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Ultrastructural observations of the life cycle stages of *Ameson atlanticum* sp. nov., a microsporidan parasitizing *Cancer pagurus* L.

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Abstract. *Cancer pagurus* (Cancridae) from the Atlantic coast of France is parasitized by a new species of microsporida *Ameson atlanticum* sp. nov. The main stages of the life cycle of the parasite are a monokaryotic and then diplokaryotic meront, tetranucleate (at least) sporont (sporogonial sporont), uninucleate sporoblast and spore. The spore (1·9×1·5 μm) possesses 11–12 coils of the polar filament and a lamellar polaroplast. Hairlike appendages are present on the surface of the sporoblastic plasmodium, sporoblast and spore. All stages take place in direct contact with the muscular tissue of the crab. Infection provokes the destruction of 80% of host muscle myofibrils.

Introduction

Sprague (1977) listed only 11 microsporida from crabs. A new species was described by Azevedo (1987) in *Carcinus maenas* from the Portuguese Atlantic coast. During a study of parasitism in European crabs, the authors found the musculature of the commercially important species *Cancer pagurus* L. to be parasitized by a microsporidan belonging to the genus *Ameson* (Sprague 1977). Based on light and electron microscopy, this present paper describes this new species of *Ameson*. An ultrastructural study of the major stages of the parasite life cycle, including the spore, and their interactions with host cells, is presented.

Materials and methods

Several infected specimens of *Cancer pagurus* from the French Atlantic coast were collected (Roscoff, northern Brittany). For light microscopy (LM), smears of infected muscle were fixed in methanol and stained with eosin and methylene blue. For transmission electron microscopy (TEM), small pieces of infected muscle were dissected out, fixed in 3% glutaraldehyde in 0·1 M sodium cacodylate buffer, pH 7·4, at 4°C for 3 h, washed overnight, postfixed in 2% osmium tetroxide in the same buffer at 4°C for 3 h, rinsed, dehydrated through an ethanol series and embedded in Epon or Spurr's embedding medium. The semithin sections for LM were stained with toluidine blue and the ultrathin sections, after staining with aqueous solutions of uranyl acetate and lead citrate, were examined with a JEOL 100 CX II electron microscope at 60 kV.

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Results

Different life cycle stages of the parasite including immature and mature spores were seen within the same section of *C.pagurus* muscle (Figs 1 & 2).

Progressive infiltration of the myofibril-containing sarcoplasm by some life cycle stages, mainly meronts and sporonts, was observed. Electron micrographs show the details of meronts (Figs 2 & 3), sporonts (Figs 4 & 5), sporoblasts (Figs 6 & 7) and spores (Figs 8–10). All stages of the microsporida develop in direct contact with the host muscle fibres.

Merogony

The earliest stages observed were uninucleated meronts. The nuclei sometimes contained a central dense mass, possibly a nucleolus. The cytoplasm was granulo-fibrillar in appearance with rough endoplasmic reticulum cisternae (RER) and groups of free ribosomes (Fig. 3). Some elongate meronts were observed containing an elongated nucleus with evidence of nuclear division (spindle plaque and cluster of polar vesicles; spindle microtubules). Others were ellipsoid with two nuclei resulting from the nuclear division of a uninucleate meront, but prior to cytoplasmic fission. Some meronts had diplokaryotic nuclei (Fig. 4). The nuclei of diplokaryotic meronts divided once more giving rise to a tetranucleate meront. All merogonial stages lay directly in the host cell cytoplasm and had no surface coat. Parasitized muscle myofibris showed evident autolysis but the cell mitochondria were well preserved.

Sporogony

The sporont was characterized by an electron-dense surface coat which developed into the exospore layer of the sporoblast wall and subsequently the spore wall. The sporont cytoplasm contained a greater amount of RER than did meronts, free and associated ribosomes, and sometimes one or two aggregates of vesicles representing the Golgi apparatus (Fig. 5). The sporonts were elongate cells measuring 12–15 μm in the long axis. Young sporonts showed a diplokaryotic nucleus. In sporonts, four nuclei could be counted in serial ultra-thin sections. The nuclei of sporonts presented a characteristic dumbbell shape prior to division (Fig. 6). The tetranucleate sporonts were presumed to divide into four sporoblasts via a moniliform stage.

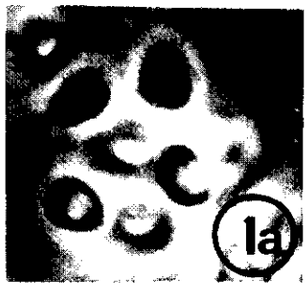
Figure 1. (a) Fresh mature spores observed in unstained wet smears (bar=2 μm). (b) Semi-thin section of the muscle tissue of *C.pagurus* containing different life cycle stages of *A. atlanticum* including mature spores. Some macrospores (arrows) are present among mature spores (×100).

Figure 2. Ultrathin section showing different developmental stages: Me=uninucleate meront; S=early sporoblast; Sb=sporoblast; Sp=spore (×16 300).

Figure 3. Binucleate meront (Me) from uninucleate meront undergoes division. Meronts are in direct contact with muscle fibres (*) (×40 000).

Figure 4. Meront: detail of a diplokaryon (×24 400).

Figure 5. Sporont with diplokaryotic nucleus, N_1N_2; hair-like appendages are visible on thick plasmalemma (arrows) (×48 000).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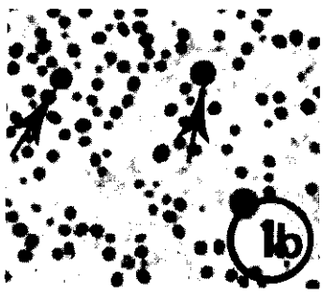
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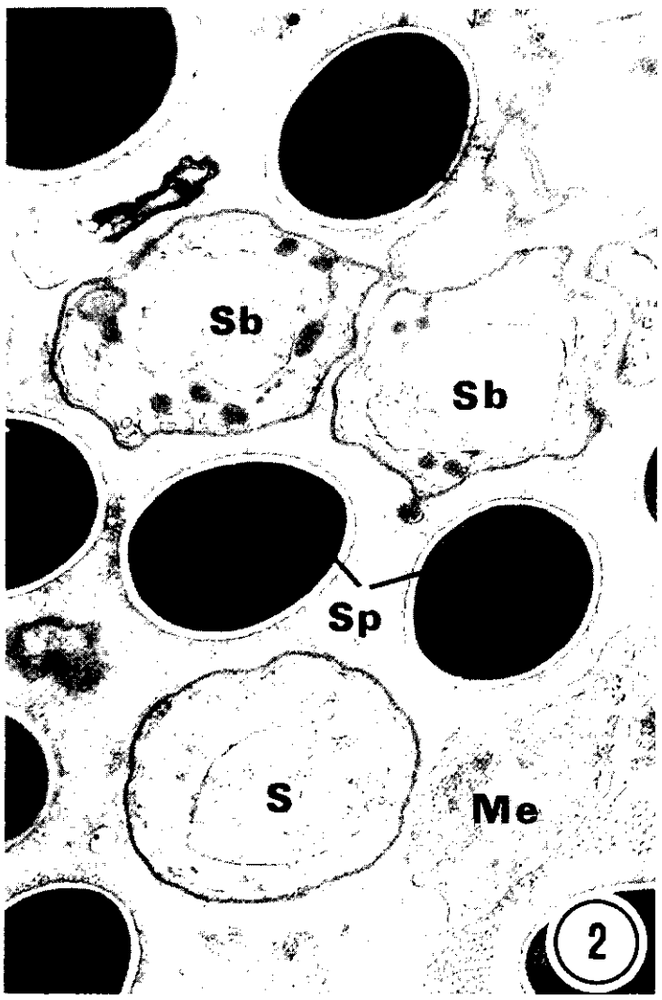
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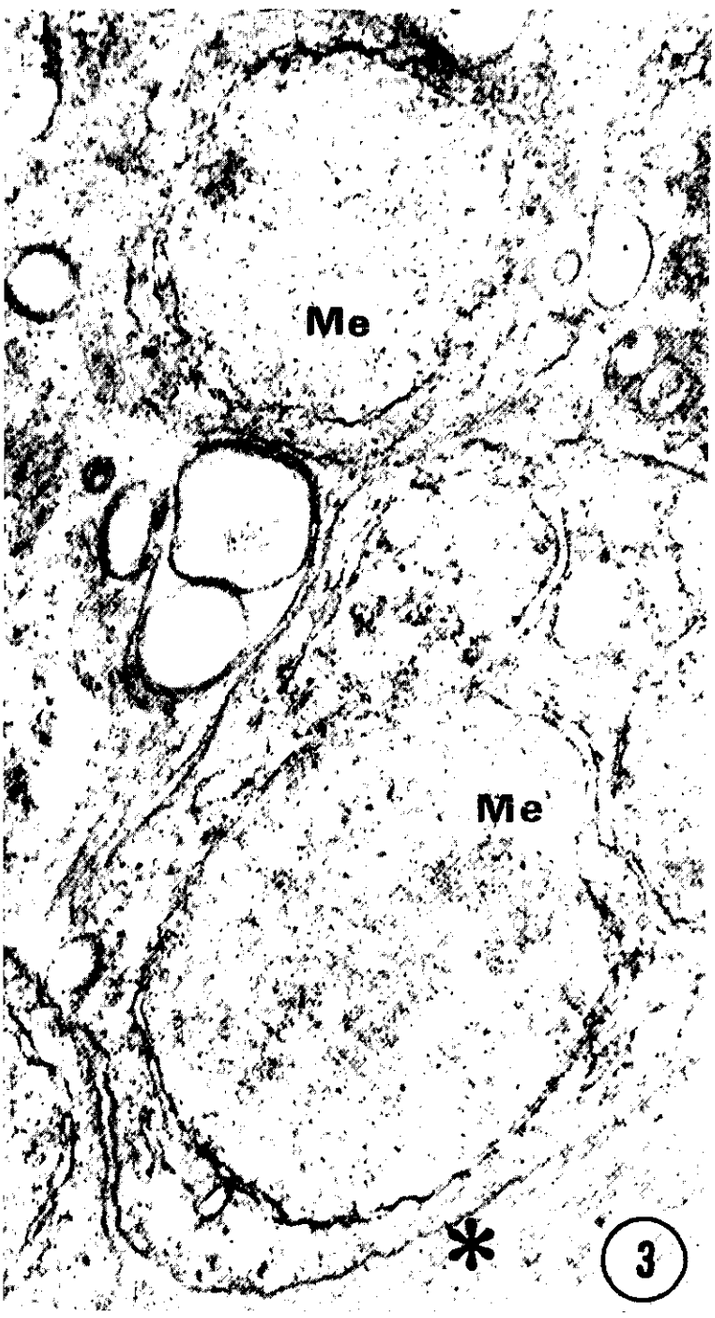
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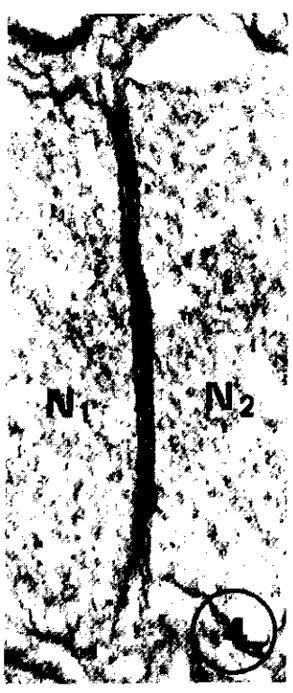
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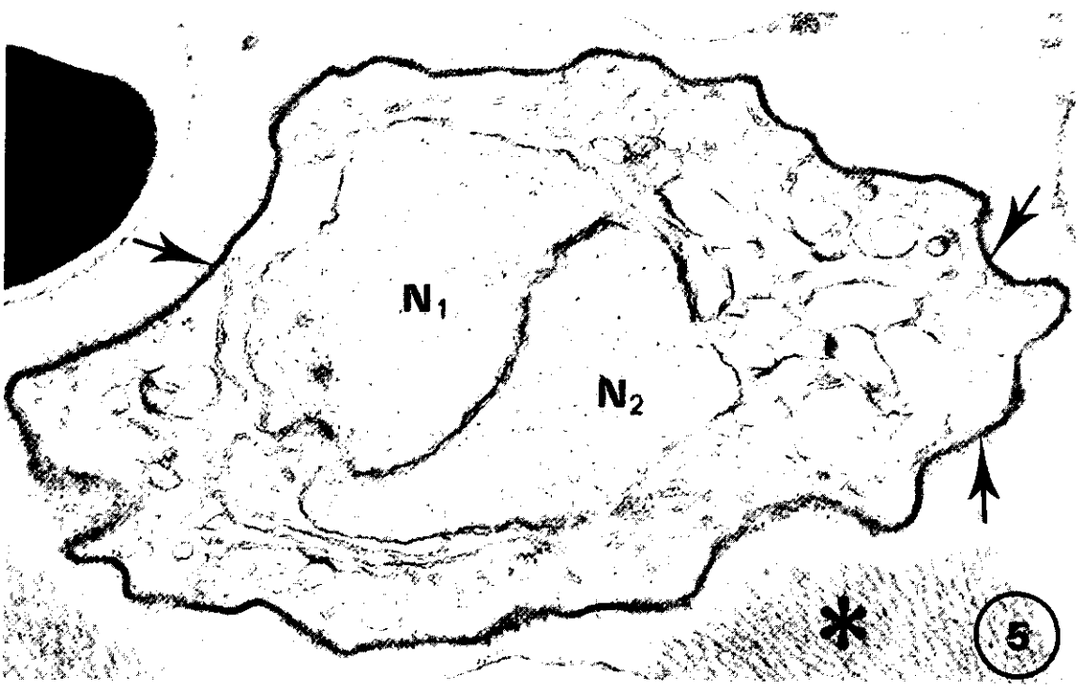
N2
4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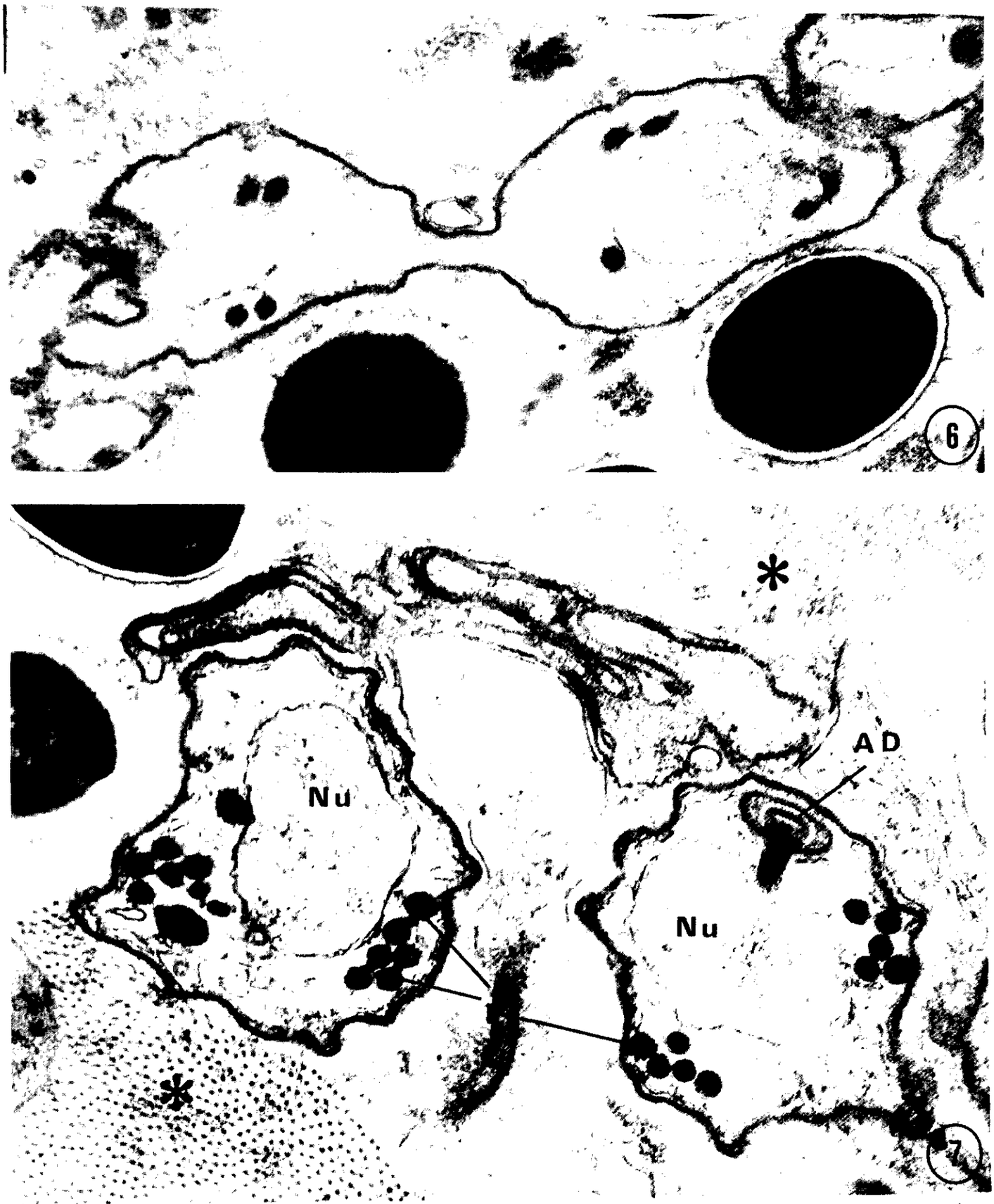


Figure 6. Portion of moniliform sporogonial plasmodium dividing into sporoblasts (4) with filamentous appendages (×26 800).

Figure 7. Uninucleate sporoblasts showing the anchoring disc (AD), the polar filament (Fi) and the nucleus (Nu); muscle fibres (*) (×32 300).

Sporoblasts were characterized by an irregular outline and by the presence of precursors of the main organelles of the mature spore: the anchoring disc, polar filament and polaroplast (Fig. 7). The sporoblast wall became denser and thicker during development and was entirely covered by short hair-like projections (Fig. 7). In some transverse sections, five to eight coils of the polar filament were visible, arranged in a disorganized group around the nucleus.

Spore

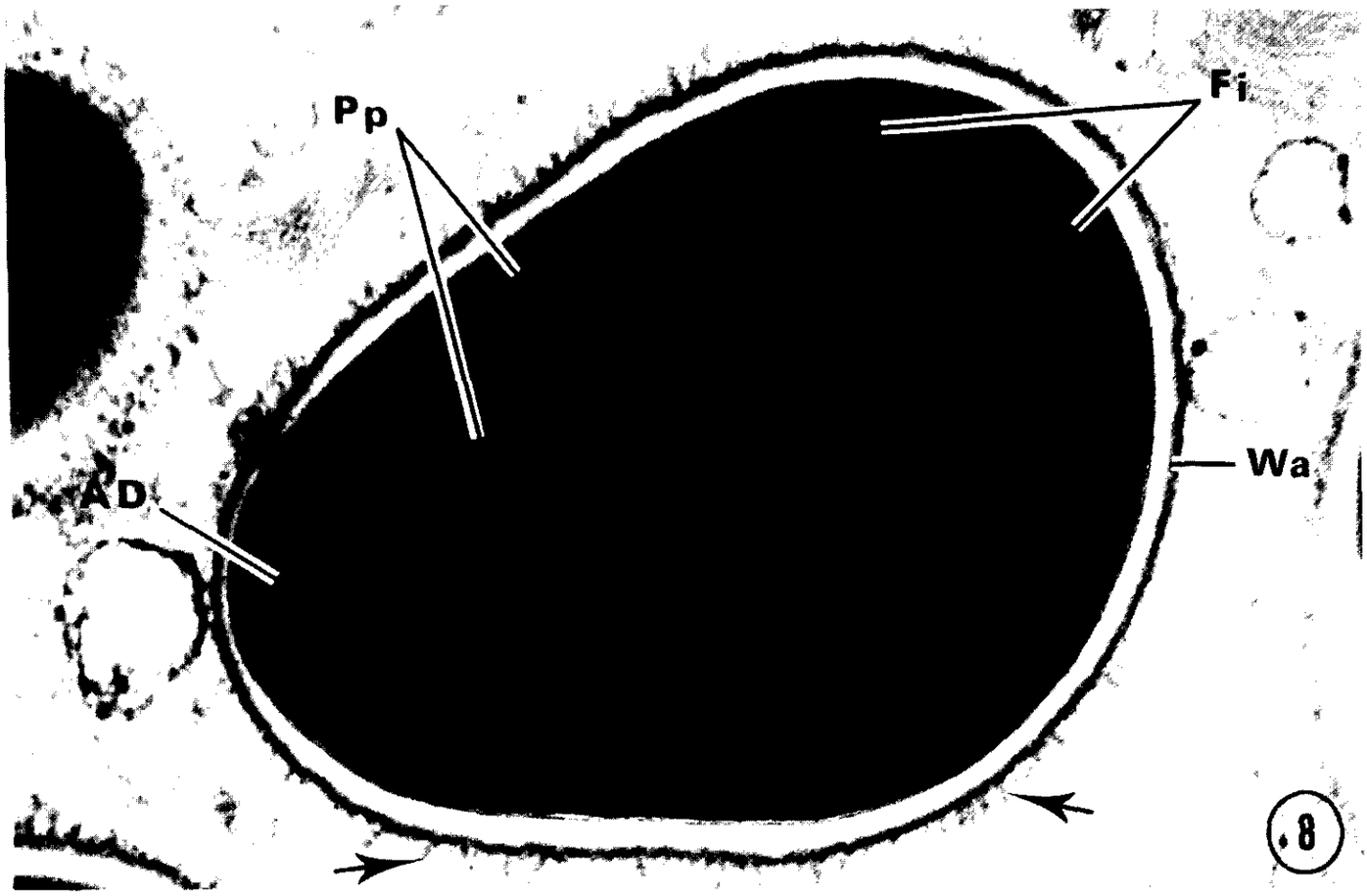
During spore maturation the wall thickens by expansion of the endospore layer and growth of the hair-like projections of the exospore to reach a maximum thickness of 140 nm (Fig. 10). Spores are ovoid, 1·98±0·40 μm (n=25 measurements of fresh material) in length and 1·51±0·12 μm in width (Figs 8 & 9). They lie in close contact with the host myofibres which sometimes show advanced autolysis. The single nucleus of the mature spore is situated at the posterior pole. The anchoring disc is situated anteriorly beneath the plasmalemma. The polar filament, 52-μm long, is uniform in diameter with 11 to 12 coils. The last one or two turns are narrower and are generally situated internally to the others (Figs 8 & 9). The polaroplast, surmounted by the polar sac (anchoring disc), consists of an anterior and a lateral region of close-packed membranes and of an internal region composed of more widely spaced membranes: all membranes seemed to be in continuity with the compacted polaroplast (Fig. 9). A posterior vacuole was not observed (nor in fresh spores). At the final phase of maturation, the fully mature spores present a very dense endosporoplasm so that it is very hard to see internal structure (Fig. 10). The hair-like appendages on the exospore are long and bipartite, consisting of a short basal portion and a relatively long and thin extension (Figs 8–10).

Discussion

The results of our study demonstrate that the ultrastructure of the different life cycle stages and the spore correspond to the phylum Microspora, class Microsporea and order Microsporida (Vávra 1976; Levine, Corliss, Cox, Deroux, Grain, Honigberg, Leedale, Loeblich, Lom, Lynn, Meinfeld, Page, Poljansky, Sprague, Vávra & Wallace 1980; Sprague 1982). The absence of a pansporoblastic membrane, so that the different life cycle stages lie directly in the host cell cytoplasm, places this microsporidan in the suborder Apansporoblastina (Sprague 1977, 1982; Levine *et al.* 1980).

Vivarès & Sprague (1979), in an ultrastructural study of the life cycle of *Ameson (Nosema) pulvis*, discussed the taxonomy of the families Unikaryonidae and Pereziidae. As the main characteristic of the family Unikayronidae is that all stages of its life cycle are uninucleate, the genus *Ameson*, with binucleate meronts and sporonts was transferred by Sprague (1982) to the family Pereziidae (Loubès, Maurand, Comps & Campillo 1977). The present study confirms this view.

The presence of hair-like appendages on the exospore is a characteristic of the genus *Ameson*. The role of these organelles is unknown though Vávra, Barker & Vivarès (1981) suggested that they could aid dispersion in aquatic environments. Various types of appendages, which appear during sporogony, were listed by Takvorian & Cali (1983) who suggested that these structures should be described in detail and classified. However, it is important to distinguish between material secreted during sporogonic development and organelles which persist on the exospore, since their roles may be different.


Pp
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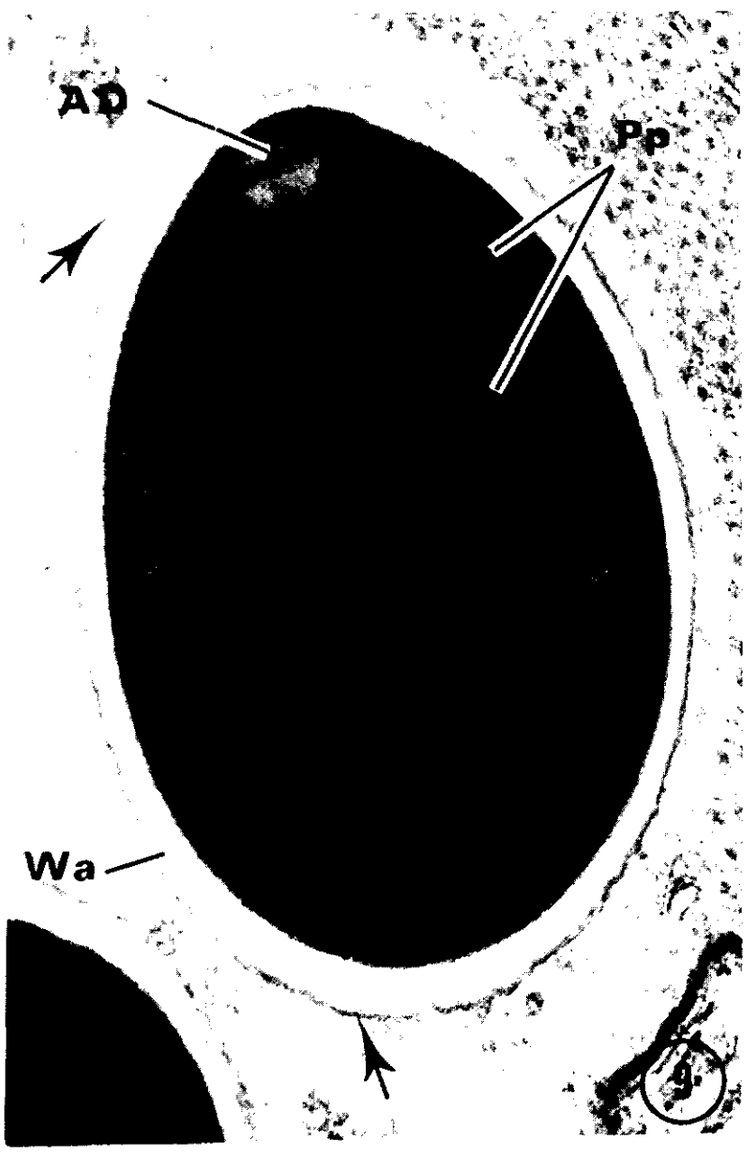
8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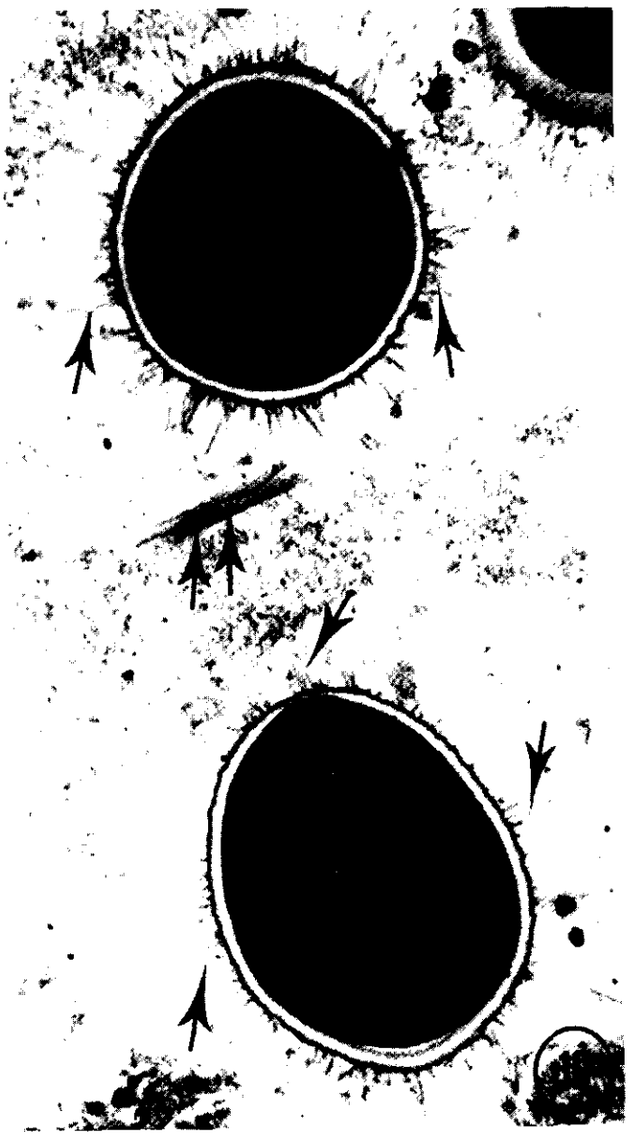

The authors believe that the presence of appendages on the spore is an important character which distinguishes the genera *Ameson* and *Perezia* The family Pereziidae is a small group, represented by the two genera *Perezia* and *Ameson* with only a few species (Sprague 1982). The parasite in this study from *Cancer pagurus* has uninucleate and subsequently diplokaryotic meronts. The sporonts are tetranucleate with diplokarya persisting. In the division into sporoblasts the diplokarya separate and sporoblasts are uninucleate in direct contact with host cytoplasm. The parasite can be classified in the genus *Ameson* within the family Pereziidae. The ultrastructural morphology of the spores (shape and size, morphology and position of the polaroplast, the length and number of turns of the polar filament) may be used as taxonomic characters to identify species. Characteristics of the species of the genus *Ameson* are presented in Table 1 from which it is concluded that the parasite described in this paper is a new species of the genus *Ameson*. The name *Ameson atlanticum* sp. nov. is proposed.

Specific diagnosis

Ameson atlanticum sp. nov.

Host. All life cycle stages take place in muscle tissue of the crab, *Cancer pagurus* L., (Crustacea, Decapoda) in direct contact with myofibrils.

Pathogenic activity. Lysis and destruction of the parasitized tissue.

Merogony. Elongate uninucleate meronts with diplokaryotic nuclei give rise to cylindrical plasmodia with four nuclei.

Sporogony. Sporonts are diplokaryotic cells. Sporogony results in four uninucleate sporoblasts, at least.

Spore. Ellipsoidal to ovoid with dimensions up to 1·9×~1·5 μm; polaroplast with two kinds of lamellae; polar filament isofilar with 11–12 coils arranged generally in a single layer (except for the two most posterior coils), and 52-μm long when extruded; a single nucleus; no posterior vacuole.

Table 1. Characteristics of the three species of the genus *Ameson*

Characters of spores	*A. michaelis*	*A. pulvis*	*A. atlanticum*
Length	1·3 μm	1·7 μm	1·9 μm
Breadth	1·0 μm	1·3 μm	1·5 μm
Number of coils of polar tube	11 (three rows)	8–9 (two rows)	12 (two rows)
Diameter of polar tube	80 nm	100 nm	170 nm
Thickness of wall	100 nm	40 nm	140 nm

Figure 8. Immature spore with a thin exospore (Wa), anchoring disc (AD), polar filament (Fi) and polaroplast (Pp). On the exospore, hair-like appendages are present (arrow) (×57 700).

Figure 9. Mature spore showing a thick exospore: AD, anchoring disc; Pp, polaroplast; Wa, spore wall; arrow, hair-like appendages.

Figure 10. Two mature spores with hair-like appendages on exospore (arrows). Between the spores some autolyzed myofibrils are present (double arrows) (×62 400).

Type material. Slides are deposited in the International Protozoa Type Slide Collection, U.S. National Museum, the Smithsonian Institution, Washington, DC 20.560, USA, and in the collection of the authors.

Only a few microsporida are known to parasitize crabs on the Atlantic Old World coasts. These are *Thelohania maenadis* (Perez 1904; Vivarès 1980), *Ameson pulvis* (Perez 1905; Vivarès & Sprague 1979), *Abelspora portucalensis* (Azevedo 1987), all in *Carcinus maenas*. Thus *Cancer pagurus* is a new host for microsporida.

Different tissues are destroyed by microsporida, i.e. muscle by *T. maenadis* (Perez 1904; Vivarès 1980), *A. pulvis* (Perez 1905; Vivarès & Sprague 1979) and *A. atlanticum* sp. nov., ovary by *T. maenadis* (Perez 1906) and hepatopancreas by *A. portucalensis* (Azevedo 1987). *C. maenas* is an edible crab but *C. pagurus*, host of *A. atlanticum*, is a crab of even greater commercial interest. In *C. pagurus*, the most significant parasite previously described is *Mesonophrys maggii* (=*Anophrys maggii*=*Paranophrys carcini*) (de Puytorac & Grolière 1979), an opportunistic histophagous ciliate. As other microsporida of crabs, *A. atlanticum* does not provoke any tissue reaction but the lysis of the muscles is very significant (~80% of the mass). Such an infection can alter the host's biochemical balance as previously described in *C. maenas* by Vivarès, Cuq, Ceccaldi & Richard (1980) and Vivarès & Cuq (1981). In crabs from a wild fishery infected muscle appears chalk-white and is aesthetically displeasing. *Ameson atlanticum* might be of significance in any culture of *C. pagurus*.

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References

Azevedo C. (1987) Fine structure of a microsporidan *Abelspora portucalensis* gen. n., sp. n. (Microsporida) parasite of the hepatopancreas of *Carcinus maenas* (Crustacea, Decapoda). *Journal of Invertebrate Pathology* **49**, 83–92.

Levine N.D., Corliss J.O., Cox F.E.G., Deroux G., Grain J., Honigberg B.M., Leedale G.F., Loeblich A.R. III., Lom J., Lynn D., Meinfeld E.G., Page F.C., Poljansky G., Sprague V., Vávra J. & Wallace F.G. (1980) A newly revised classification of the Protozoa. *Journal of Protozoology* **27**, 37–58.

Loubès C., Maurand J., Comps, M. & Campillo A. (1977) Observations ultrastructurales sur *Ameson nelsoni* (Sprague, 1950) microsporidie parasite de la crevette *Parapenaeus longirostris* Lucas conséquences taxonomiques. *Revue des Travaux de l'Institut des Pêches Maritimes* **41**, 217–222.

Perez C. (1904) Sur une Microsporidie parasite du *Carcinus maenas*. *Comptes Rendus de la Société de Biologie* **57**, 214–215.

Perez C. (1905) Sur une nouvelle Glugeidae parasite de *Carcinus maenas*. *Comptes Rendus de la Société de Biologie* **58**, 146–148.

Perez C. (1906) Sur un cas d'envahissement de l'ovaire par *Thelohania maenadis*. *Comptes Rendus de la Société de Biologie* **60**, 1091–1092.

de Puytorac P. & Grolière C. (1979) Sur le Cilié *Anophryoides salmacida* (Mugard, 1949) nov. gen. *Protistologica* **15**, 223–230.

Sprague V. (1977) Classification and phylogeny of the Microsporida. In: *Comparative Pathobiology*, Vol. 2 (ed. by L. A. Bulla Jr. & T. C. Cheng), pp. 1–30. Plenum Press, New York.

Sprague V. (1982) Microspora. In: *Synopsis and Classification of Living Organisms*, Vol. 1 (ed. by S. P. Parker), pp. 589–594. McGraw Hill, New York.

Takvorian P.M. & Cali A. (1983) Appendages associated with *Glugea stephani*, a microsporidian found in flounder. *Journal of Protozoology* **30**, 251–256.

Vávra J. (1976) Structure of microsporidia. In: *Comparative Pathobiology*, Vol. 1. *Biology of Microsporidia* (ed. by L. A. Bulla Jr. & T. C. Cheng), pp. 1–85. Plenum Press, New York.

Vávra J., Barker R.J. & Vivarès C.P. (1981) A scanning electron microscopic study of a microsporidian with a pansporoblast: *Thelohania maenadis*. *Journal of Invertebrate Pathology* **37**, 47–53.

Vivarès C.P. (1980) Etude ultrastructurale de *Thelohania maenadis* Pérez (Microspora, Microsporida) et données nouvelles sur le genre *Thelohania* Henneguy. *Archiv für Protistenkunde* **37**, 47–53.

Vivarès C.P. & Cuq J.L. (1981) Physiological and metabolic variations in *Carcinus mediterraneus* (Crustacea, Decapoda) parasitized by *Thelohania maenadis* (Microspora, Microsporida): an ecophysiopathological approach. *Journal of Invertebrate Pathology* **37**, 38–46.

Vivarès C.P., Cuq J.L., Ceccaldi H.J. & Richard P.J. (1980) Influence d'une microsporidiose sur les acides aminés libres de *Carcinus mediterraneus* Czerniavsky, 1884 soumis à diverses salinités et à des valeurs extrêmes de température. *Journal of Experimental Marine Biology and Ecology* **43**, 207–220.

Vivarès C.P. & Sprague V. (1979) The fine structure of *Ameson pulvis* (Microspora, Microsporida) and its implications regarding classification and chromosome cycle. *Journal of Invertebrate Pathology* **33**, 40–52.