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SURFACE PATTERN OF SOME MICROSPORIDIAN SPORES AS SEEN IN THE SCANNING ELECTRON MICROSCOPE

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Abstract. Microsporidian spores of several insect- and fish-invading species have been studied with the scanning electron microscope (SEM). This technique reveals minute details of their surface. In some species the spores have wrinkles of different size and arrangement, in others they appear smooth or display distinct ridges. Spores of some species appear crumpled, probably due to preparation techniques, while the shape of others is well preserved. This first, preliminary study indicates that the use of a SEM may supply additional features for species differentiation providing a standard preparation technique is employed to ensure the comparability of the results.

Microsporidian taxonomy is based largely on the morphology of the spores. In most of the genera, however, the spores seem to be very uniform. With the increasing number of microsporidian species being continuously recorded from various hosts, particularly arthropods, species differentiation based on spore morphology becomes more difficult. Light microscopy reveals hardly more than the shape and size of the whole spore and of its posterior vacuole in the living state and nuclei after staining. The helpful presence of various surface projections such as, e.g., caudal appendages (*Mrázekia*—Léger Hesse 1918, *Caudospora*—Weiser 1946), membranous collars (*Weiseria*—Doby and Saguez 1964) or circular thickenings (*Weiseria sommermanae*—Jamnback 1970) is exceptional. Transmission electron microscopy records the exact number of coils of the polar filament and, in some instances, also the surface structure, if these structures are of a more constant character (tubules on *Plistophora debaisieuxi* Vávra 1965 or spines on *Nosema* sp. spores — Weidner 1970). The scanning electron microscope (= SEM) with its ability to reveal minute details of the surface, might well prove to be of great help in recognizing differences in the individual microsporidian species. This communication presents the first investigation of such possibility.

MATERIAL AND METHODS

We examined spores of several microsporidian species from fish and insect hosts. Spores from fish hosts were kept for some time in refrigerator at +4 °C in a small amount of pond water. Prior to fixation, the spores were simply concentrated by sedimentation. Insect species were preserved in unfixed, desiccated tissues of dead insect bodies or in a water suspension in the refrigerator. In the former case, they were briefly soaked

in distilled water and then crushed so that the spores were freed as a heavy suspension mixed with tissue debris. Finally, by means of a fine Pasteur pipette the spores were isolated from the tissue fragments.

For the preparation of specimens for the SEM we used a technique based essentially on that devised by Marszalek and Small (1968). A drop of thick spore suspension in distilled water was deposited on a microscope slide. After several minutes, the spores settled down to form a dense layer on the surface of the slide. The water was gently removed as not to disturb the layer of spores; a new, larger drop of distilled water was added and gently stirred with a pipette and by tipping the slide. This was repeated several times and resulted in removing the gross impurities. Afterwards, a drop of Párducz fixative (2 parts of 2% $HgCl_2$ and 6 parts of 2% osmic acid) was added for about 5 minutes. After a thorough washing with distilled water, drops of spores were transferred to specimen stubs coated with a thin layer of xylene-dissolved adhesive from a double-coated Scotch tape (3 M corporation). Then the procedure continued as described by Marszalek and Small. After sublimation, the specimens were coated with gold and examined with the Cambridge stereoscan electron microscope model Mark III operated at accelerating voltages of up to 20 kV*). Special care was taken to follow exactly the same procedure for all specimens in order to reduce any possible variation of the results.

OBSERVATIONS

A. MICROSPORIDIA FROM FISH

Their rather soft-shelled spores often collapse partly during the preparation; however, even in such cases, the surface pattern was preserved.

1. *Glugea hertwigi* Weissenberg from *Osmerus mordax* (Pl. I.**), Fig. 1). The outer layer of the spore wall in elongated spores is finely wrinkled into short irregular folds.

2. *G. weissenbergi* Sprague et Vernick from *Apeltes quadracus* (Pl. I., Fig. 4). The surface of the elongated spores reveals a pattern resembling the preceding species. However, the wrinkles seem to be oriented in a more scale-like manner, perpendicular to the longitudinal axis of the spore.

3. *Nosema lophii* Doflein from *Lophius americanus* (Pl. I., Fig. 3). This species has been studied only with the transmission electron microscope. A tangential section of the spore wall shows an irregular network of fine meandering folds which seems to be much more delicate than in *Glugea hertwigi*.

4. *Nosema notabilis* Kudo invading the myxosporidian *Sphaerospora polymorpha* parasitic in *Opsanus tau* (Pl. I., Fig. 2). The surface is not wrinkled being made only slightly uneven by very small wart-like structures.

B. MICROSPORIDIA FROM INSECTS

1. *Nosema bombycis* Nägeli, 1857, from *Bombyx mori* (Pl. II., Fig. 1). The broadly oval, stubby shape of the spores is well visible: the surface bears a coarse pattern of broad irregular flat wrinkles.

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**) The Plates I—VI will be found at the end of this issue.

2. *Plistophora schubergi* Zwölfer, 1927 from *Euproctis chrysorrhoea* (Pl. II., Fig. 2). Spores broadly oval with less prominent, rather fine and long wrinkles on their surface. The spore shape is well preserved.

3. *Nosema muscularis* Weiser, 1957 from *Lymantria dispar* (Fig. 7). The surface of the oval spores almost smooth, with very shallow indistinct wrinkles in circular arrangement. Spore wall rigid, undeformed.

4. *Nosema heliothidis* Lutz et Splendore, 1903 from *Plusia chalcites* (Pl. III., Figs. 1, 2). Elongated or slender cigar-shaped spores, sometimes slightly curved, bear very coarse surface folds, mostly longitudinal, sometimes with Ane wrinkles. Interior spore wall layer very rigid, the spores do not crumple.

5. *Nosema heliothidis* Lutz et Splendore, 1903 from *Heliothis* sp. (Pl. III., Fig. 3). Spores of this population are visibly different in type with broad rounded ends and less elongated. They are covered with finer, rather transverse wrinkles of the outer spore wall.

6. *Nosema lymantriae* Weiser, 1957 from *Lymantria dispar* (Pl. III, Fig. 4). Spores of this species are smooth, without apparent wrinkles, considerably crumpled. The interior spore wall layer is not rigid, i.e., the spores are rather thin-walled.

7. *Nosema tortricis* Weiser, 1956 from *Tortrix viridana* (Pl. III, Fig. 5). The elongated spores have surface pattern of very coarse ridges with additional fine, irregular, flat wrinkles.

8. *Thelohania similis* Weiser, 1957 from *Lymantria dispar* (Pl. III., Fig. 6). Regularly oval spores with a clear, smooth surface, without a rigid interior spore wall. One pole of the spore shows a flat depression on the polar cap.

9. *Nosema mesnili* (Paillot, 1918) from *Pieris rapae* (Pl. IV., Fig. 1). The elongated spores of this species have a surface pattern consisting of rather fine, irregular wrinkles. Spore wall partially deformed, with longitudinal irregular pits and ridges.

10. *Nosema* sp. from *Pseudoaletia correcta* (Pl. IV., Fig. 2). Spores of a regularly elongated oval shape, surface wrinkled into an irregular network of folds. The spore shape is well preserved.

11. *Plistophora balbiani* Veber, 1961 from *Antherea pernyi* (Pl. IV., Fig. 3). Oval spores with a coarsely wrinkled surface pattern on the exterior spore wall layer.

12. *Nosema* sp.*) from *Antherea pernyi* (Pl. IV., Fig. 4). The spores were partly collapsed and corrugated as the result of specimen preparation. Their surface shows irregular mostly transverse wrinkles.

13. *Nosema plodiae* Kellen et Lindegren, 1968 from *Plodia interpunctella* (Pl. V., Figs. 1, 2). In addition to spores fixed in the usual way, one sample of spores was treated differently: live spores were directly freeze-dried without fixation. These spores were crumpled and the surface pattern was less distinct (Fig. 2) than in the sample prepared according to the standard technique (Fig. 1). In these preparations the spores were wrinkled longitudinally and transversally while their shape was well preserved. The spores manifested a variability typical of this species (stubby ones 2.5×1.6 and cigar-shaped 3.8 by 1.5 μ).

14. *Nosema* sp. from *Euproctis chrysorrhoea* (Pl. V., Figs. 3, 4). Spores extremely variable in size and shape, similar to *N. plodiae*. Their shape ranges from tiny, stubby spores of about 1.6 by 1 μ to long cigar-shaped spores of 6 by 1.6 μ in size.

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*) This is a new species to be described later.

15. *Weiseria* sp.*) from *Ephemerella ignita* (Plate VI.). Elongated sausage-shaped spores with deformations revealing a rather plastic interior spore wall layer. Surface smooth, one longitudinal suture ending in a polar field is limited by other sutures branching from the longitudinal row.

DISCUSSION

Light microscopy reveals two kinds of structures on the surface of microsporidian spores: appendages, membranous, tail-like or filamentous in appearance, or ridge-like elevations of various form and layers of adhering, amorphous substance.

The former structures, if present, are constant attributes of spores of a given species and characteristic of some genera (e.g., *Weiseria, Caudospora*). They are visible in live spores, are not altered by fixation, and, hence, of great taxonomic importance.

Mucous envelopes or adhering gelatinous substances are generally amorphous (however, on the electron microscopic level the former might have a fibrous substructure — Vávra 1968). In general, adhering coatings have a monotonous appearance and may dissolve in water after a prolonged storage; this decreases their taxonomic importance in comparison with that of the first group.

Observation in the SEM is a relatively easy method to detect fine surface structure in spores appearing to be quite smooth in the light microscope. In some species, we could detect seam- or suture-like ridges which are essentially of the kind observed above, i.e., ridge-like elevations—only that they were minute. This applies to *Weiseria* sp. from *Ephemerella ignita* in Plate VI. Most of the other species examined bear surface structures in the form of fine to coarse wrinkles. Such wrinkles are in fact undulations of the outer layer of the spore wall ("exospore", "exosporium") seen considerably less distinctly also in sectioned spores. The wrinkles may be due to fixation-induced shrinkage—but not entirely, since a) they are visible also in unfixed spores (Pl. V., Fig. 2) though less pronounced, and b) they have a distinct pattern in some species (Pl. I., Fig. 3) or they may be almost absent. To a certain degree, they might be perhaps compared to villi-like projections of the surface layer of some spores. The osmiophobic thick inner layer of the spore wall, which is the chitinous case proper, is evidently rigid enough in some species to withstand deformation or crumpling, while in other species it is elastic.

Our results suggest that the SEM might be of help in distinguishing closely related species and/or populations thereof, thus contributing to the scarce knowledge of species variability of microsporidian spores. In the series investigated above, *Plistophora schubergi* and *P. balbiani* seem to be identical; on the other hand, *Nosema heliothidis* from *Plusia chalcithes* and *Heliothis* sp. should have the same appearence, but the SEM picture shows clear differences.

Careful examination of our series of pictures shows that in spite of many species looking almost alike in the SEM, there are pronounced differences distinguishing them from other species. This means that the application of the SEM in microsporidian taxonomy has a good prognosis, provided a standard technique is employed to assure comparability of the results achieved by different authors. In addition, the SEM pictures clearly demonstrate the actual variability in size and shape of spores in some infections (Pl. V., Figs. 3, 4; not taking into account spores which were scanned at an oblique angle, resulting in an axial distortion).

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*) This is a new species to be described later.

СТРУКТУРА ПОВЕРХНОСТИ НЕКОТОРЫХ СПОР МИКРОСПОРИДИЙ ПОД СТЕРЕОСКАНИРУЮЩИМ ЭЛЕКТРОННЫМ МИКРОСКОПОМ

Й. Лом и Я. Вайзер

Резюме. Споры нескольких, поражающих насекомых и рыбы видов микроспоридий изучались под стереосканирующим микроскопом. Этот метод обнаруживает малейшие детали их поверхности. У некоторых видов споры имеют морщины разных размеров и порядка, у других они гладкие или выказывают отчетливые борозды. Споры некоторых видов кажутся помятыми, вероятно в результате препарационной обработки, в то время как форма других хорошо сохранена. Настоящий первый предварительный очерк показывает, что применение стереосканирующего электронного микроскопа может дополнить характеристику для видовой дифференциации, при условии, что применяются стандартные препарационные методы, обеспечивающие сравнение полученных результатов.

REFERENCES

DOBY J. M., SAGUEZ F., Complément à l'étude morphologique et biologique de *Weiseria laurenti* Doby et Saguez 1964 (Microsporidie). Bull. Soc. zool. France 89: 777—784, 1964.

JAMNBACK H. A., *Caudospora* and *Weiseria*, two genera of Microsporidia parasitic in blackflies. J. inverteb. Path. 16: 3—9, 1970.

LÉGER L., HESSE E., *Mrázekia*, genre nouveau de microsporidies, parasite à spores tubuleuses. C. R. Soc. Biol. 79: 345—348, 1916.

MARSZALEK D. S., SMALL E. B., Preparation of soft biological specimens for scanning electron microscopy. In: Scanning Electron Microscopy Proc. 2nd An. Scanning Electron Microscope Symposium. IIT Res. Inst., Chicago 1969.

VÁVRA J., Etude au microscope électronique de la morphologie et du développement de quelques microsporidies. C. R. Ac. Sci. 261: 3467—3470, 1965.

WEIDNER E., Ultrastructural study of microsporidian development. I. *Nosema* sp. Sprague, 1965 in *Callinectes sapidus* Rothbun. Z. Zellforsch. 105: 33—54, 1970.

WEISER J., Studie o mikrosporidiích z larev hmyzu našich vod. Věst. Čsl. zool. spol. 10: 245—272, 1946.

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THE PROTOZOAN FLORA OF POTABLE WATER FROM NATURAL SOURCES AND ITS SUB-CLINICAL IMPLICATIONS

Water from natural sources, used for drinking by man, in rural areas in the southwestern zone of India, was studied for its protozoan flora, since routine methods of chlorination has no effect on it, and there exists the distinct possibility of their pathogenicity in man, at least in a sub-clinical form. The people in these areas, including the literate, do not boil or purify drinking water by using filters or alum precipitation techniques, (Maxcy K. F., Rosenau Prev. Med. and Publ. Health., Appl. Cent. Crofts. N.Y. VIII ed: 1956), prior to drinking but depend upon the chlorination done by the Public Health Departments at regular intervals. With the zone Sirur, as the base for study, surveys, location and mapping of the sources of potable water were done.

Samples of water from the surface and deeper levels were collected in sterile test-tubes, examined as fresh preparations, then stained and mounted for identification of the protozoan. Wherever possible the protozoans were cultured on infusoria of hay and other protozoological media. Although two to three or at times even four different types of protozoans were found in these water sources used for drinking, for

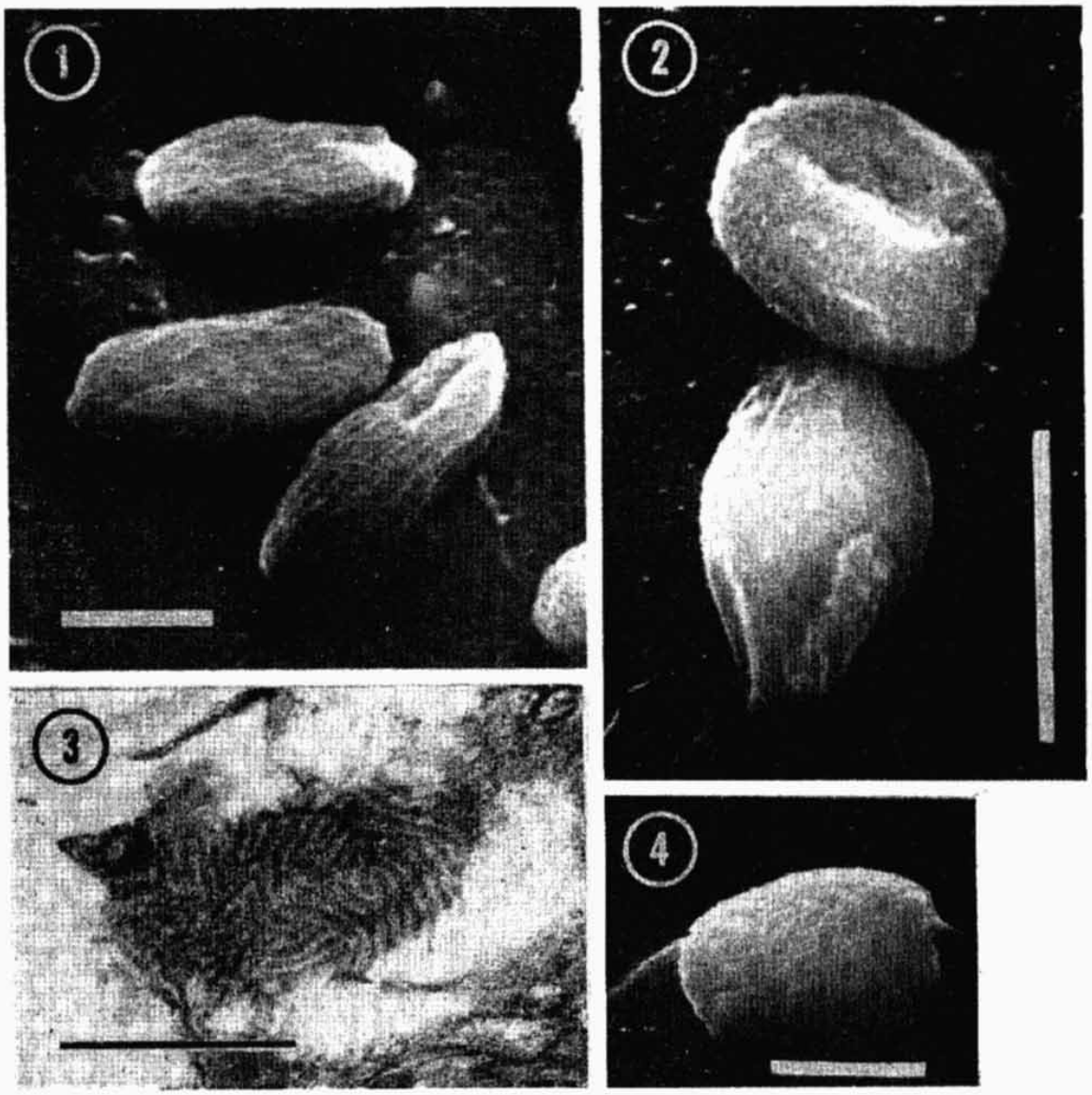


Fig. 1. *Glugea hertwigi* spores. × 1,0000. All pictures, with the exception of Fig. 3. are taken with the scanning electron microscope. In Figs. 1, 2 and 4 the scale equals 2 μ; in Fig. 3 it is 0.5 μ. — **Fig. 2.** *Nosema notabilis*. × 20,000. — **Fig. 3.** Tangential section of the wrinkles in the surface layer of the spore of *N. lophii*. × 30,000. This is the only transmission electron micrograph; all others in this paper are scanning electron micrographs. — **Fig. 4.** *Glugea weissenbergi*. × 10,000.

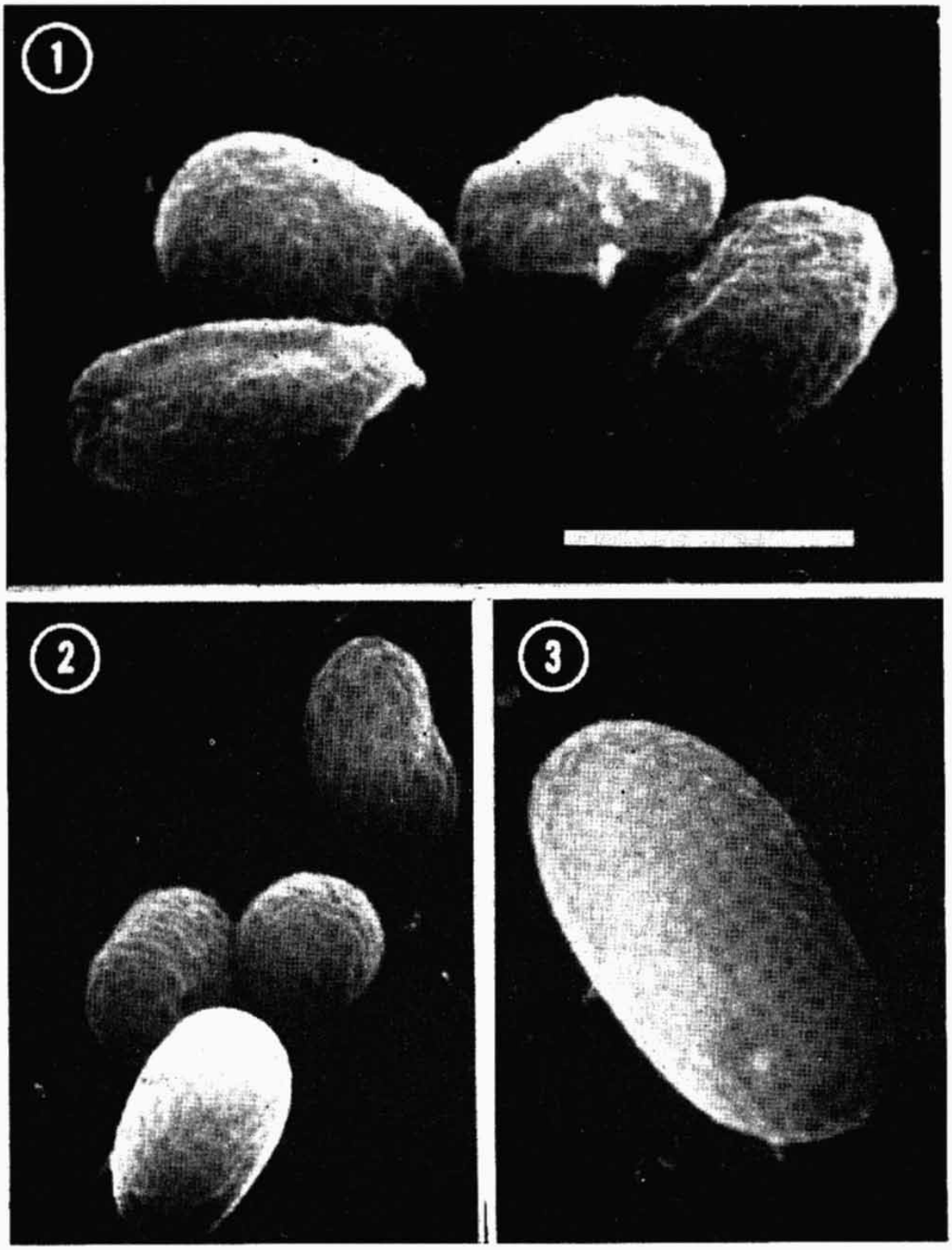
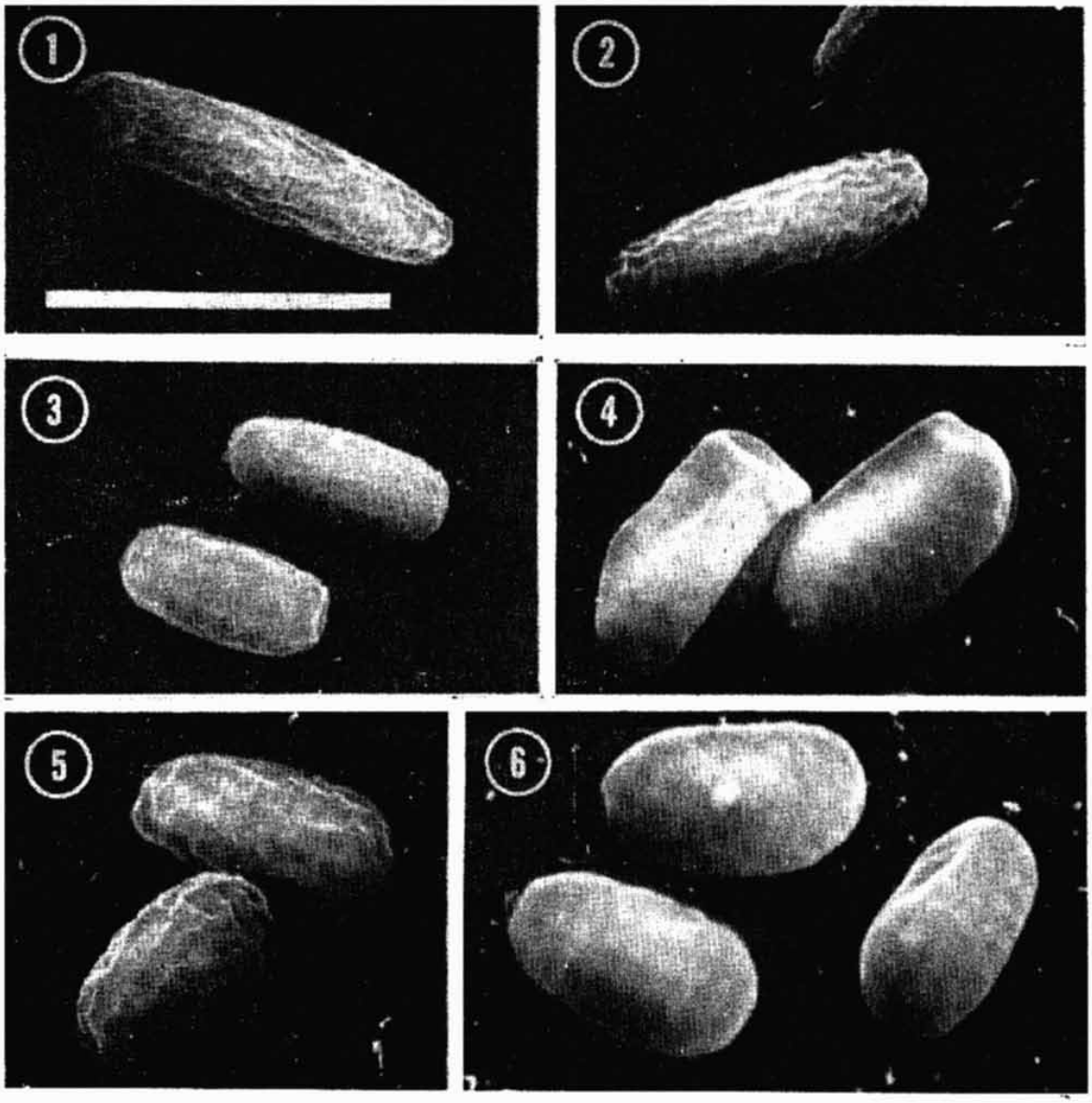


Fig. 1. *Nosema bombycis.* ×20.000. — **Fig. 2.** *Pleistophora schubergi.* ×20.000.—**Fig. 3.** *Nosema muscularis.* ×20.000. In all figures, the scale equals 2 μ.



Figs. 1 and **2.** *Nosema heliothidis* from *Plusia chalcites.* × 10,000. — **Fig. 3.** *N. heliothidis* from *Heliothis* sp. × 10,000. — **Fig. 4.** *N. lymantriae.* × 10,000. — **Fig. 5.** *N. tortricis.* × 10,000. — **Fig. 6.** *Thelohania similis.* × 10,000. In all figures the scale equals 2 μ.

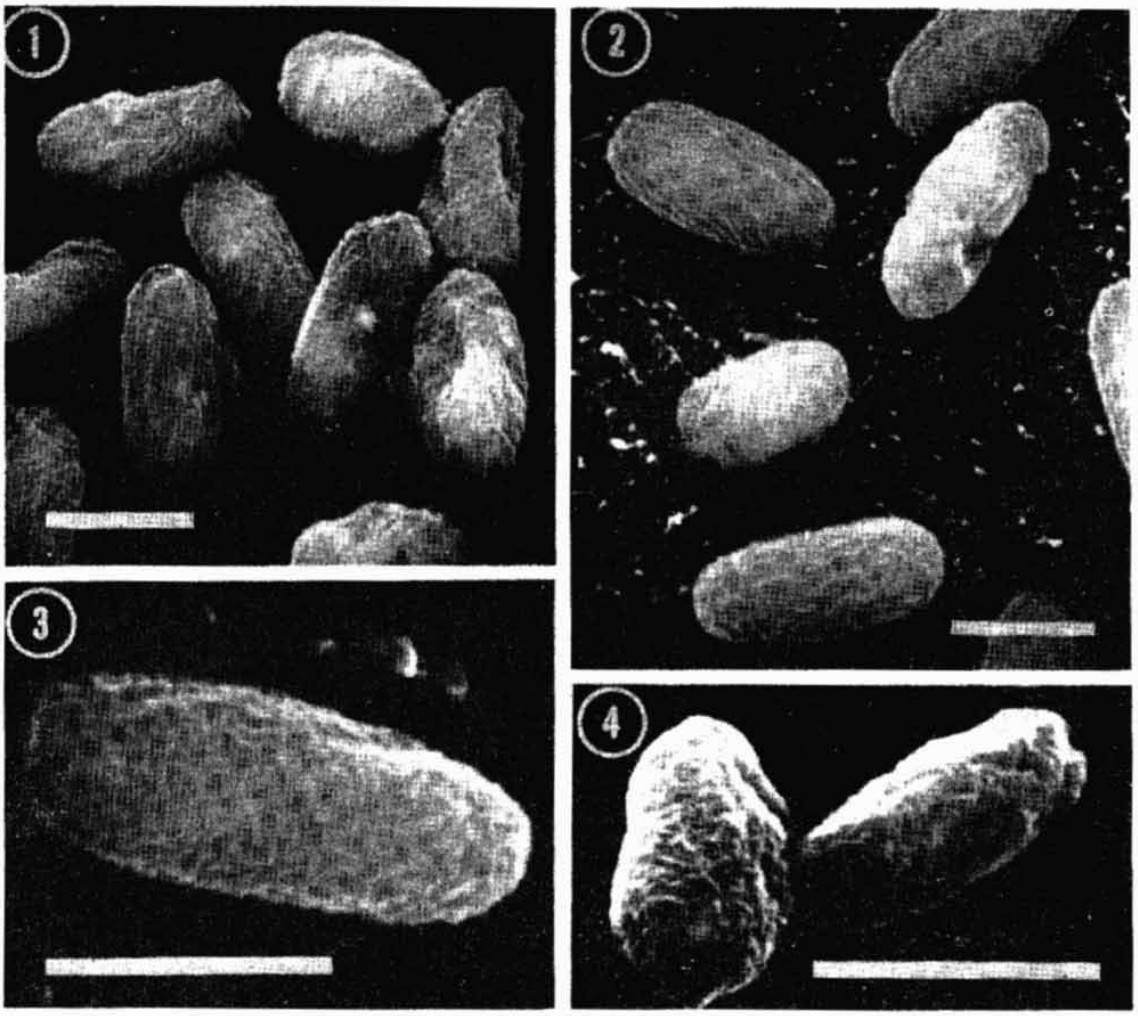


Fig. 1. *Nosema mesnili*. × 10,000. **Fig. 2.** *Nosema* sp. from *Pseudoleptia convexa*. × 10,000. **Fig. 3.** *Pleistophora halbiani*. × 20,000. — **Fig. 4.** *Nosema* sp. from *Anthrena perezi*. × 20,000. In all figures the scale equals 2 μ.