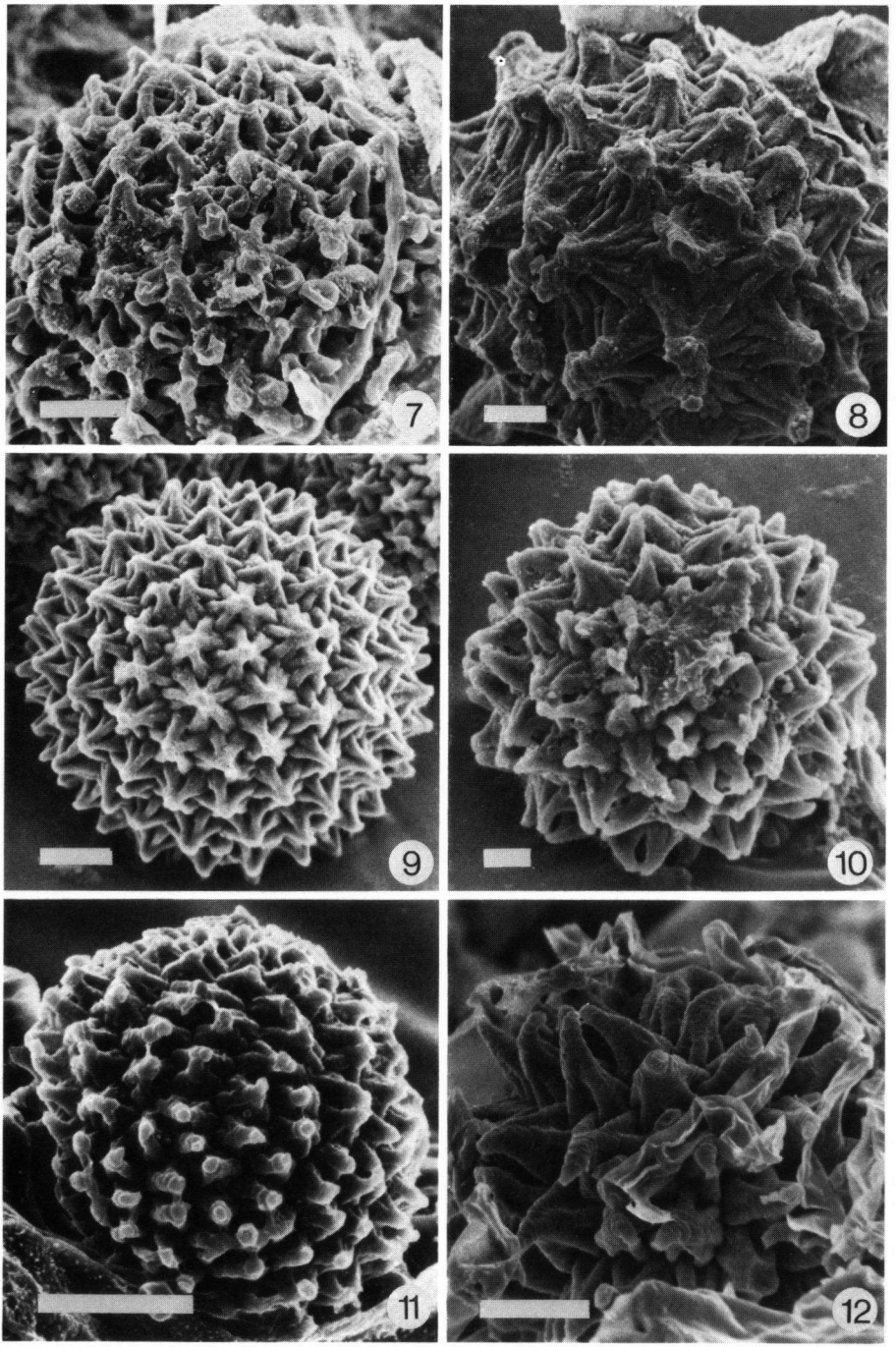
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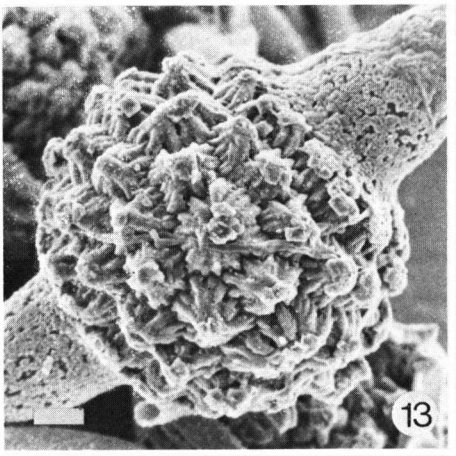
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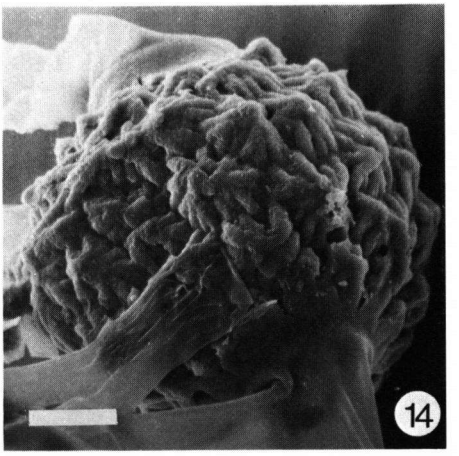
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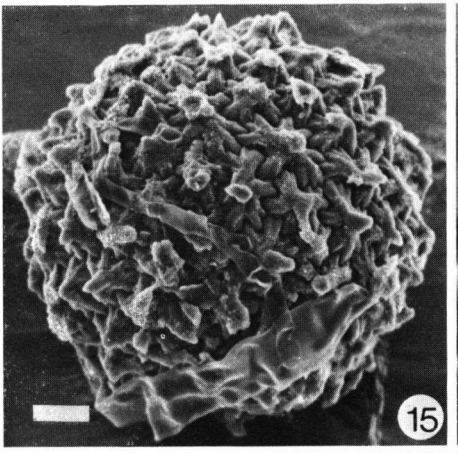
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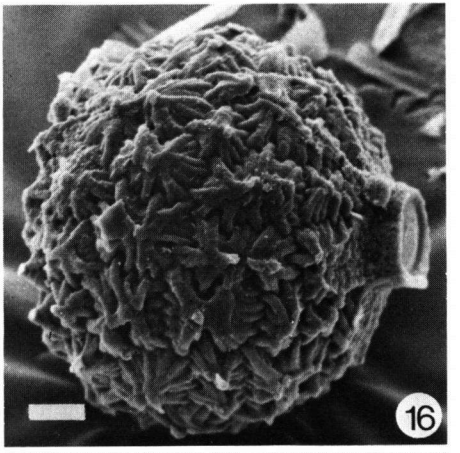
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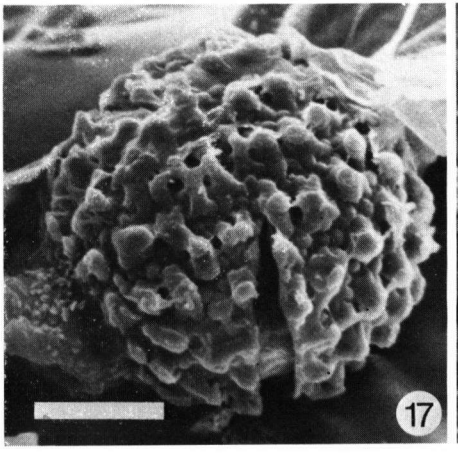
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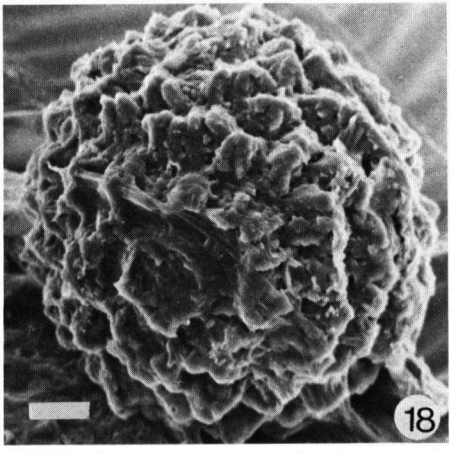
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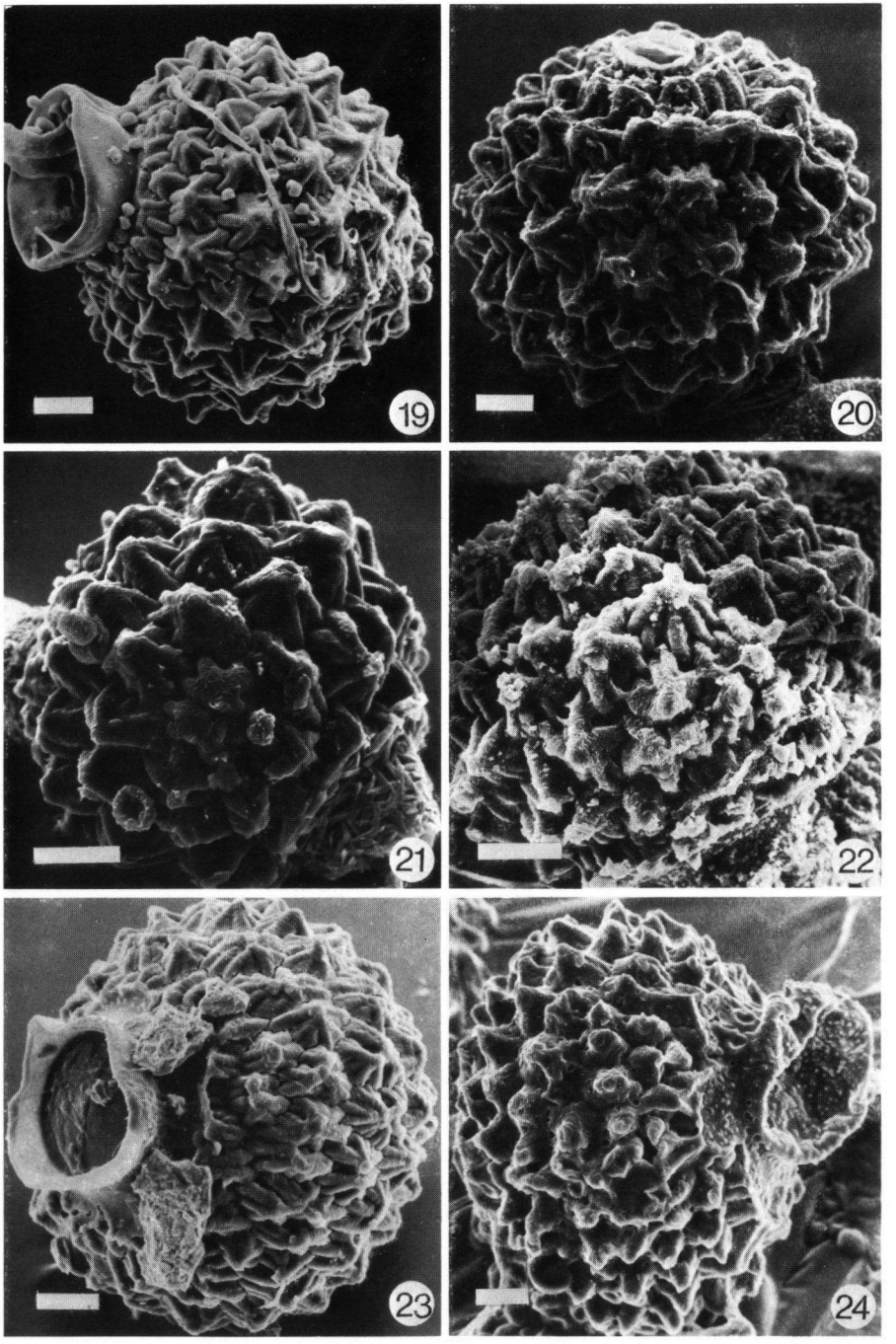
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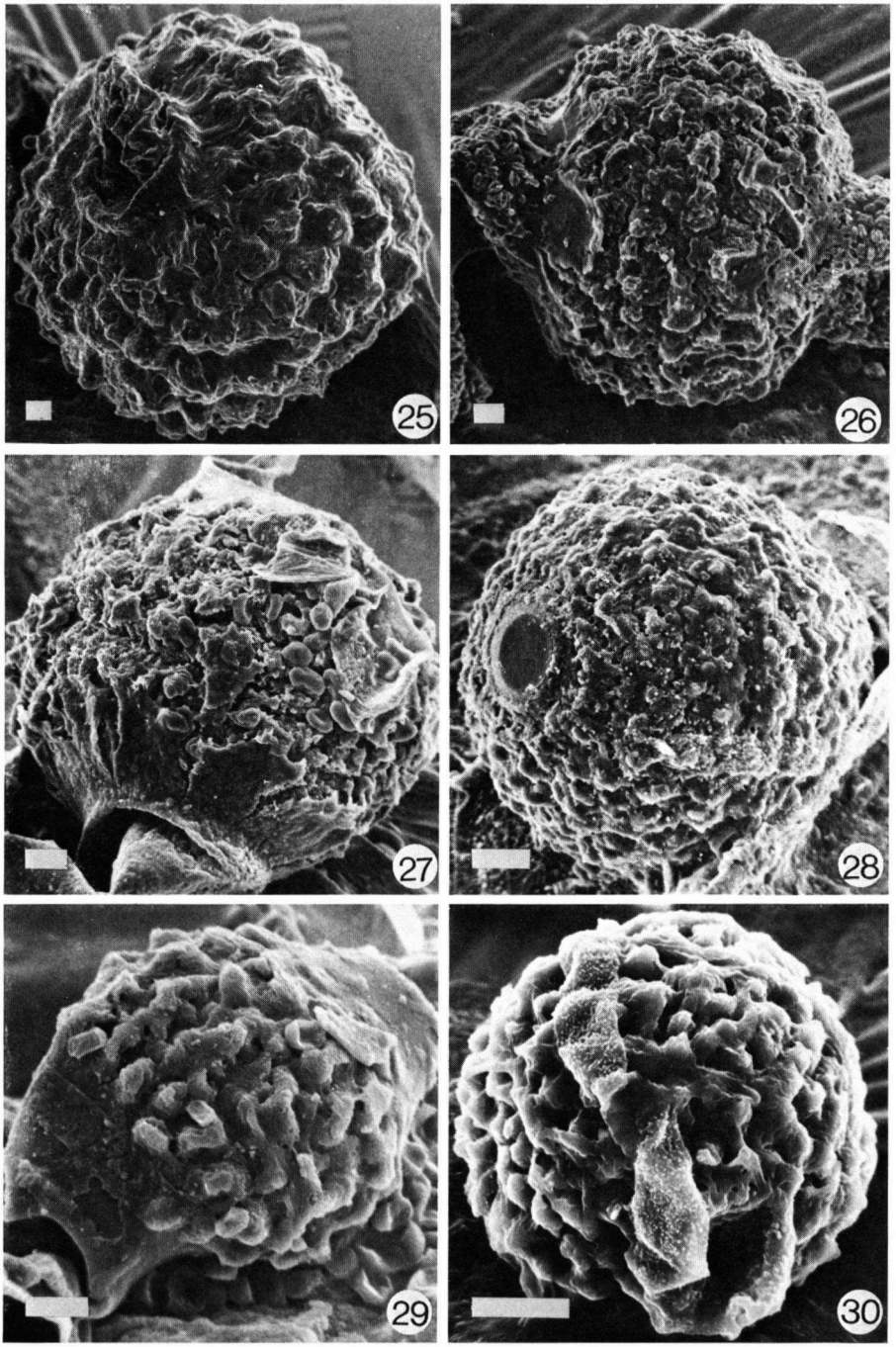
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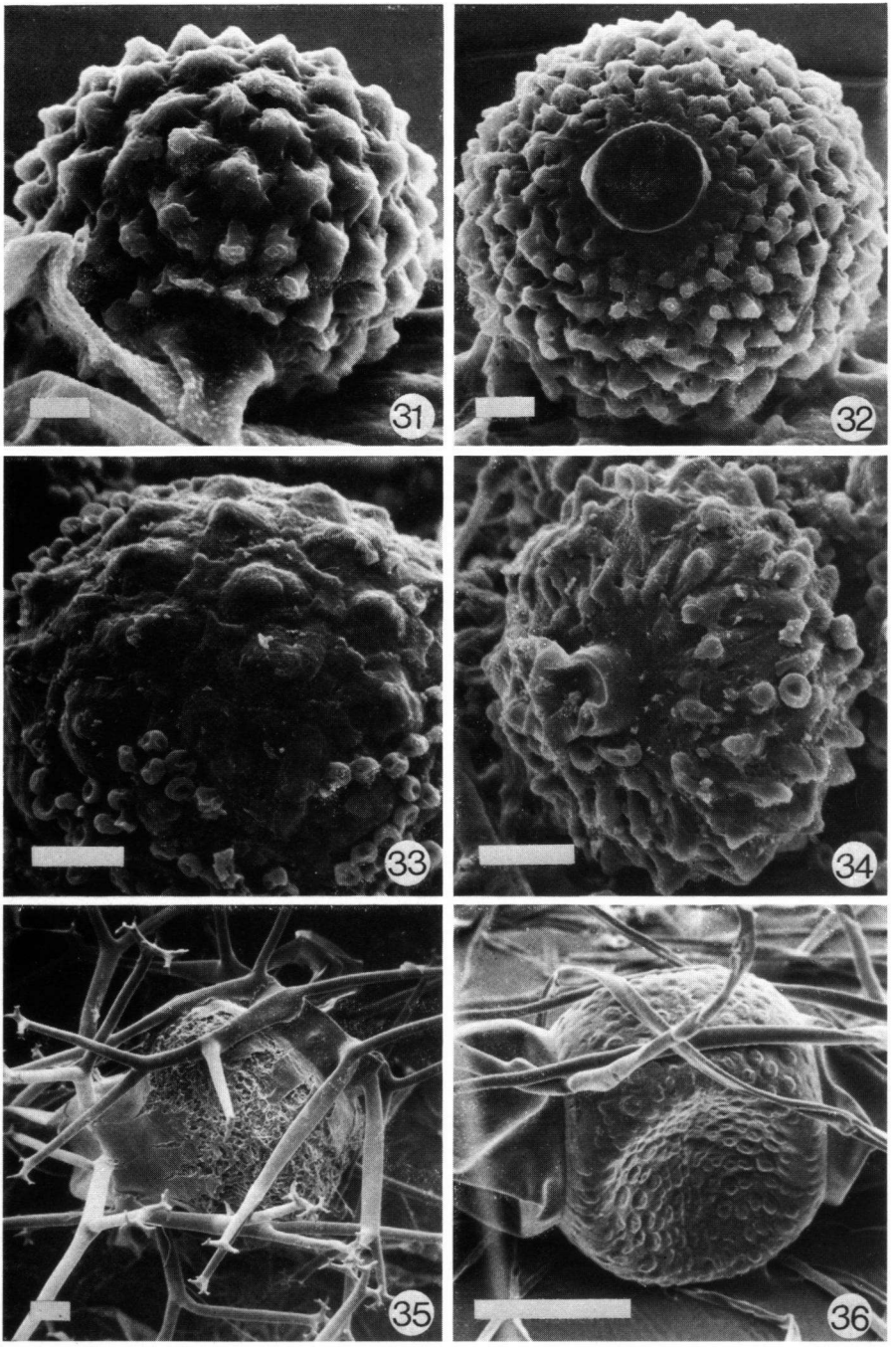
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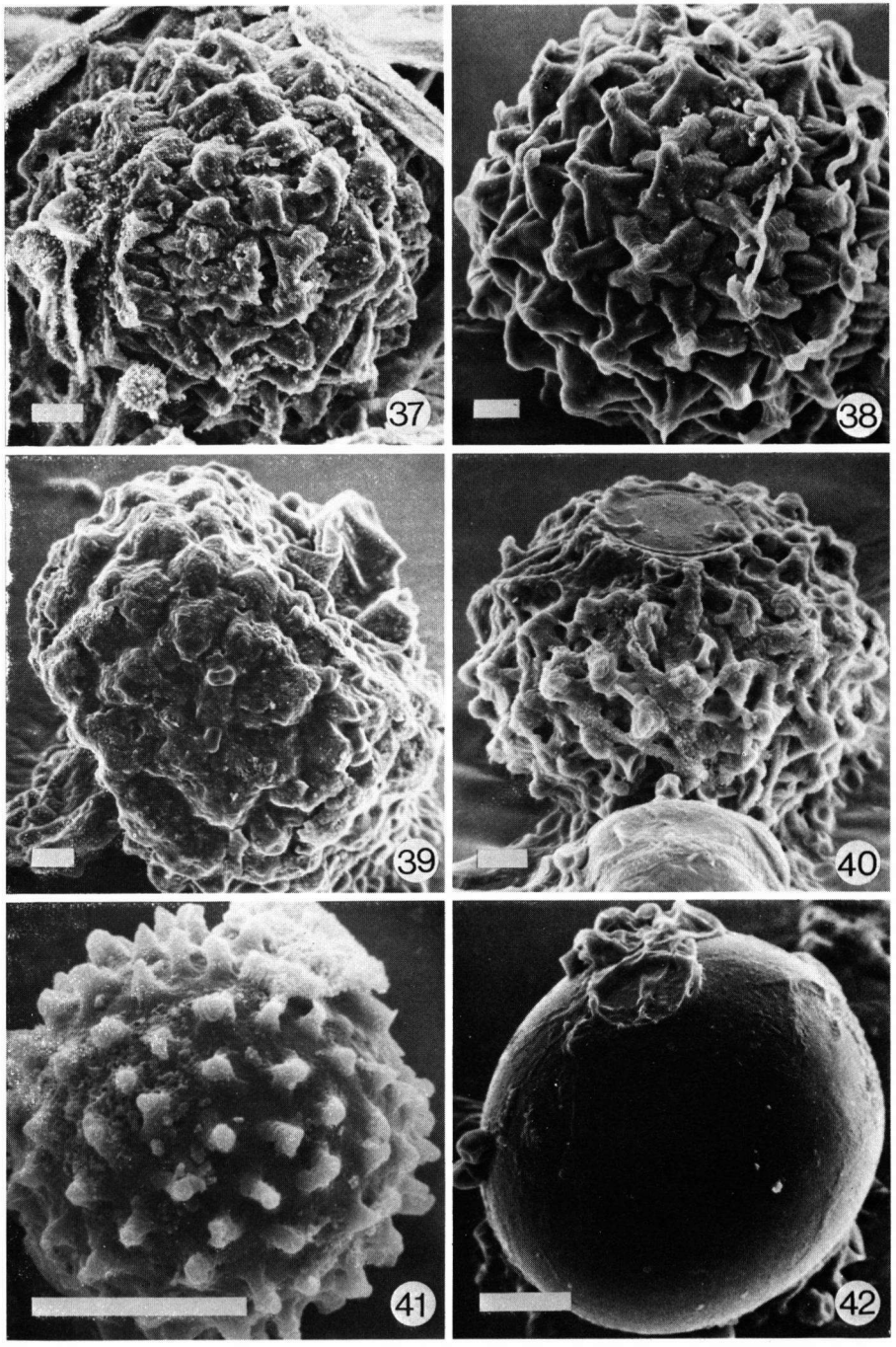
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