

# Fine Structure of the Microsporidan *Abelspora portugalensis* gen.n., sp.n. (Microsporida) Parasite of the Hepatopancreas of *Carcinus maenas* (Crustacea, Decapoda)

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A new species of a microsporidan, *Abelspora portugalensis*, was found in the hepatopancreas of *Carcinus maenas*, forming white xenomas. Each xenoma seems to consist of an aggregate of hypertrophic host cells in which the parasite develops and proliferates. This cytozoic microsporidan being characterized by one uninucleate schizont giving rise to two sporonts, each originating two sporoblasts, resulting in two spores within a persistent sporophorous vacuole (pansporoblast) should be included in a new family *Abelsporidae*. In fresh smears most spores were 3.1–3.2  $\mu\text{m}$  long and 1.2–1.4  $\mu\text{m}$  wide. Fixed, stained, and observed in SUS mature spores measured  $3.1 \pm 0.08 \times 1.3 \pm 0.06 \mu\text{m}$  ( $n = 25$  measurements). Spore cytoplasm was dense and granular, polyribosomes were arranged in helicoidal tape form. The polar filament was anisofilar and consisted of a single coil with 5–6 turns. The anchoring disc and the anterior zone of the filament are surrounded by the polaroplast composed of two usual zones. In the anterior zone, the membrane of the polar filament is in continuity with the membranes of the polaroplast. The appearance of a microsporidan with described nuclear divisions in life cycle, spores shape and size, polaroplast and polar filament morphology and identity of the host suggests that we may erect a new genus *Abelspora* and a new species *A. portugalensis* (Portugal = Portucalem). © 1987 Academic Press, Inc.

**KEY WORDS:** *Abelspora portugalensis* gen. n., sp. n.; *Abelsporidae* fam. n.; microsporida; ultrastructure; life cycle; hepatopancreas; *Carcinus maenas*, Crustacea, Decapoda.

## INTRODUCTION

Several species of the order Microsporida occur as hyperparasites (Canning and Nicholas, 1974; Canning et al., 1974; Canning, 1975; Sprague, 1982; Azevedo and Corral, 1985) or, as parasites in several animal groups (Canning, 1976; Sprague, 1977; Canning et al., 1982). Most of these microsporidians were found to be pathogenic to their hosts causing destruction of the cellular organization.

Some of the most important taxonomic characters are the multiplication phases of the parasite (merogony and sporogony) and consequently, the final number of the resulting spores. These characters are usually used as significant particularities in the taxonomic determinations of the family (Sprague, 1982).

The present study described some light and ultrastructural aspects of the life cycle and spore maturation phases of a new

species of a microsporidan, *Abelspora portugalensis*. Its specific morphological characters and its taxonomic positions are discussed.

## MATERIALS AND METHODS

Infected adult specimens of *Carcinus maenas* (Crustacea, Decapoda) used in this study were collected at low tide from the lake region of Aveiro (Portuguese town, 60 km South of Oporto), during the last 3 years.

The specimens were dissected and the infected hepatopancreata containing the xenomas (cysts) were removed. For ultrastructural studies, small fragments of the cysts, containing numerous spores and different life cycle stages, were fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer in filtered sea water, pH 7.6, at 4°C for 3 hr, rinsed overnight in the same buffer, and postfixed in 2% OsO<sub>4</sub> in the same buffer at

4°C for 3 hr. After dehydration in a graded ethanol series the fragments were transferred to propylene oxide and embedded in Epon. Semithin sections for light microscopy were stained with methylene blue-azur II. Ultrathin sections (UTS) were stained with uranyl acetate and lead citrate and were examined in a JEOL 100B and a JEOL 100CXII TEM, operated at 80 kV.

Some spores fixed under the same previously described conditions were dehydrated in a graded ethanol series, dried by critical-point freezing and coated with gold and examined and photographed in a JEOL 35S scanning electron microscope (SEM) operated at 20 kV.

## RESULTS

### *Light Microscopy*

A great number of *Carcinus maenas* were parasitized by a large quantity of spores and different life cycle stages of the parasite, contained in white cysts (xenomas) (Figs. 1, 2). These xenomas are irregularly dispersed in the hepatopancreas more frequently observed at the periphery. They contain numerous microsporocysts generally spherical (Fig. 1), consisting of a great quantity of hypertrophic host cells in which the parasite develops and proliferates. The proliferative forms of the different stages of the life cycle develop directly within the host cytoplasm (Fig. 2).

Observed at high magnification in semithin sections the spores are contained two by two in a cytoplasm vacuole of the host cell (Fig. 2). Live mature spores observed in smears without stain are ellipsoid, 3.1–3.2  $\mu\text{m}$  long and 1.2–1.4  $\mu\text{m}$  wide.

### *Electron Microscopy*

In all ultrathin sections (UTS) it was possible to observe the different life cycle stages embedded in the host cell cytoplasm near the mature spores (Fig. 3). The schizonts are uninucleate, the nucleus showing sometimes an irregular nucleolus and few chromatin. Later, centriolar plaques consisting of an electron dense

disc, begin their differentiation around the nucleus (Fig. 3). During further development the nucleus of the schizont divides by binary fission and the two daughter nuclei migrate to the opposite pole of the cell (Fig. 4). This nuclear division is immediately followed by cytokinesis, giving rise to two sporonts that progressively get isolated in the host cytoplasm. This schizogony is more frequently observed at the periphery of the xenomas (Fig. 4). The cytoplasm, containing numerous free ribosomes and RER, is limited by a thin plasmalemma in close contact with the host cell cytoplasm (Fig. 4).

The sporonts were ultrastructurally characterized by the appearance of an incomplete coat of amorphous electron-dense material external to the plasmalemma (Fig. 5). During this stage several great vesicles appeared in close contact with the plasmalemma. These vesicles of different diameters contain a matrix of flocculent material with one to two cores of dense material (Fig. 5).

At high magnification it is possible to observe the region of contact with the vesicle membrane and plasmalemma. The wall material is deposited between two of these membranes (Fig. 5, inset). These vesicles seem to be the primordia of the sporophorous vacuole (pansporoblast), that begins its differentiation at this stage (Fig. 5).

During this stage the uninucleate sporont begins its nuclear division as signalled by the appearance of mitotic figures. The nucleus becomes elongated and a microtubular manchette is differentiated in the nucleoplasm. The bundle of microtubules is attached in each extremity to the spindle pole dense bodies located in the inner nucleoplasm near the internal nuclear membrane (Fig. 5). In the cytoplasm a well-developed RER, numerous ribosomes and some vesicles are developed (Fig. 5). After nuclear division followed by cytokinesis, each sporont gives rise to two sporoblasts within a sporophorous vacuole (Fig. 6).

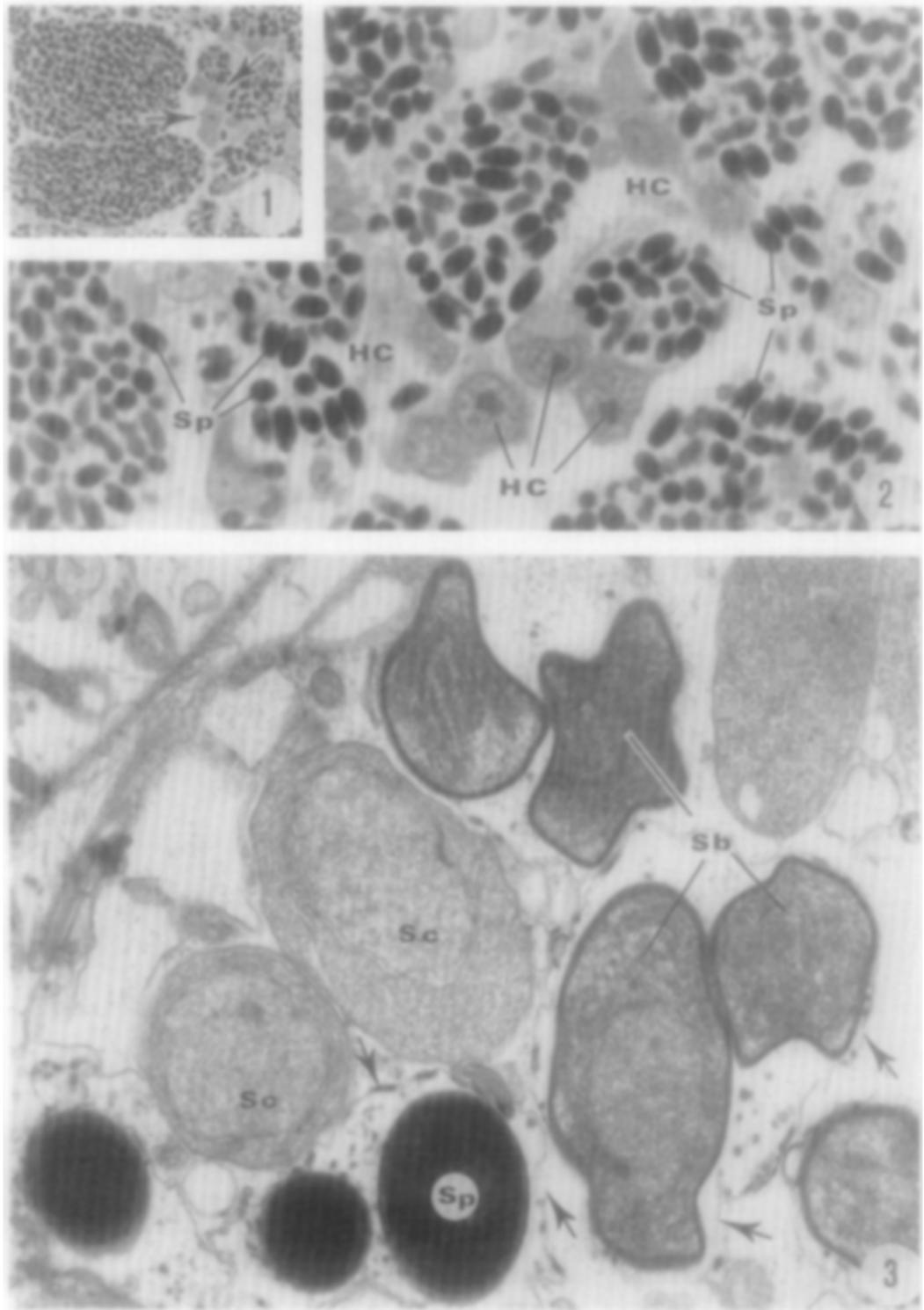


FIG. 1. Semithin section (STS) of several spore cysts, each containing different groups of numerous spores and host cells (arrows).  $\times 60$ .

FIG. 2. STS of some host cells (HC) showing some spores (Sp) within pansporoblastic vacuole.  $\times 230$ .

FIG. 3. Ultrathin section (UTS) showing different stages of life cycle: Sc—schizonts; Sb—sporoblasts; and Sp—spores surrounded by pansporoblastic membranes (arrows).  $\times 19,600$ .

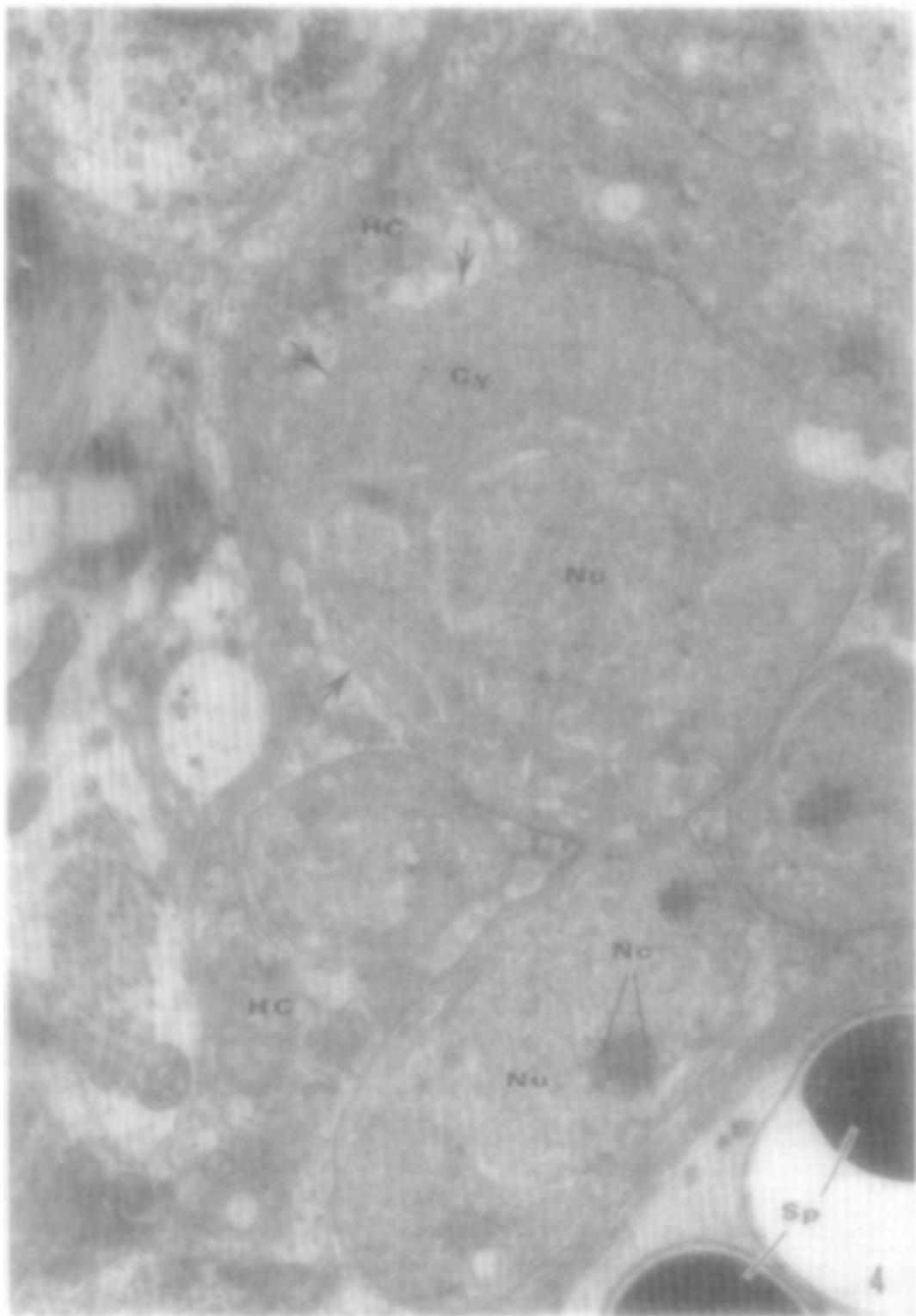


FIG. 4. UTS of a dividing binucleate schizont showing its strangulated shape during cytokinesis. The nuclei (Nu) and the nucleolus (Nc) are well evident. Cy—cytoplasm; Sp—spores.  $\times 24,000$ .

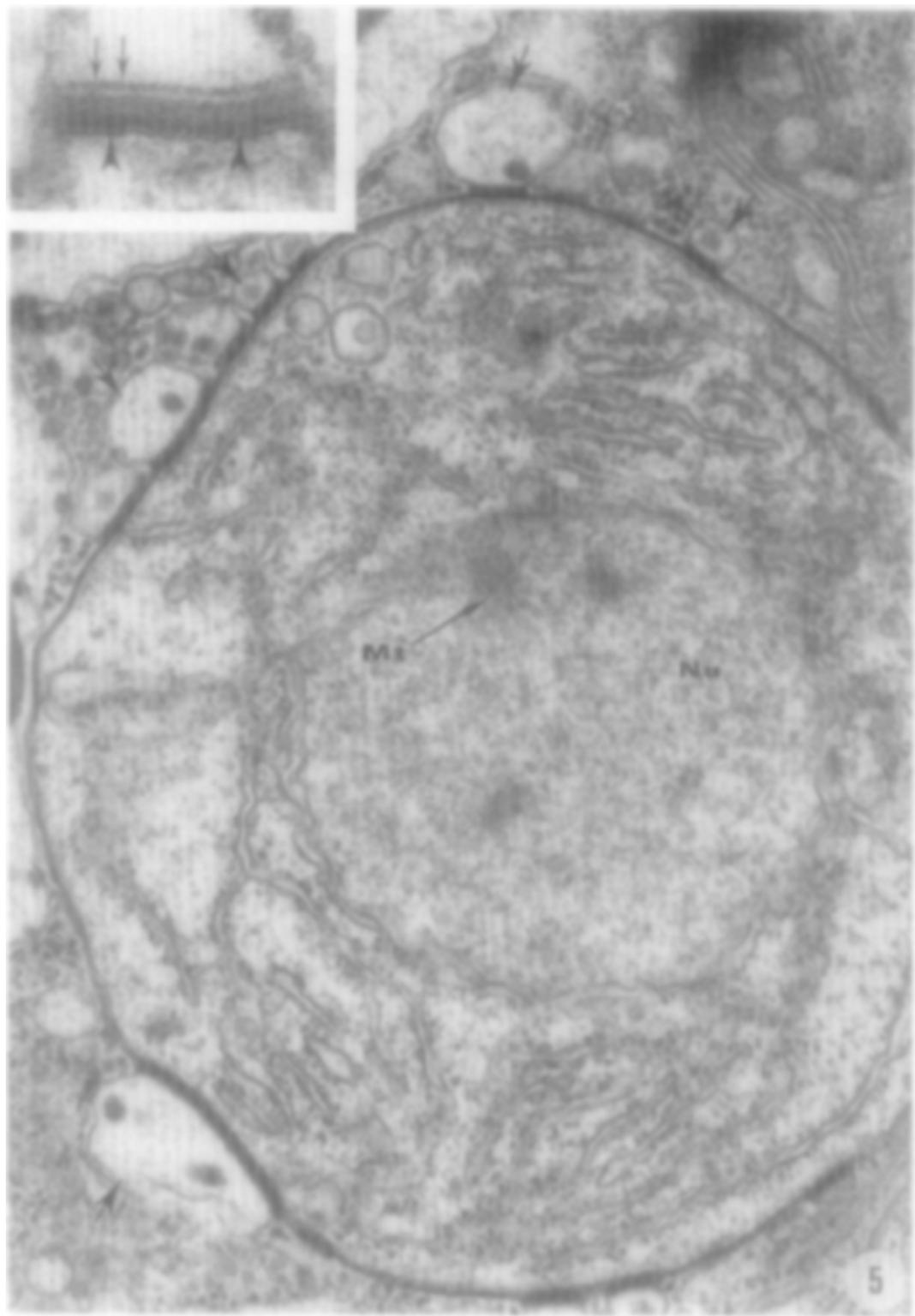


FIG. 5. UTS of a sporont showing the beginning of the nuclear division characterized by the differentiation of a nuclear microtubule manchette (Mt). In contact with the wall it is possible to observe some well-developed vesicles (arrows), that seem to be the primordia of the envelope of the sporophorous vacuole (pansporoblast).  $\times 52,700$ . Inset. Detail of the peripheral vesicles showing the internal wall membrane (arrow heads) and the vesicular membrane (double arrows).  $\times 210,000$ .

Each sporoblast becomes elongated and contains an irregular nucleus with a central nucleolus. The cytoplasm presents some vesicles irregularly distributed among numerous ribosomes. The RER is less evident. The wall consists of a complete and dense thick layer in contact with the light matrix of the sporophorous vacuole (Fig. 6). The cytoplasm of the host cell presents numerous vesicles and vacuoles of different diameters (Fig. 6). The sporoblasts developed directly into spores.

During the maturation phase the *two spores* enclosed in the sporophorous vacuole become gradually denser. At the final phase of maturation the spore has an ellipsoid form which when observed in fresh preparations or in SEM measures  $3.0 \times 1.4 \mu\text{m}$  in diameter (Fig. 7) and when observed in ultrathin serial sections (UTS) (25 measurements),  $3.1 \pm 0.08 \times 1.3 \pm 0.06 \mu\text{m}$  (Fig. 8). This species shows a similar organization described in different microsporidan species. The most important features are: (a) The spore wall is composed of two layers. It is  $\sim 40 \text{ nm}$  thick. The external layer is denser than the inner one, which is in close contact with the plasmalemma (Figs. 8–12). (b) The extrusion apparatus is composed of the apical anchoring disc and the polar sac which overlies the manubroid portion of the PF (Figs. 8, 9). This last structure is completely surrounded by the dense lamellar structure, the polaroplast, which is connected with the PF. At high magnification it is possible to observe that the manubroid part of the PF is in continuity with the polaroplast membrane, forming a well-organized membranous structure (Figs. 9, 12).

A central nucleus is surrounded by four to five layers of helically arranged aggregates of polyribosomes which lay peripherally in a relative dense cytoplasm near the plasmalemma (Fig. 10).

The posterior part of the PF was coiled in a single layer beneath the spore wall. The filament was anisofilar with 5–6 coils, the diameter of the largest one being  $\sim 50$

nm, and gradually tapers to  $\sim 40 \text{ nm}$  (Fig. 11).

## DISCUSSION

The ultrastructural organization observed in the spores of the species herein described, corresponds to the phylum Microspora, class Microsporea and order Microsporida (Levine et al., 1980). On the other hand, the life cycle schematic drawing in Figure 13 shows the differentiation of a well-developed and persistent sporophorous vacuole which contains two spores, permitting us to identify the species with the suborder Pansporoblastina (Sprague, 1977; Levine et al., 1980). We have used the term vacuole (pansporoblastic vacuole), instead of vesicle, frequently used in other works (Canning and Hazard, 1982; Canning et al., 1982; Sprague, 1982; Larsson, 1983), because it seems to be ultrastructurally more correct. In our material, as in other described species this microsporidan structure, attains great diameters (sometimes 8–10  $\mu\text{m}$ ) and so, according to the classic ultrastructural nomenclature the term used should be vacuole. Besides, the diagnostic features of our observations included uninucleate schizonts, sporoblasts arising from binucleate schizonts, two uninucleate spores arising from an uninucleate sporoblast within a pansporoblastic membrane, and finally the host-cell hypertrophy. So, our results suggest that this parasite does not fit into any of the known microsporidan families (Sprague, 1977). For this reason, I propose a new family *Abelsporidae* and a new genus and new species *Abelspora portucalensis*.

### Family Generic and Specific Diagnosis

*Abelsporidae*. Microsporida are characterized by one uninucleate schizont giving rise to two sporonts, each giving rise to two sporoblasts within a sporophorous vacuole circumscribed by a persistent pansporoblastic membrane. This family is repre-

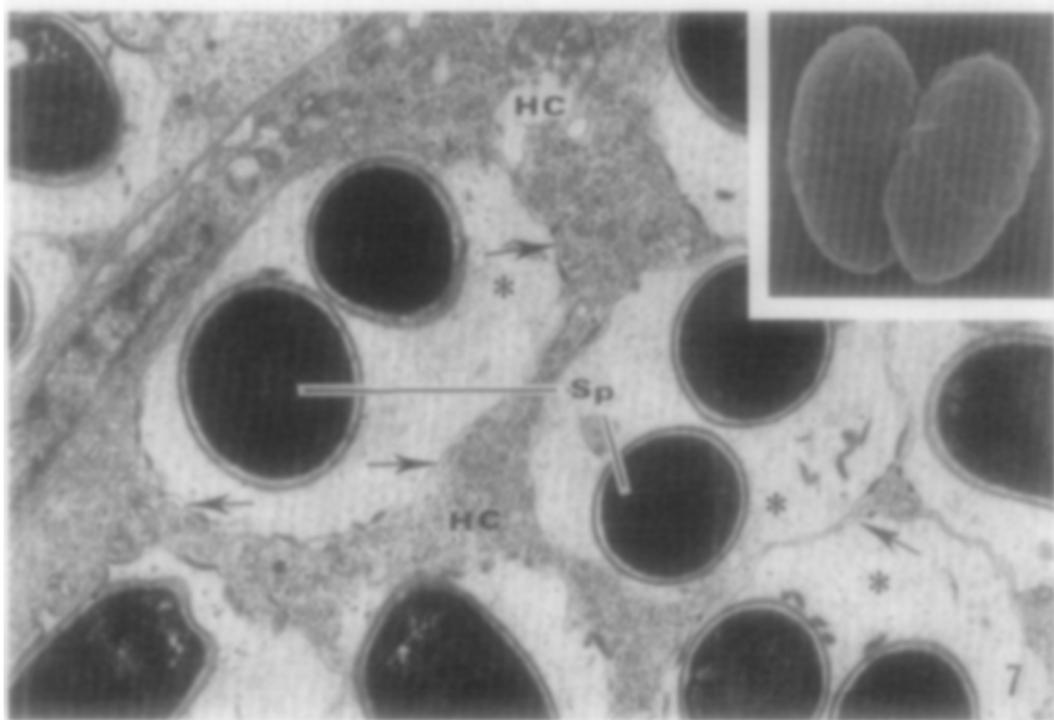
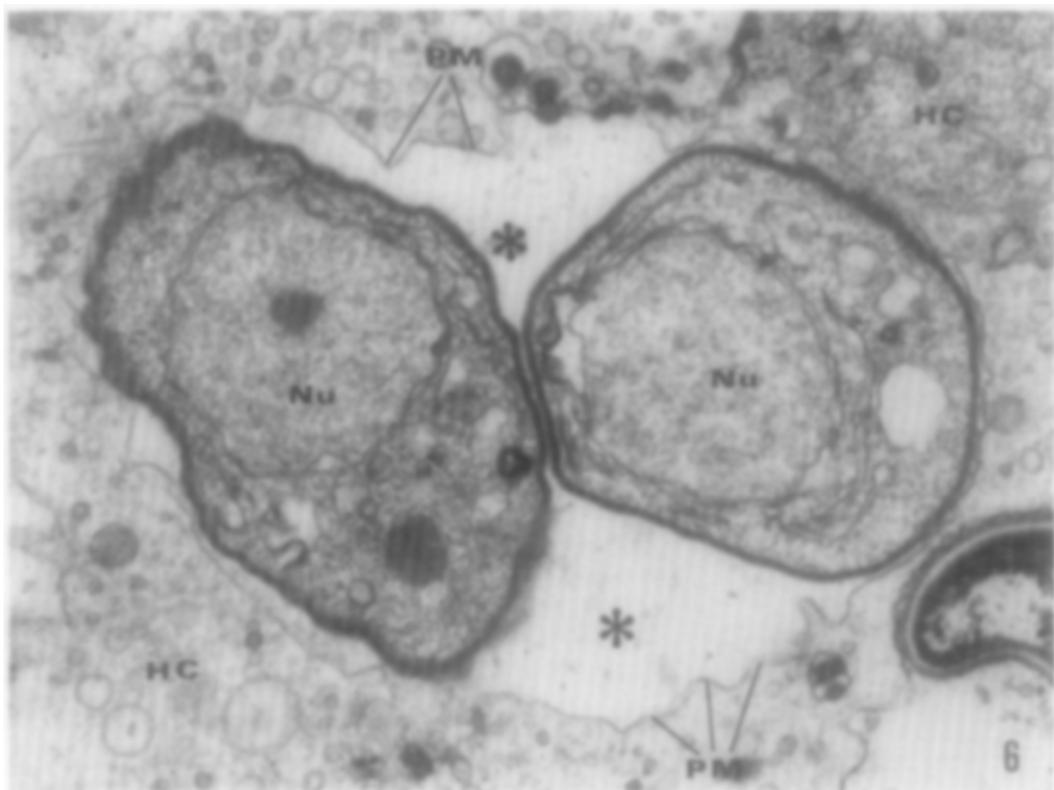
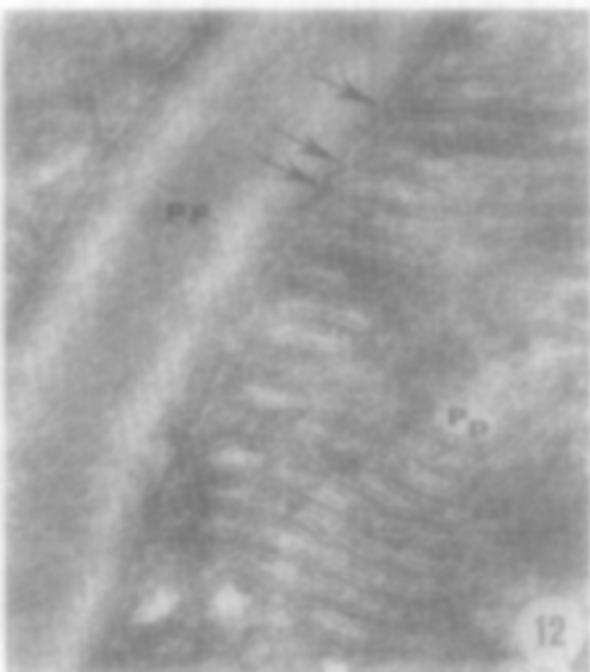
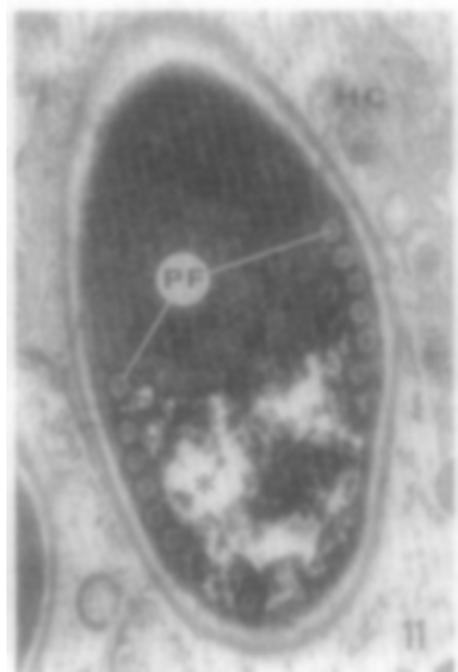
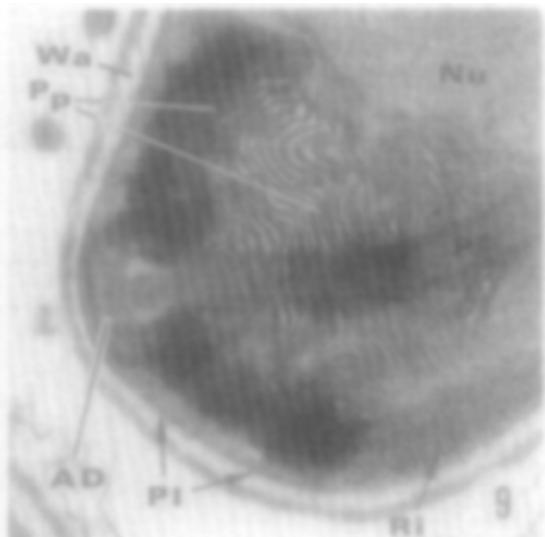


FIG. 6. UTS of two sporoblasts surrounded by the same pansporoblastic membrane (PM). The sporophorous vacuole presents a light matrix (\*) and the host cell (HC) shows several vesicles among other organelles.  $\times 28,300$ .

FIG. 7. UTS of some groups of two spores (Sp), two by two surrounded by the pansporoblastic membrane (arrows). The host cell (HC) shows a granular morphology.  $\times 16,580$ . Inset. Two mature spores of the same pansporoblastic vacuole observed in SEM.  $\times 10,600$ .



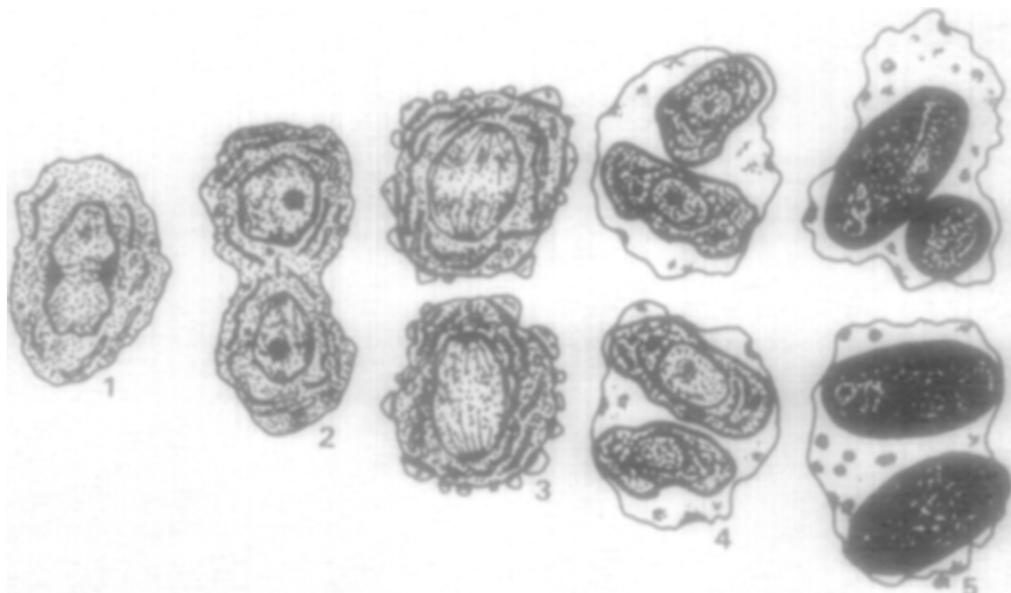


FIG. 13. Schematic drawing of the life cycle of *A. portocalensis*. 1-2—schizonts; 3—sporonts; 4—sporoblasts; 5—spores.

sented by a new genus (type genus) and a new species.

*Abelspora portocalensis* has the following salient features: uninucleate life cycle stages; spores are ellipsoid and measure  $3.1-3.2 \times 1.2-1.4 \mu\text{m}$  ( $n = 25$ ); the polar filament is coiled in a single row of 5–6 turns and is  $\sim 52 \mu\text{m}$  long; type host: hepatopancreas of *Carcinus maenas* (Crustacea); etymology: the generic name *Abelspora* is derived from Abel [name of an eminent Portuguese scientist named Abel Salazar (1889–1946)] + spora; the specific name *portocalensis* is obtained from the word portucalem—Portugal; type slide:

slides will be deposited in International Protozoan Type Slide Collection, Smithsonian Institution, Washington, DC. 20560, USA, and in the collection of the author.

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FIG. 8. Another aspect of a UTS of a mature spore in a favorable section showing the anchoring disc (AD), polar filament (PF), and polaroplast (Pp).  $\times 34,600$ .

FIG. 9. UTS of a detail of the anterior end of the spore, where it is possible to observe the wall (Wa), plasmalemma (Pl), anchoring disc (AD), polar filament (PF), polaroplast (Pp), and polyribosomes (Ri).  $\times 59,200$ .

FIG. 10. UTS showing a detail of the polyribosomes (Ri) organized in helical tape-like structure situated in the vicinity of the plasmalemma (Pl) and the wall.  $\times 36,000$ .

FIG. 11. Ultrathin longitudinal section of a mature spore that shows the transverse section of the polar filament (PF), situated laterally near a great vacuole (\*).  $\times 37,400$ .

FIG. 12. UTS of the polaroplast (Pp) showing its relationship with the polar filament (PF). Its membranes (arrows) are in continuity with the polaroplast lamellae.  $\times 296,000$ .

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