

## Variability of Spores of *Ceratomyxa appendiculata* Thélohan, 1892 (Myxozoa, Bivalvulida)

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**Summary.** Spore variability of *Ceratomyxa appendiculata* Thélohan, 1892, found in the gallbladder of black angler-fish, *Lophius budegassa* from NE Spain were studied by light microscopy and, for the first time, by scanning electron microscopy. More than 18,000 spores of *C. appendiculata* were examined, classified and quantified. Of this, 98.31% were bivalvular normal spores, the rest were abnormal spores (1.63% were trivalvular spores and 0.06% were tetra-valvular spores). The monthly evolution of these spores was followed for ten months. Differences among the proportions of trivalvular spores in different months were significant. Percentage of trivalvular spores ranged from 9.90% (May) to 0.00% (December). The highest percentage of tetra-valvular spores was reached in november (0.39%). Some polysporous trophozoites can produce simultaneously normal and abnormal spores.

**Key words:** *Ceratomyxa appendiculata*, gallbladder, Myxozoa, abnormal spores.

### INTRODUCTION

Scientists have been aware of the variability in the spores of Myxosporidia since the first studies were undertaken on this group of organisms. Thélohan (1895) found that the spores of *Myxobolus ellipsoides* were highly varied. This variability was observed in all aspects of the spores: in their typical form, the presence or absence of iodophilic vacuoles, the presence of extra valves and/or extra polar capsules, etc. (Shul'man 1990). This phenomenon can be found in almost all of the studied species of Myxosporidia, and has been cited

on different occasions in reference to spores belonging to the species of the genus *Ceratomyxa* (Auerbach 1912, Meglitsch 1960, Sitjà-Bobadilla *et al.* 1995, Yokohama and Fukuda 2001, Cho *et al.* 2004, Aseeva 2003, Maíllo and Gracia 2007).

However, very few studies have focused exclusively, or at least with any degree of detail (Thélohan 1895), on such spores. Studies that undertake to quantify abnormal spores are almost nonexistent, though Parisi (1912), Meglitsch (1960) and Shul'man (1990) carried out important work.

This paper presents the results of a quantitative and qualitative study of the abnormal spores of *Ceratomyxa appendiculata*, a Myxosporidia parasite found in the gallbladder of *Lophius budegassa*. For the first time, the monthly evolution of these spores was followed for al-

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most a year. Scanning electron microscopy (SEM) was used to observe abnormal spores with extra valves.

## MATERIALS AND METHODS

Over a period of 10 months, 579 specimens of black anglerfish (*Lophius budegassa*) caught in the Mediterranean Sea off the coast of Barcelona (NE Spain) were purchased from fishermen, transported to the laboratory at 4°C and examined immediately. A drop of bile fluid from the gallbladder of each fish was observed in a bright field and phase contrast microscope, to detect, classify and quantify abnormal spores of *C. appendiculata*.

For the scanning electron microscopy (SEM) study, spores were fixed in 2.5% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.2, 4°C), washed in the same buffer, postfixed in 2% OsO<sub>4</sub> (12 h), dried in air and coated with gold-palladium. Observations were made on a SEM Hitachi S 2300 at 15 kV.

Two  $\chi^2$  tests were carried out (one for bivalvular spores and the other for trivalvular spores) to analyse whether there were significant differences among the proportions of spores in samples from different months. The  $\chi^2$  test was not undertaken for tetra-valvular spores, as validity conditions were not met, due to the low number of spores observed (Martín and Luna 2004).

## RESULTS

The results are shown in Table 1. A total of 18,326 spores of *C. appendiculata* were examined, classified and counted. Of these, 98.31% were bivalvular spores (normal) (Fig. 1); 1.63% trivalvular spores (Fig. 2); and just 0.06% of the total were tetra-valvular spores (Fig. 3). Three-valved spores were the most common abnormal form. They were found in all months of the study, apart from December. In some months, they constituted a significant percentage of the total number of spores. For example, in May and November they reached almost 10% of the total. There were considerably less tetra-valvular spores. No tetra-valvular spores were detected in 6 out of 10 of the months studied. Their highest numbers were reached in November, when they represented 0.39% of the monthly total.

The results of the  $\chi^2$  tests were as follows: bivalvular spores:  $\chi^2_{\text{exp}} = 15.24$  and trivalvular spores:  $\chi^2_{\text{exp}} = 875.21$ . The monthly differences between trivalvular spores were significant.

Table 1 shows that during the coldest months of the year, the proportion of trivalvular spores remain at relatively low and constant levels, and even disappear completely (0.0% in December). The rest of the year a stage

begins with strong ascents and sudden decreases, with maximums every three months (May: 9.90%, August: 4.41% and November: 9.83%).

The study of these spores with the aid of a scanning electron microscope revealed that trivalvular spores had two dehiscence lines that merge into one line at a given point (Fig. 4). These spores have a totally smooth valve surface, as do normal bivalvular spores. The apices of the valves are rounded off. We could not observe the ultrastructure of tetra-valvular spores, as no such spores were found in the samples taken for SEM.

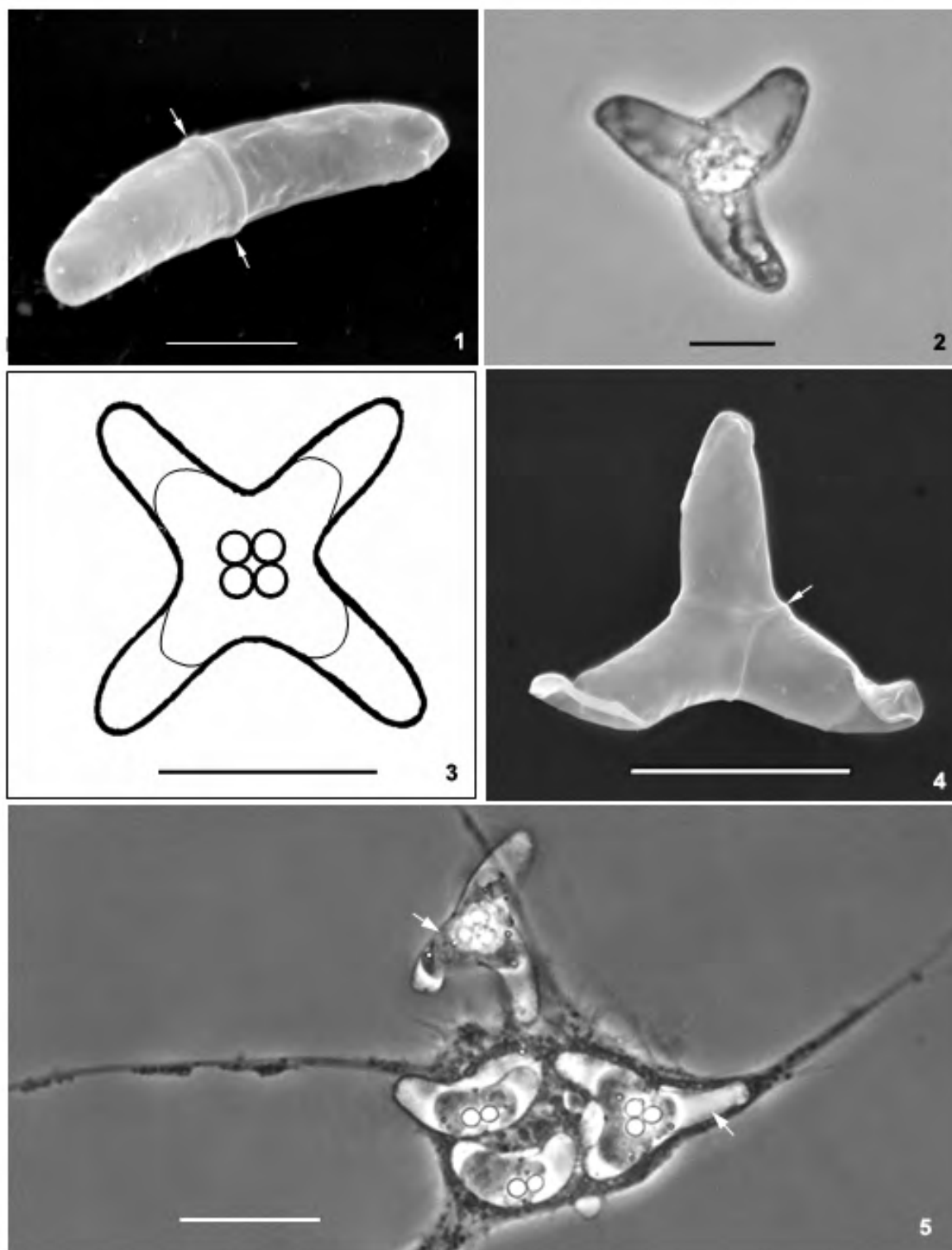
Occasionally some polysporous trophozoites produce spores with three or four valves together with normal spores (Fig 5).

## DISCUSSION

Abnormal spores of Myxosporidia can have very varied forms, as shown in a detailed study by Thélohan (1895). According to this author, the following types of anomalies can be found in Myxosporidia spores: 1) anomalies in the spore covering, 2) anomalies related to the polar capsules (in the position, in the number, in the protoplasm) and 3) anomalies that affect all of the elements of the spore simultaneously.

Intriguingly, in his classification of anomalies, Thélohan did not refer to the existence of spores with extra valves – the focus of this paper. However, we know for certain that he observed such spores, as he refers to them in his work (1895). In addition, his paper contains an illustration of a typical trivalvular spore, similar to those that we have studied (Figs 2, 4). It is an abnormal spore from the species *Ceratomyxa truncata*, found by Thélohan in the gallbladder of the sardine. This author confirms that abnormal spores are particularly common in the genus *Ceratomyxa*. However, Thélohan did not mention the existence of anomalous tetra-valvular spores.

Meglitsch (1960) stated that the presence of tri-valvular spores (which he calls triads) is a common anomaly in the genus *Ceratomyxa*. He detected triads in 6 species (*C. minuta*, *C. castigatoides*, *C. hama*, *C. vepallida*, *C. constricta* and *C. polymorpha*) of the 28 included in his study, i.e., triads appeared in 21.4% of the analysed species. This author asserts that triads are more frequent in some species than in others. He also discovered that triads were common in the cold months and much less common in the hotter months – at least in some of the species studied. This data is, to a certain



**Figs 1–5.** Variability of *Ceratomyxa appendiculata* spores. **1** – bivalvular spore (normal spore). Valve ends are always rounded, with smooth wall. The suture line (arrow) forms a thick strand that goes around the central part of the spore. SEM; **2** – trivalvular spore. Phase contrast. Bar; **3** – line drawing of a tetravalvular spore; **4** – trivalvular spore. Valve ends are rounded, with smooth wall. Note sutural lines that merge into one line (arrow). SEM; **5** – sporulated polysporous trophozoite with two normal bivalvular spores and one trivalvular spore (arrow) with three polar capsules. Note another trivalvular spore (arrow) next to the trophozoite. Phase contrast. Scale bars: 5  $\mu\text{m}$  (1), 10  $\mu\text{m}$  (2, 3, 4), 20  $\mu\text{m}$  (5).

**Table 1.** Classification and quantification of different types of *C. appendiculata* spores. bivalv. – bivalvular spores, trivalv. – trivalvular spores, tetra-val. – tetra-valvular spores, % – percentage.

Month	Total spores	Total bivalv.	Total trivalv.	Total tetra-val.	% bivalv spores	% trivalv. spores	% tetra-val. spores
February	1,000	982	17	1	98.20	1.70	0.10
March	1,656	1,629	27	0	98.37	1.63	0.00
April	204	201	3	0	98.53	1.47	0.00
May	980	880	97	3	89.80	9.90	0.31
June	2,308	2,296	12	0	99.48	0.52	0.00
July	812	799	11	2	98.40	1.35	0.25
August	363	346	16	1	95.32	4.41	0.28
October	9,900	9,864	35	1	99.64	0.35	0.01
November	763	685	75	3	89.78	9.83	0.39
December	340	340	0	0	100.00	0.00	0.00
TOTAL	18,326	18,022	293	11	98.31	1.63	0.06

extent, in contrast with our results (Table 1). We found that the frequency of trivalvular spores was at its lowest in the coldest month (December). This variation in results may be due to differences in the biology of the hosts or to physical-chemical factors in the environment. Meglitsch (1960) also provided data on the frequency of triads in some species. In *C. minuta* they represented 5% of the population of total spores. In some winter samples of *C. polymorpha*, numbers reached between 60–70% of the total. In some cases, triads disappeared completely in the summer. Parisi (1912) found 20% of triads and 70% of tetrads in *C. truncata*. As we can see, these frequencies are much higher than those we found in *C. appendiculata*, in which the average number of triads was only 1.63% of the total spores, and the highest value did not reach 10% of the total.

With respect to the tetra-valvular spores (or tetrads, according to Meglitsch's naming system), Meglitsch states that they are never common and have never been found to be widespread in a particular species. In fact, he only mentions their appearance in *C. polymorpha*, which represents an incidence in only 3.6% of the analysed species. However, although never abundant, they reached up to 5% of the total spores in some of analysed samples. In contrast, in *C. appendiculata* we found a maximum of only 0.39% four-valved spores.

In our observations, all of the three-valved spores of *C. appendiculata* had three polar capsules and all four-valved spores had four. This corresponds to the most normal situation, and can be considered a general

characteristic in the case of extra structures in the genus *Ceratomyxa*. In fact, Meglitsch (1960) states that he found this situation in all of the thousands of triads and tetrads that he examined. This suggests that these two spore characteristics are not independent. Instead, any factor that leads to the formation of an extra valve also leads to the appearance of a corresponding extra polar capsule. These results are in contrast with cases of spores that have a normal number of valves but extra polar capsules, as mentioned by Thélohan. However, Thélohan's examples were from the genera *Chloromyxum* and *Myxobolus*. Thus, spore development in different genera may facilitate the appearance of certain types of anomalies.

Our observation that one trophozoite can simultaneously be the source of triads and normal spores (Fig. 5) is also confirmed by Meglitsch, who nevertheless states that such a situation is not normal (he only observed it in 6 cases). According to Meglitsch, in species in which triads are common, when a trophozoite contains a triad it normally also contains another one.

The reasons for these aberrations are not clear. According to Shul'man (1990), in some cases they could be due to an atypical location of the cyst or to parasitisation of an inappropriate host. Shul'man (1990) considers that the many aberrations and anomalies in Myxosporidia constitute a major source of variability that could have played an important role in the evolution of the species. This author theorises that the appearance of tri- and tetra-valvular spores in *Ceratomyxa* may

have facilitated their ability to float (one of the main lines of evolution of the Myxosporidia) so that they can spend more time between two bodies of water and sink more slowly to the bottom. This fact might facilitate the ingestion of the spores for the possible intermediary hosts, since it happens with *Ceratomyxa auerbachii* and the sabellid polychaete *Chone infundibuliformis* (Køie *et al.* 2008).

For Shul'man, some of the current genera of marine multivalvulids, such as *Trilospora* (with three valves) and *Kudoa* (with four valves) could have originated from ancestral forms of *Ceratomyxa*, given their similarity to the triads and tetrads of current species of *Ceratomyxa*. In short, according to this author, the variability of the Myxosporidia has a high ecological significance, as it has enabled these organisms to occupy a wide range of ecological niches. Thus, for example, a simple change in the form or size of a spore can increase its ability to float, facilitating the completion of its life cycle. In Shul'man's opinion, the wide variability in these organisms has great evolutionary significance.

**Acknowledgements.** This study was supported by Generalitat of Catalonia's Aquaculture R&D and innovation Reference Network. Professor Dr. M<sup>a</sup> del Pilar Gracia from Department of Animal Biology of the University of Barcelona (Spain) is warmly acknowledged for her unconditional help. The author also wish to express his sincere gratitude to the staff of the Scientific-Technical Services of the University of Barcelona for their technical assistance.

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Received on 12<sup>th</sup> September, 2008; revised version on 26<sup>th</sup> December, 2008; accepted on 17<sup>th</sup> January, 2009

