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Scanning Electron Microscopic (SEM) Observations on Some Members of Actinosporean (Phylum Myxozoa) Collective Groups and Possible Usage of SEM in Type Description

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Abstract: In this study, eleven different actinosporean types belonging to the collective groups synactinomyxon, aurantiactinomyxon, echinactinomyxon, raabeia and siedleckiella were observed under a scanning electron microscope (SEM) for the first time. The results obtained showed that surface structures and their ornamentation on the spore body and branches seen on the caudal processes of actinosporean spores are of importance in determination of the spores, at least at the collective group level.

Key Words: Myxozoa, actinospore, Scanning Electron Microscope

Aktinospor (Filum: Myxozoa) Kollektif Gruplarına Ait Bazı Bireylerin Tarayıcı Elektron Mikroskopundaki Gözlemler ve Tarayıcı Elektron Mikroskopunun Tip Tanımlamasında Muhtemel Kullanımı

Özet: Bu araştırmada, synactinomyxon, aurantiactinomyxon, echinactinomyxon, raabeia ve siedleckiella kollektif gruplarına ait 11 farklı aktinospor tipi Tarayıcı Elektron Mikroskopu'nda ilk defa incelendi. Elde edilen sonuçlar spor vucudundaki yüzeyel yapılar ve ayak uzantılarındaki dallanmaların, en azından, kollektif grubunun belirlenmesinde önemli olabileceğini gösterdi.

Anahtar Sözcükler: Myxozoa, aktinospor, Tarayıcı Elektron Mikroskopu

Introduction

Actinosporeans are parasites of aquatic oligochaetes and have been known for a century (1). So far, more than 100 species or types of actinosporeans have been identified (2).

The taxonomy of actinosporeans is based on spore morphology and the taxonomy has been revised several times by several authors (3-7) Recently Kent et al. (8) redefined the phylum Myxozoa Grasse, 1970 to solve the taxonomic and nomenclatural problems arising from the two host life cycle of myxozoans as first described by Wolf and Markiw (9). The distinction between the two previously recognised classes Actinosporea and Myxosporea disappeared and the class Actinosporea was suppressed, becoming a synonym of the class Myxosporea Bütschli, 1881. The generic names of actinosporeans were retained as collective group names, and it was proposed that they be used to characterize different morphological types of actinosporeans. Actinosporeans, for which the myxosporean stage is not known, are to be

retained as species inquerandae until their specific identity is established. Quite recently, it was proposed by Lester et al. (10) that the International Code for Zoologean Nomenclature should be applied to newly described actinosporeans, so that genera and species of actinosporeans should be named even if no myxosporean stage is known. However, in a previous paper, Özer and Wootten (2) described actinosporeans as "types" with numbers according to Kent et al. (1994) to prevent future confusion in life cycle studies and for uniformity with other recently published descriptions of actinosporeans. The same description is also used in the present study for the reasons given above.

This study shows some additional observations obtained under SEM for several actinosporean types belonging to 5 different collective groups.

Materials and Methods

Oligochaetes infected with different actinosporean types were collected from an Atlantic salmon farm

located in the extreme north of Scotland. Samples of actinosporeans were fixed in 1% glutaraldehyde at 4°C for 1 h and then transferred to 3% glutaraldehyde at 4°C for 2-3 days. Before post-fixation in 1% osmium tetroxide for 2 h, water suspensions were syringed through an acetone resistant polyamide millipore filter with pore size 0.45 µm (Sartorius Ltd). Following post-fixation in osmium tetroxide, the filter was dehydrated in a graded ethanol series. The filters containing actinosporean samples were then transferred into HMDS (hexamethyldisilazane) for 5 min and air-dried at room temperature in the fume cupboard. Finally, filters were mounted on stubs, coated with gold and examined and photographed in a Philips 500 Scanning Electron Microscope.

Results

The results on the spore morphology obtained SEM showed that the individual spores of individual type could be differentiated from the other types in the same collective group as well as in the others. Apart from the measurement data, surface ornamentation on the spore body and the branches on caudal processes seen under SEM were also shown to be important additional characteristics for actinosporean collective groups.

Collective group synactinomyxon

In the present study, three different actinosporean types were studied: synactinomyxon type 1, type 2 and type 3, the first two of which are very close to each other in terms of the spore dimensions and appearances,

belonging to the collective group synactinomyxon (see Özer and Wootten (2) for type description). Under SEM, they were also similar in terms of surface ornamentation (Figs 1, 2). Their connection points, where the characteristic eight spores join each other, were also in the same manner (Fig. 2). However, the third type of synactinomyxon differed from the other two by the spore dimensions and appearances. Synactinomyxon type 3 had the largest episporic dimensions and its morphological appearance was very similar to the members of the collective group echinactinomyxon, but differed from them by the union of eight individual spores. Its surface structures seemed to be more solid than the other two synactinomyxon types under study (Fig. 3). Very solid looking surface ridges surrounded the spore body.

Collective group aurantiactinomyxon

Two different aurantiactinomyxon types were examined under SEM in the present study. Aurantiactinomyxon type 1 and type 3 (see Özer and Wootten (2) for type description) were similar in appearance but their morphological measurements differed. Aurantiactinomyxon type 1 had much less elongated caudal processes than type 3. Under light microscopy, the morphological appearance and the dimensions of Aurantiactinomyxon type 3 were also very similar to those of the members of the collective group Echinactinomyxon. However, at the SEM level, it was possible to obtain much more data on its morphology. The members of the collective group

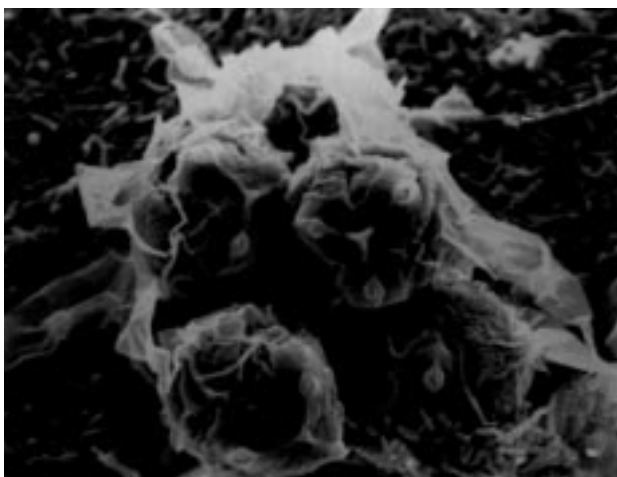


Fig. 1. Synactinomyxon type 1 with surface ornamentation on the spore body and three polar capsules. (x2500)

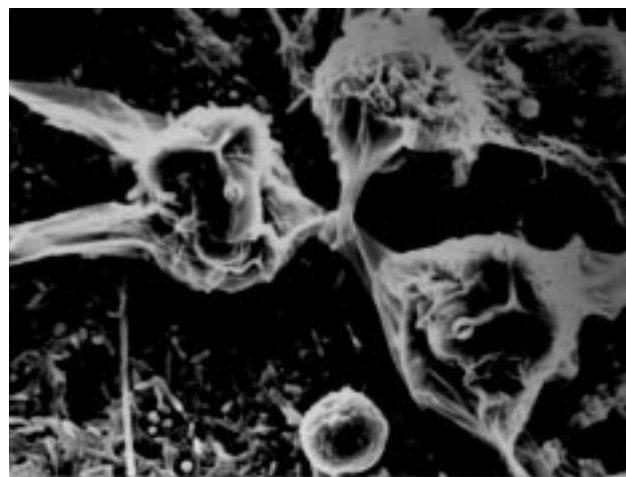


Fig. 2. Caudal process connection point of synactinomyxon type 2 and the surface ridges on the spore body (x2500)

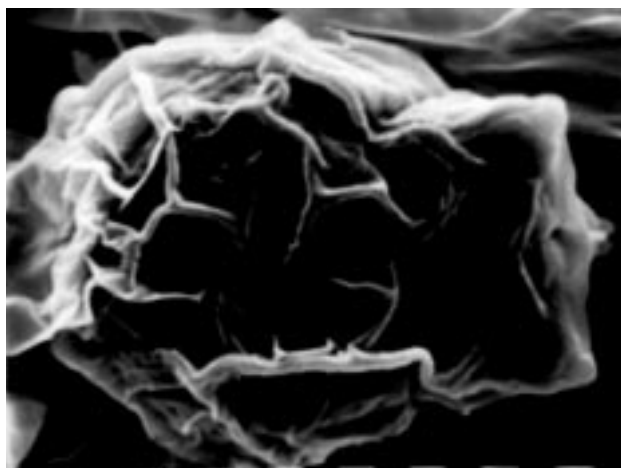


Fig 3. Surface ridges with net-like appearance on the surface spore body of a synactinomyxon type 3 spore ($\times 3000$)

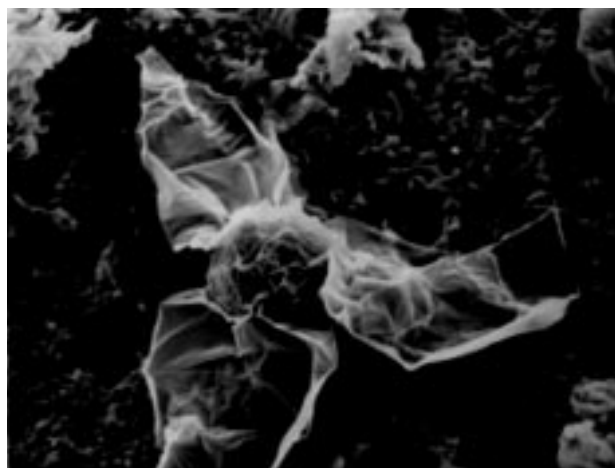


Fig. 4. Surface ornamentation on the spore body and three caudal processes of a aurantiactinomyxon type] spore. ($\times 2500$)

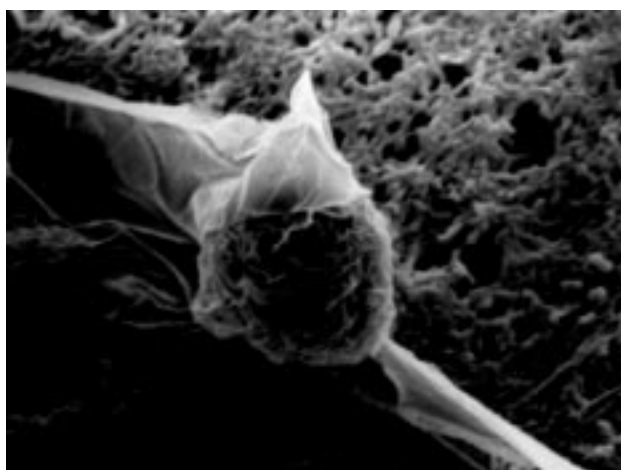


Fig 5. An individual spore of aurantiactinomyxon type 3 with many surface foldings on the spore body ($\times 2500$)

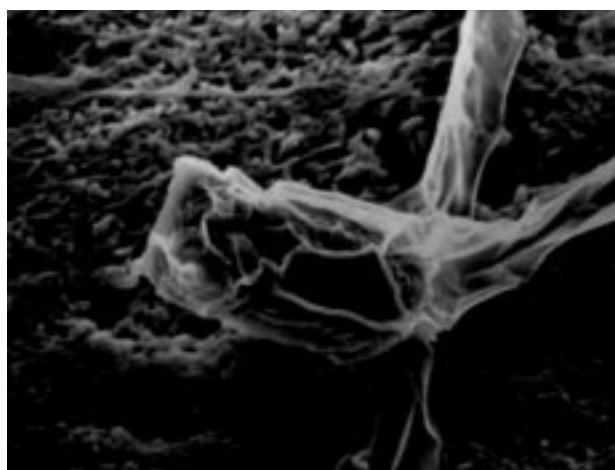


Fig. 6. Spore body of echinactinomyxon type 2 with solid surface ridges. ($\times 2500$)

aurantiactinomyxon had a different surface ornamentation on the spore body and the surface ridges were very thin and in web-like structures. The surface ornamentation of the ridges can be seen in Fig. 4 for aurantiactinomyxon type 1 and in Fig. 5 for type 3.

Collective group echinactinomyxon

In the present study, of the six different echinactinomyxon types identified (see Ozer and Wootten (2) for type description), echinactinomyxon type 2 and type 5 were observed under SEM. Echinactinomyxon type 5 was recently shown to be the actinosporean stage of *Sphaerospora truttae* by Ozer and Wootten, (11). The spore body formed by the union of three valvogenic cells of echinactinomyxon type 2 and type 5 were observed to

be the only major ridges. There were not many minor surface ridges between the major ones on the spore body of both echinactinomyxon type 2 (Fig. 6) and echinactinomyxon type 5 (Fig. 7). In addition to the general appearance, both types also differed from each other due to the ornamentation of major and minor ridges on the spore body.

Collective group raabeia

In the present study, of the six different raabeia types identified (see Özer and Wootten, (2) for type description), raabeia type 1, type 3 and type 4 were observed at the SEM level. They were the representatives of three different appearances of the collective group.

Raabeia type 1 was observed to have a shorter spore body (Fig. 8) and four branches at the tips of three caudal processes (Fig. 9). However, raabeia type 3 was observed to be characteristic in appearance, with a very elongated spore body and caudal processes. Polar capsules were elongated and located on top of the spore body (Fig. 10). Another morphological aspect of the collective group was the presence of twig-like structures on the caudal processes. Raabeia type 4 had these structures on three caudal processes (Fig. 11). The spore body of raabeia type 4 had only one or two minor surface ridges apart from the major ridges formed by valvogenic cells on the suture as was seen in raabeia type 1.

Collective group siedleckiella

Siedleckiella silesica was the only species belonging to the collective group *siedleckiella* in the present study (see Ozer and Wootten (2) for type description). Their characteristic web-like appearance was observed under SEM. Individual spores had three caudal processes connecting individual spores to one another, an elongated style and a spore body with three polar capsules (Fig. 12). Three polar capsules located on top of the spore body and occupied nearly half of it (Fig. 13). The discharge canal for polar filaments could also be seen on top of the polar capsules.

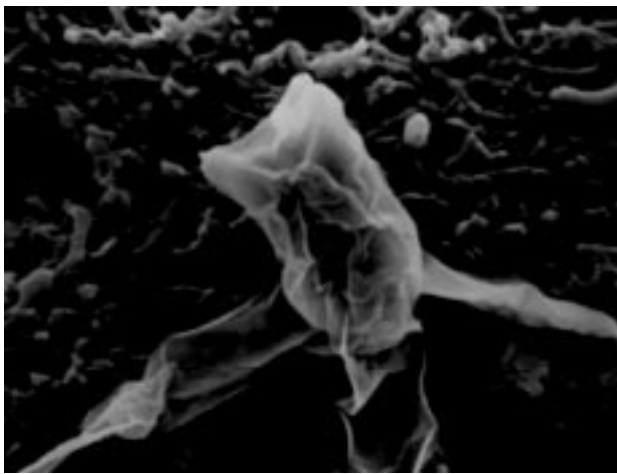


Fig. 7. Spore body of echinactinomyxon type 5 with very dense surface ridges. (x2500)

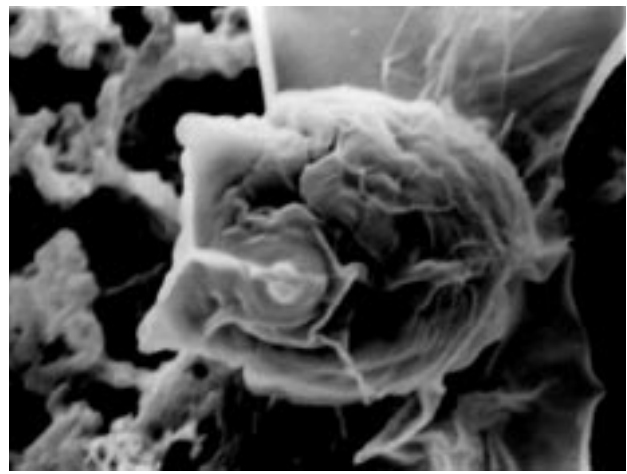


Fig. 8. Side apical view of the spore body of a raabeia type 1 spore. Apart from major appearance of the sutural line formed by valvogenic cells, there are also several secondary surface ridges. (x2500)

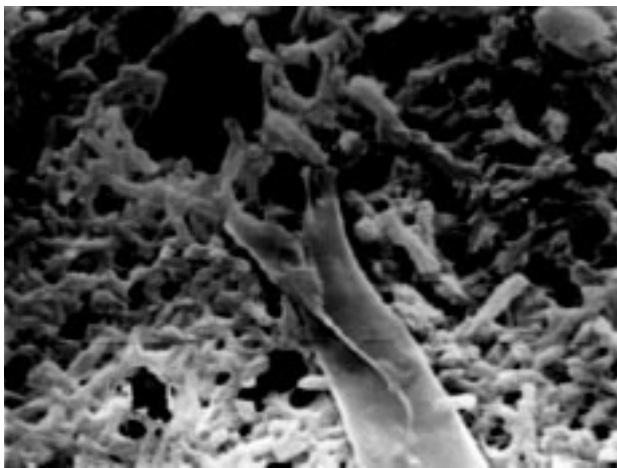


Fig. 9. Four branches at the tip of one of the caudal processes of a raabeia type 1 spore. (x5000)

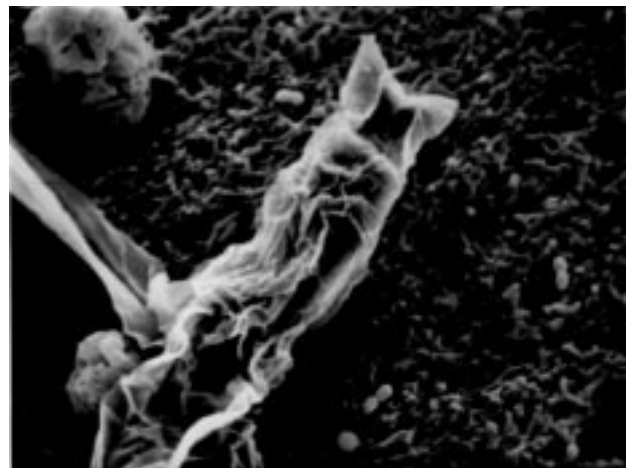


Fig. 10. An individual raabeia type 3 spore. Very elongated spore body with some surface ridges (x2500)

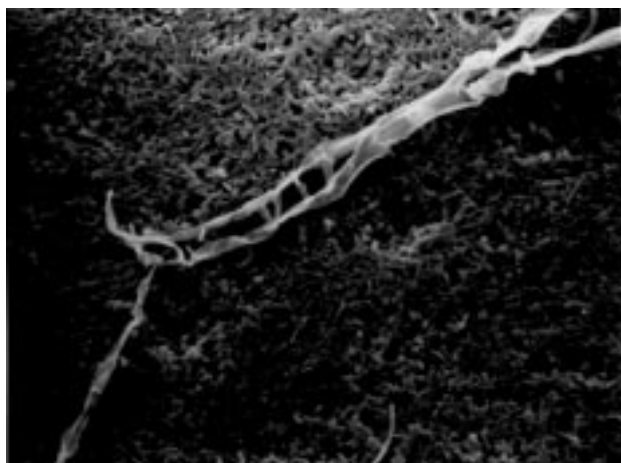


Fig. 11. Several branches on one of the caudal processes of a raabeia type 4 spore. (x5000)



Fig. 12. An individual spore of *Siedleckiella silesica*. Three caudal processes, an elongated style and three polar capsules are located on top of the endospore cavity. (x640)

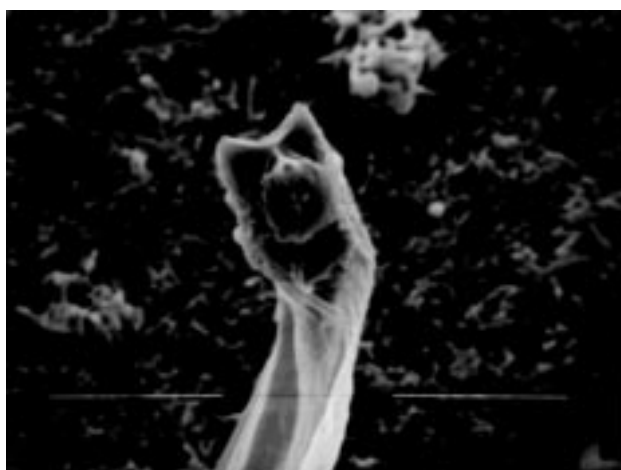


Fig. 13. Closer look at the three polar capsules and the surface structures of an individual *S. silesica* spore. Note the polar filament discharge canal appearance. (x2500)

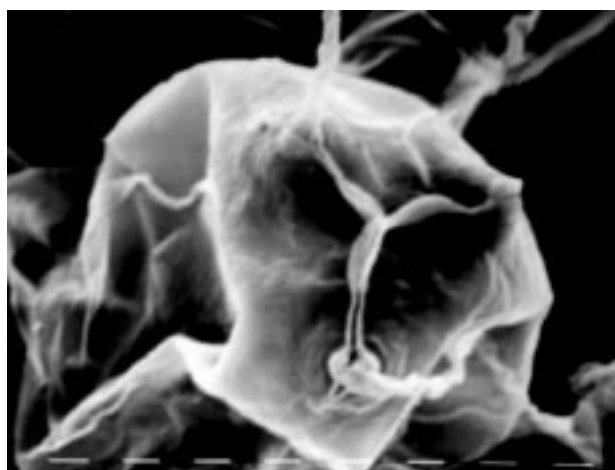


Fig. 14. Apical view of three polar capsules and two polar filaments already released. Note the opening between the septate junctions of three polar capsules of synactinomyxon type 3 spore (x5000)

Release of sporoplasm from the spore body

In the sporoplasm release process, at first, a polar filament release was observed (Fig. 14) and this was followed by the discharge of sporoplasm. Sutures of valves between the polar capsules opened and the sporoplasm discharged from this opening (Fig. 15) and surface ridges present on the spore body disappeared (Fig. 16).

Discussion

The results obtained in this study at the electron microscopic level suggested for the first time that SEM

could be a useful tool in the identification of actinosporean types, at least at the collective group level, by revealing details of the surface ornamentation on the spore body and the branches on the caudal processes.

The connection between the valves of the shell were prominent sutural ridges in all the actinosporean types examined in the present study and as was reported in the triactinomyxon stage of *M. cerebrealis* (12). However, some secondary ridges on the shell were obvious under SEM and may have potential in the identification of actinosporean collective groups. Under the light microscope, aurantiactinomyxon type 3 spores had similar features to members of the

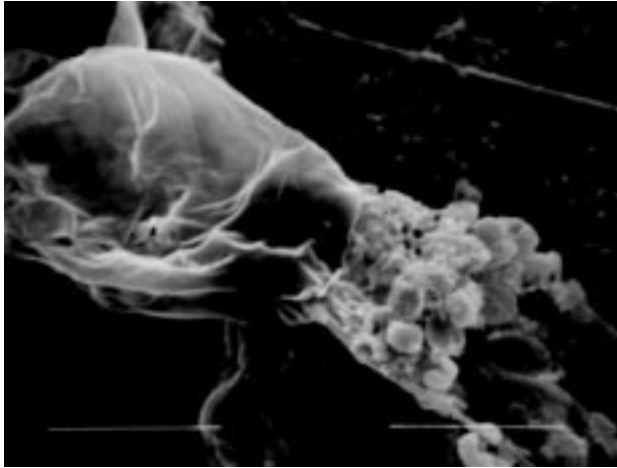


Fig. 15. An individual spore of synactinomyxon type 3 with a nearly discharged sporoplasm (x2500)

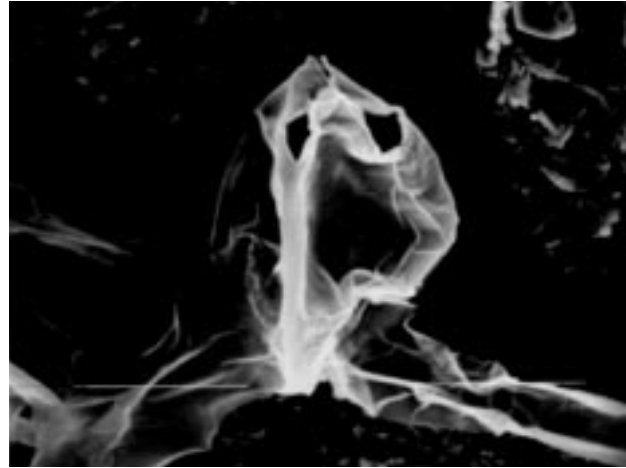


Fig. 16. Individual spore of echinactinomyxon type 2 after the sporoplasm was discharged. Note the plane surface of spore body apart from the valvogenic suture line. (x2500).

collective group echinactinomyxon. However, these secondary surface ridges on the spore body seen under SEM were minor in echinactinomyxon, while they were more abundant and numerous on aurantiactinomyxon spores, even obscuring the sutural ridges between the shell valves. The shape and the position of the polar capsules of aurantiactinomyxon type 3 were also different from the other actinosporean collective groups studied here. Thus, surface ornamentation might be of some value in differentiating actinosporean types. The secondary surface ridges were much heavier in synactinomyxon type 3 than in synactinomyxon type 1. In apical view, the three sutures of the shell valves formed a Y-shape at their intersection in all actinosporean types studied here, as was seen in the triactinomyxon stage of *M. cerebralis*

(12). The tips of the polar filaments were visible at the apertures at this intersection.

The patterns of the polar capsule and sporoplasm release observed in this study were similar to those observed from the triactinomyxon stage of *M. cerebralis* (12); polar filaments were first discharged, sutural ridges were opened and the sporoplasm released. During this sporoplasm release process, the spore body of synactinomyxon type 3 and the triactinomyxon stage spores of *M. cerebralis* lost the characteristic secondary surface ridges and the spore body became smoother. In all actinosporean types after full discharge of the sporoplasm, the three polar capsules were also released leaving empty spaces on top of the spore body, at which point recognition of the actinosporean type became impossible.

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