# Spore Structure of *Minchinia chitonis*

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*ABSTRACT— The salient features of the fine structure of the spore of* Minchinia chitonis *(Lankester, 1885) Labbé, 1896 are reviewed. The morphological characteristics are similar to those of the spores of other members of the family Haplosporidiidae, particularly the hinged operculum covering the anterior orifice, the "spherule" and the haplosporosomes.*

## Introduction

The genus *Minchinia* was established by Labbé (1896) for a haplosporidian parasite of the chiton, *Lepidochitona cinereus.* The parasite, originally named *Klossia chitonis* by Lankester in 1885, is the type species (Sprague, 1963). *Minchinia chitonis* was the object of special studies by Pixell-Goodrich (1915), Debaisieux (1920), and King (1926) and has recently been reexamined by Ball and Neville (1979) and Ball (1980). This paper briefly reviews the information about the structure of the spore of this parasite, since the fine structural investigations have been directed toward this stage in the life cycle rather than the developmental stages.

The impetus to study the ultrastructure of the haplosporidia was the discovery of the pathogenicity of *H. costale* (Andrews et al., 1962; Wood and Andrews, 1962) and *H. nelsoni* (Haskin et al., 1966) to the oyster, *Crassostrea virginica.* The history of these oyster diseases has been reviewed by Andrews (1979). It is to Ormières and de Puytorac (1968), Perkins (1968, 1969), and Rosenfield et al. (1969) that we owe our first clear understanding of the morphology of the spores of *Minchinia* spp. and their similarity in structure. Electron microscope studies have revealed a number of new structures which help to confirm the interrelation of the genus *Minchinia* and other genera within the class Stellatosporea Sprague, 1979, formerly called Haplosporea.

## Materials and Methods

Infected chitons were collected from the Plymouth area. For light microscopy, infected digestive gland was fixed in aqueous Bouin's solution and stained with Mallory's Triple stain. For electron microscopy, small pieces of infected digestive gland and foot were fixed in 2.5 percent (volume/volume) glutaraldehyde in 0.1M sodium cacodylate buffer at pH 7.2 for 2 hours at 4°C and processed as previously described (Ball, 1980).

## Results

The basic structure and organelles of the spore *M. chitonis* as revealed by electron microscopy are depicted in Figure 1.

The spores are oval with a flattened pole covered by a hinged lid which rests on a circumferential flange of the spore wall (Fig. 2, 3, 6). The mature spores are remarkably uniform in size measuring 9.0-11.0 $\mu$m in length and 6.0-8.0 $\mu$m in width. Heavily infected chitons can be diagnosed macroscopically by areas of brown coloration on the foot and gills due to aggregates of sporocysts containing mature spores.

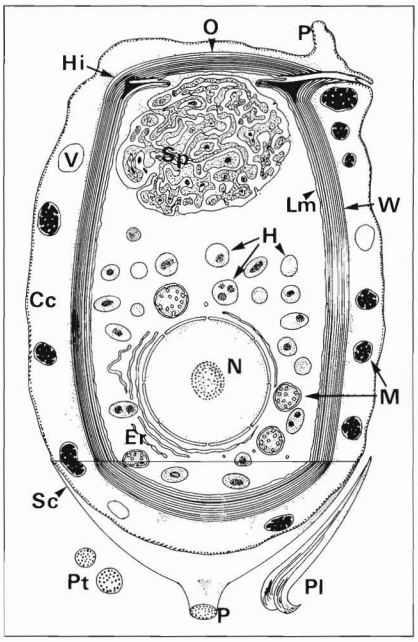


Figure 1.—Diagrammatic representation of the spore of *M. chitonis.*

In section, the smooth resistant laminated wall of the spore appears to be of uniform thickness of approximately 300 nm (Fig. 4). The outer cytoplasmic envelope contains mitochondria in the maturing spore and is strengthened by short microtubule-like filaments (Fig. 6). The extraspore cytoplasm is extended at both the poles to produce two long projections (Pixell-Goodrich, 1915; Debaisieux, 1920; Ball and Neville, 1979).

The nucleus of the spore is typically eukaryotic with a two membrane envelope, nuclear pores, and a prominent nucleolus and the cytoplasm contains smooth endoplasmic reticulum, mitochondria, and ribosomes (Fig. 1, 4, 6). Close to the lid is the organelle identified by light microscopy and called the "spherule." Its fine structure is a twisted laminated double membrane or vesicular organelle (Fig. 5, 6) considered by Perkins (1968, 1969) and Rosenfield et al. (1969) to possibly represent a Golgi apparatus.

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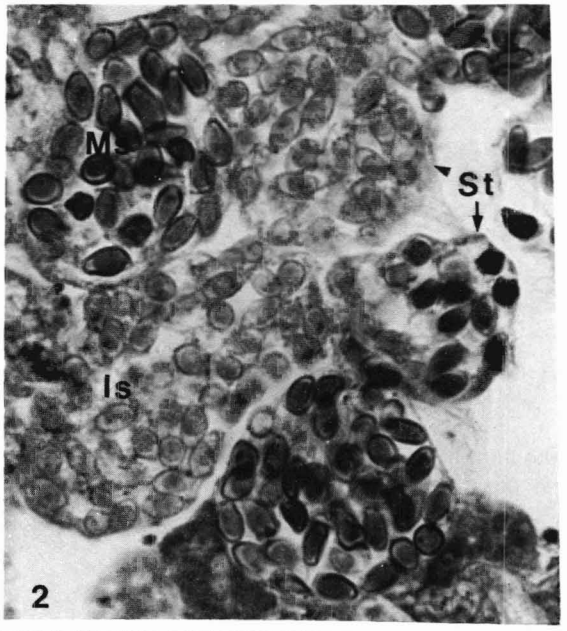
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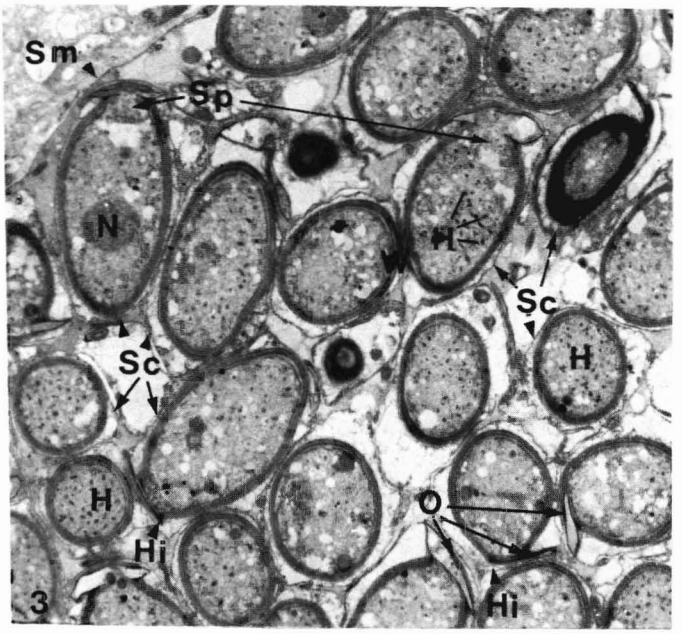
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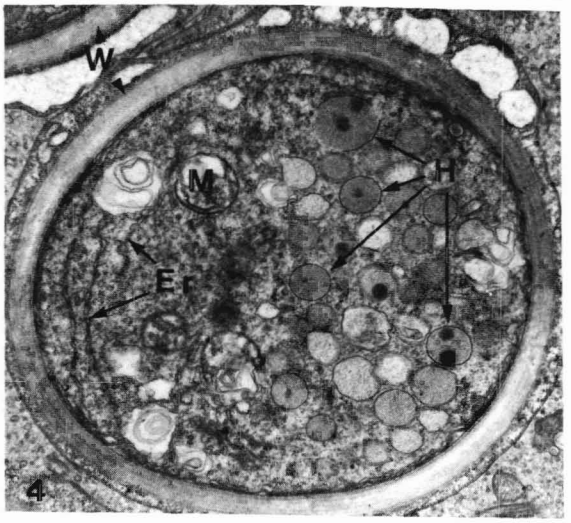
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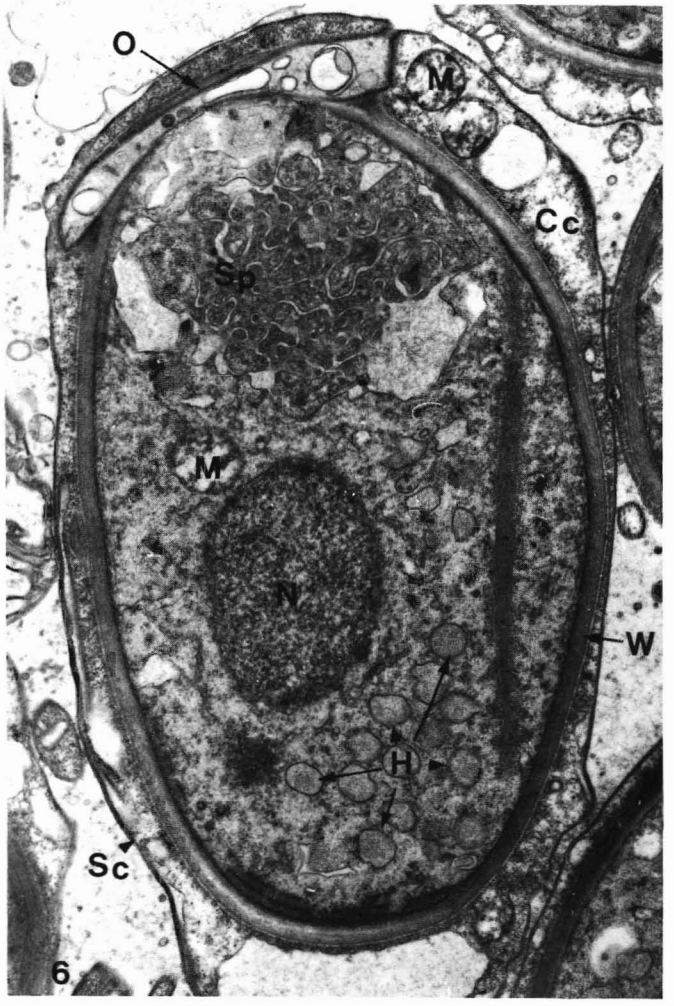
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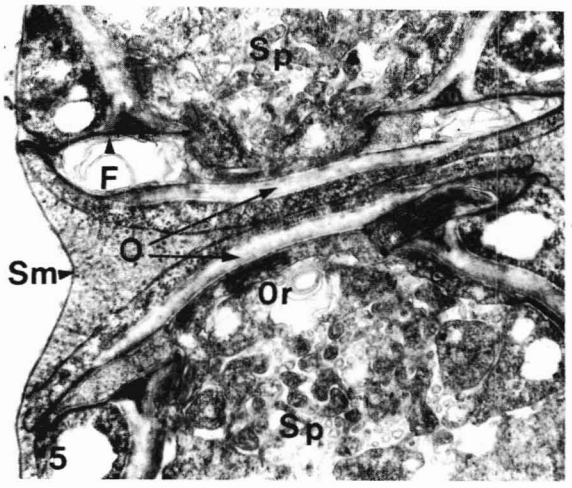
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Figure 2.—Light micrograph section of spores and sporocysts in the digestive gland of the chiton (600×).

Figure 3-6.—Electron micrographs of nearly mature spores of *M. chitonis.*

Figure 3.—Section of part of sporocyst showing developing spores each surrounded by a cytoplasmic capsule (3,000×).

Figure 4.—Cross section showing haplosporosomes and endoplasmic reticulum (15,600×).

Figure 5.—Sagittal section through anterior region showing lids, flanges, and sperules (18,000×).

Figure 6.—Slightly oblique longitudinal section to show haplosporosomes, spherule, and capsule (16,000×).

Abbreviations used in labelling. Cytoplasm of spore capsule (Cc); endoplasmic reticulum (Er); flange (F); haplosporosomes (H); hinge (Hi); immature spores (Im); limiting membrane of spore cytoplasm (Lm); mitochondrion (M); mature spores (Ms); nucleus (N); operculum (O); orifice of spore (Or); projection (P:t = transverse, l = longitudinal); spore capsule (Sc); sporocyst membrane (Sm); spherule (Sp); sporocyst (St); vacuole (V); wall (W).

Haplosporosomes (Perkins, 1971) are a constant feature scattered throughout the cytoplasm of the maturing (Fig. 3, 4, 6) and mature spore. They are electron pale vesicles often containing a single or occasionally up to three osmiophilic core(s). In section, the profiles of the haplosporosomes were either spherical, with a diameter ranging from 250 to 583 nm ($\bar{x}$ = 373 nm; $n$ = 50), or oblate spheroid with a length from 267 to 563 nm ($\bar{x}$ = 413 nm; $n$ = 50) and a width range of 233 to 444 nm ($\bar{x}$ = 329 nm; $n$ = 50).

## Discussion

In the last 12 years, studies of the fine structure of the Stellatosporea, particularly of the spores, have produced a number of new findings and advances in the knowledge of this group. Although the haplosporidian cell has many organelles similar to those in other cells, it has certain ultrastructural characteristics that distinguish it from other protozoans. These typical fine structural features have so far been found predominantly in the spore stage and are the operculum, "spherule," and haplosporosomes. The discovery of these fine structures and organelles resulted in their characteristics being used to reinforce the systematic position of this group of protozoans and as a basis for determining the relationship of various genera. The close relationship between the genera *Minchinia, Urosporidium,* and *Haplosporidium* has been established (Perkins, 1979) and the order Haplosporida to which they belong has been renamed Balanosporida by Sprague (1979).

Although the spore of *M. chitonis* is similar in its fine structure to the spores of other balanosporidans, the characteristic which distinguishes it from all but *M. armoricana* found in *Ostrea edulis* by van Banning (1977) is its two long extensions of the extraspore cytoplasm. In addition, both these parasites color the host brown when the spores are present in large numbers.

In 1963, Sprague reestablished the genus *Minchinia* and transferred several species to it from the genus *Haplosporidium.* He pointed out in 1970 that the electron microscope studies on the development of the spores of some members of these two genera had shown a similarity between them which invalidated his criteria for distinguishing them on the difference of the origin of the lids. In 1978, he reaffirmed an earlier suggestion (Sprague, 1970) and transferred the species with spores without projections (tails) to the genus *Haplosporidium.* This leaves only two species in the genus *Minchinia,* namely *M. armoricana* and *M. chitonis,* presumably defined as balanosporidans of the family Haplosporidiidae having spores with both orifice and operculum and two projections from the extraspore cytoplasm.

The mechanism of wall formation has not yet been determined and the significance of the wall ornamentation and the two projections of the wall is not known. Perkins (1979) pointed out that in the *Minchinia* spp. which he has examined the extraspore cytoplasm disperses leaving the spore surrounded by threads or ribbons. He made the interesting suggestion that the substructure of the wall ornaments might be species specific. The microtubule-like filaments seen in the extraspore cytoplasm of *M. chitonis* have not been recorded from other haplosporidans, but dispersal of the cytoplasm revealing this ornamentation has not so far been observed.

*Minchinia chitonis* appears to be host specific and is found in various organs and tissues of *L. cinereus* where it causes displacement of cells and destruction by volume alone. However, there is little obvious evidence of pathogenicity although the reproductive potential of infected hosts may be adversely affected. The many unanswered questions concerning *M. chitonis* are the same as those for the other balanosporidans, the main one being the elucidation of the life cycle with particular reference to the infectivity of the spore and its transmission.

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