Journal of Invertebrate Pathology **78,** 194–200 (2001)
doi:10.1006/jipa.2001.5072, available online at http://www.idealibrary.com on IDEAL®

Ultrastructural Aspects of a New Species, *Vavraia mediterranica* (Microsporidia, Pleistophoridae), Parasite of the French Mediterranean Shrimp, *Crangon crangon* (Crustacea, Decapoda)

Carlos Azevedo

Department of Cell Biology, Institute of Biomedical Sciences and CIIMAR, University of Oporto, 4099-003 Porto, Portugal

E-mail: azevedoc@icbas.up.pt

Received January 30, 2001; accepted December 17, 2001

The life cycle stages of a new species of the genus *Vavraia* (Microsporidia, Pleistophoridae), which parasitizes the shrimp *Crangon crangon* (Crustacea, Decapoda), were examined by light and electron microscopy. This parasite was monomorphic with polysporous sporogony and developed in the skeletal muscle of the host. The multinucleate sporogonial plasmodium divided by plasmotomy and multiple division into uninucleate sporoblasts. All stages were surrounded by a thick and amorphous dense coat external to the plasmalemma. This structure gradually became a merontogenetic sporophorous vacuole (MSV) where the sporonts developed into sporoblasts. The MSV was filled with episporontal granular secretory products and eventually contained up to 50 uninucleate spores. During spore morphogenesis, these episporontal granular products within the MSV became organized as episporontal tubular-like structures. In transverse sections, these structures showed a mean diameter of 1.0 μm, but disappeared during the final phase of the spore maturation. Mature spores were ellipsoidal to slightly pyriform and measured 2.30 × 1.41 μm. The polar filament was anisofilar and consisted of a single coil with six to seven turns (rarely five). This new species is named *Vavraia mediterranica* n. sp. © 2001 Elsevier Science (USA)

Key Words: **ultrastructure; life cycle; *Vavraia mediterranica* n. sp.; parasite; merontogenetic sporophorous vacuole; shrimp; *Crangon crangon*.**

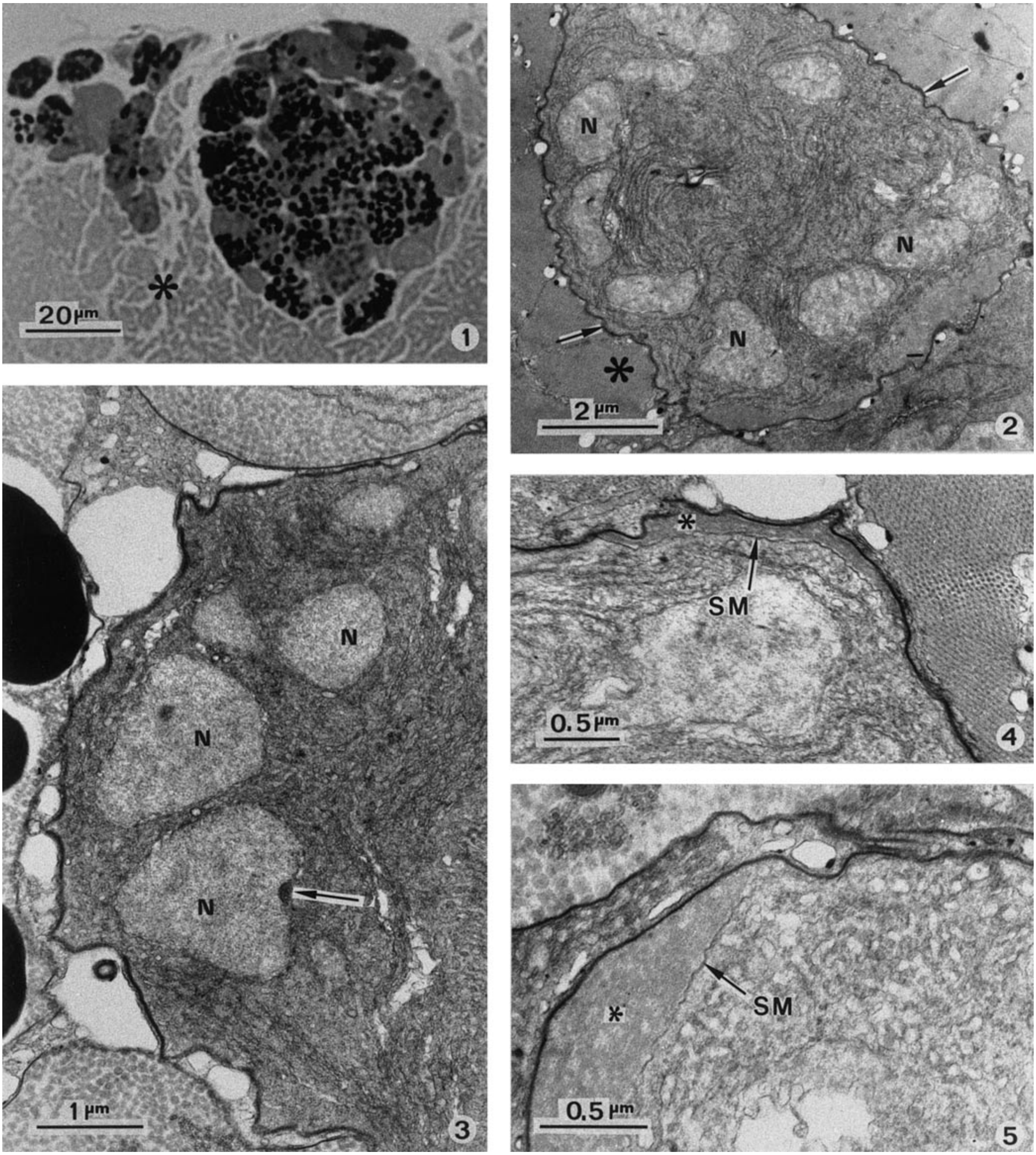
INTRODUCTION

The microsporidia are obligate intracellular parasites found in a wide range of vertebrate and invertebrate hosts (Canning and Hazard, 1982; Canning and Nicholas, 1980; Larsson, 1999; Sprague, 1977; Sprague *et al.*, 1992). Microsporidia are especially common in insects, crustaceans, and fish, comprising about 1300 named species (Larsson, 1999).

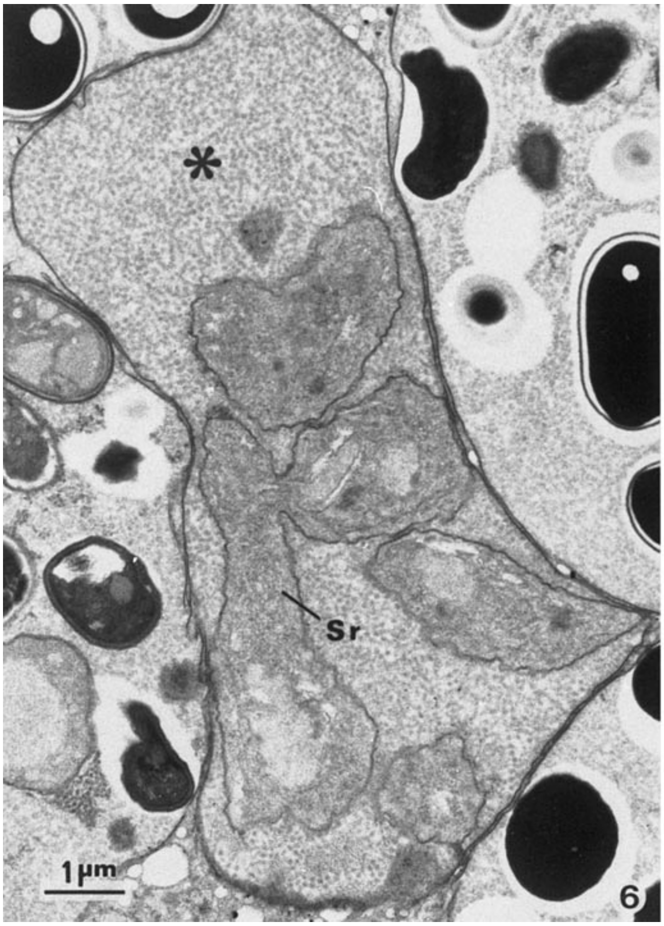
Recently, I obtained from France material of a microsporidian species infecting the skeletal muscle of the Mediterranean shrimp *Crangon crangon*. Preliminary investigations indicated that this species undergoes multisporous sporogony in sporophorous vacuoles, which historically has typified the group of microsporidia referred to as *Pleistophora*-like (Canning and Hazard, 1982) or *Pleistophora* complex that embraces nine genera (Canning *et al.*, 1991). Numerous species of microsporidia have been described from decapods (Azevedo, 1987; Azevedo *et al.*, 2000; Larsson, 1999; Larsson and Voronin, 2000; Overstreet and Weidner, 1974; Sprague *et al.*, 1992; Street and Sprague, 1974; Vivarès, 1975; Vivarès and Azevedo, 1988; Voronin and Melnikova, 1984) and a number of genera have shrimp as the type host, including *Thelohania*, *Inodosporus*, *Agmasoma*, and *Orthothelohania* (Larsson, 1999; Sprague, 1977; Sprague *et al.*, 1992). None of these genera, however, fit the developmental features observed for the present species but a number of microsporidia from shrimp have been assigned to the genus *Pleistophora* (Street and Sprague, 1974). The family Pleistophoridae currently contains two genera, *Pleistophora* and *Vavraia* (Sprague *et al.*, 1992). It has recently been suggested that species of *Pleistophora* reported from invertebrates do not belong in this genus but rather belong in *Vavraia* (Larsson, 1999). I have, therefore, undertaken an ultrastructural investigation of this species from *C. crangon* to provide details on its development and to determine its generic placement.

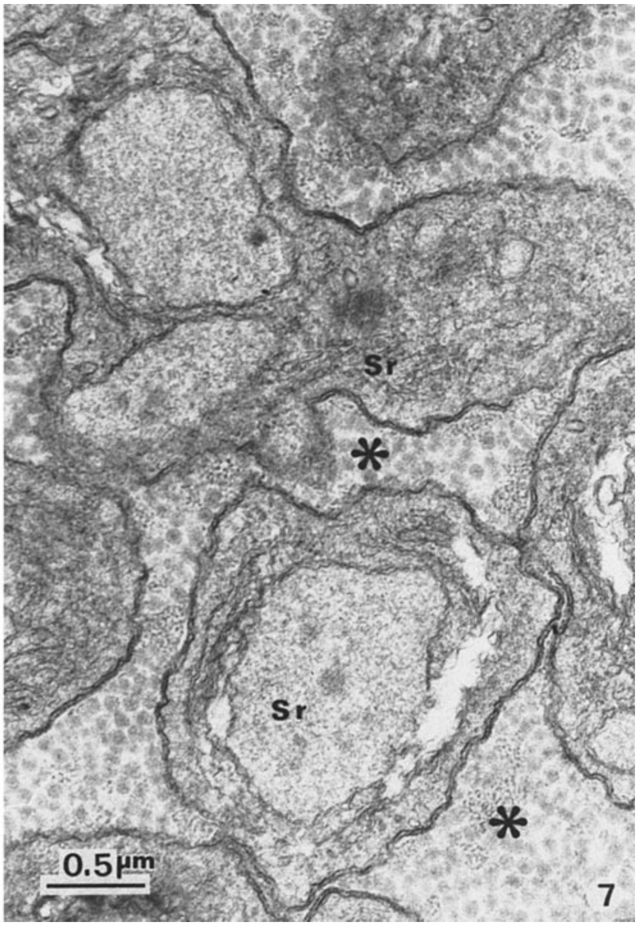
The ultrastructural features of the developmental stages of a new microsporidian species infecting the skeletal muscle of the Mediterranean shrimp *C. crangon* are reported in this study. Based on fundamental differences from other microsporidia, which undergo multisporous sporogony in merontogenetic sporophorous vacuoles (MSV), we propose the creation of a new species.

0022-2011/01 $35.00
© 2001 Elsevier Science (USA)
All rights reserved.



FIGS. 1–5. Light and electron micrographs showing different life cycle stages of the parasite *Vavraia mediterranica*. n. sp. Fig. 1. Semithin section showing some cysts containing developmental stages of the parasite in the muscle tissues (*). Fig. 2. Ultrathin section of a multinucleate meront, showing several nuclei (N) and the cytoplasm containing abundant ribosomes and rough endoplasmic reticulum. The plasmalemma is externally reinforced by a dense coat (arrows) in contact with myofibrils (*). Fig. 3. Ultrastructural detail of the periphery of a multinucleate meront, showing some nuclei (N), one of them with a spindle pole body (arrow). Figs. 4 and 5. Two sequential phases of the initial phases of the separation of the sporont membrane (SM) from the merontogenetic sporophorous vacuoles. Note the granular secretory products within the episporontal space (*).





FIGS. 6, 7. Ultrastructural aspects of dividing life cycle stages within the MSV of *V. mediterranica* n. sp. Fig. 6. A plurinucleate sporont (Sr), with some dividing cells. The MSV is occupied by numerous episporontal amorphous substances (*). Fig. 7. Details of dividing sporonts (Sr) showing the matrix of the MSV situated among the dividing sporonts occupied by several episporontal amorphous substances (*).

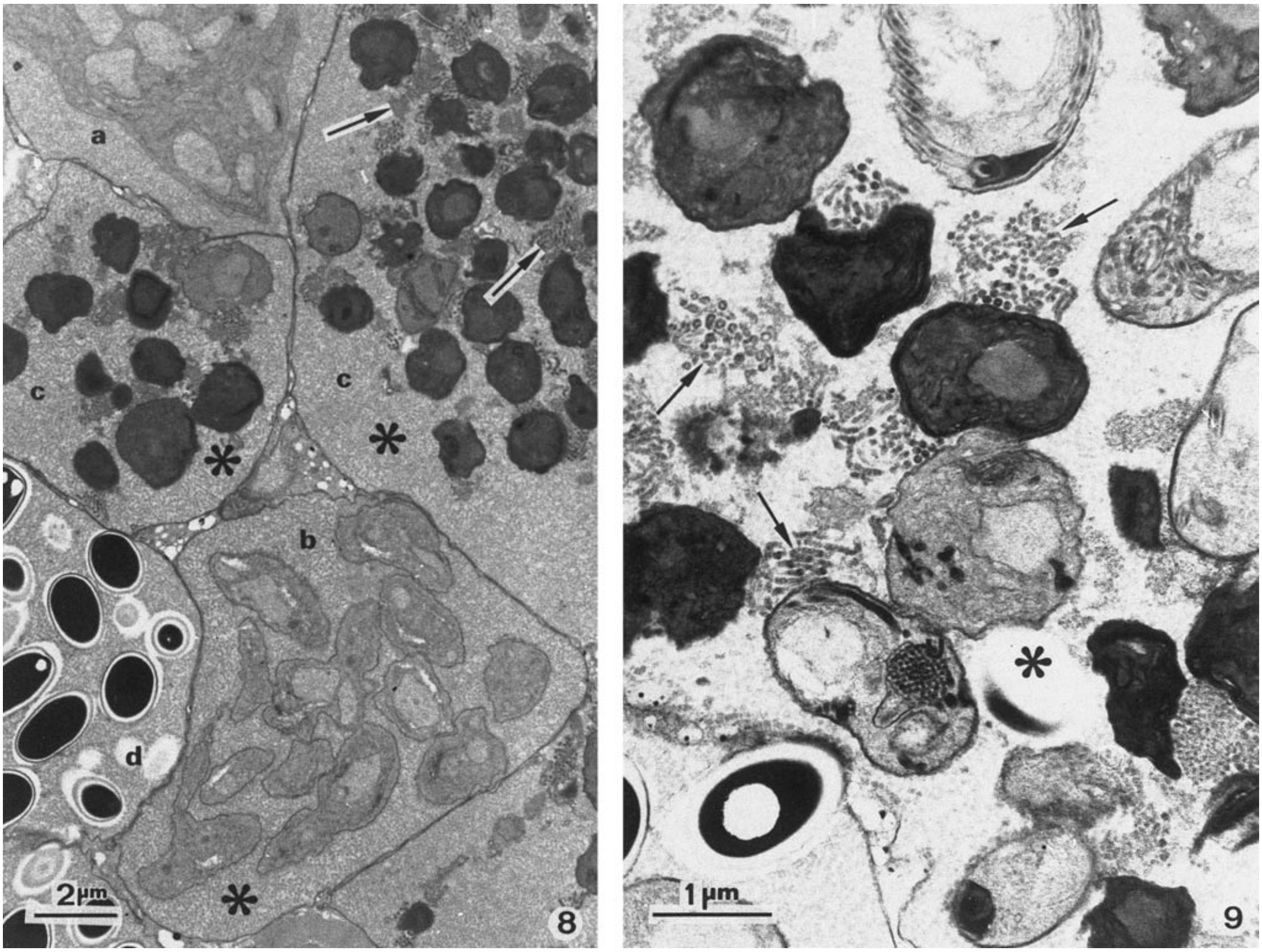
MATERIALS AND METHODS

A microsporidian parasite obtained from the skeletal muscle of several adult shrimps, *C. crangon* (Crustacea, Decapoda), collected in the intertidal zone of the French Mediterranean coast near Banyulus, was prepared for observation by light (LM) and transmission electron microscopy (TEM). For TEM studies, small pieces of infected skeletal muscle were fixed in 3% glutaraldehyde buffered with 0.2 M sodium cacodylate (pH 7.4) at 4°C for 5 h, held overnight at 4°C in the same buffer, postfixed in buffered 2% OsO_4 at 4°C for 3 h, dehydrated in an ascending series of ethanol, and embedded in Epon. Semithin sections for LM were stained with methylene blue-azur II, and the ultrathin sections were double-stained with uranyl acetate and lead citrate and then observed and photographed with a JEOL 100CXII TEM, operated at 60 KV.

RESULTS

Light microscopy studies. Infections were only detected in the skeletal muscles, which had a white appearance. Isolated fresh mature spores were ellipsoid to ovoid in shape. Among the myofibrils, the spores were observed in groups with a variable number of spores inside the irregular cysts, of which the biggest one measured 70–80 μm in diameter and contained some thousand of spores (Fig. 1). There was a tendency for younger stages to be located at the peripheral regions of the cysts (Fig. 1).

Electron microscopy studies. The earliest stages were irregular, multikaryotic meronts with unpaired nuclei often mixed with different life cycle stages (Figs. 2 and 3). There was a thin, electron-dense amorphous layer present on the external surface of the meront plasmalemma (Figs. 2 and 3). The meront cytoplasm contained numerous ribosomes and cisternae of rough endoplasmic reticulum. The nuclear division of the meronts was characterized by the presence of spindle plaques on the outer surface of the nuclear envelope. Merogony occurred by binary fission, producing multinucleate merogonial plasmodia. The nucleoplasm was less dense than the cytoplasm and no synaptonemal complexes were observed (Fig. 3).

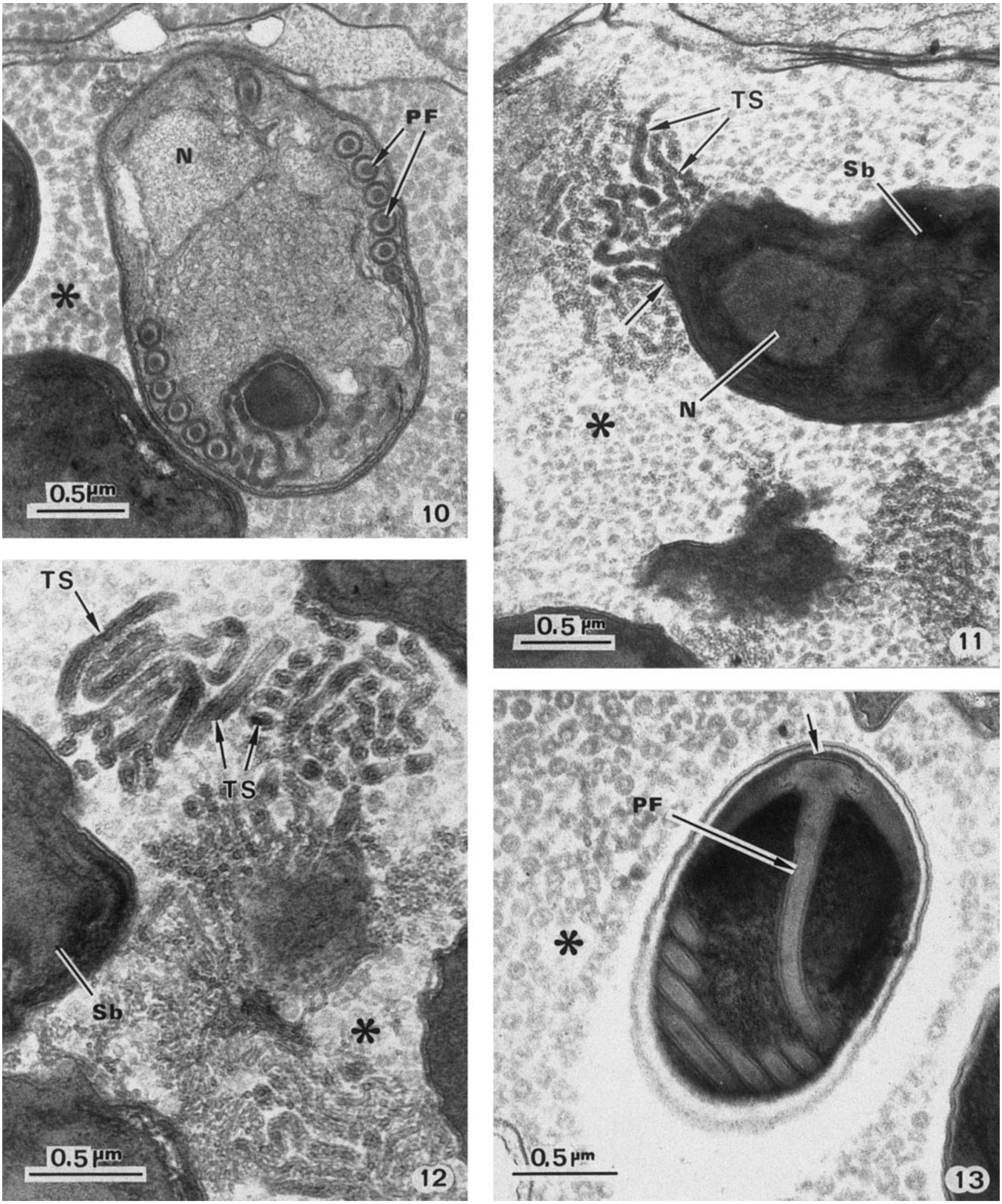


FIGS. 8, 9. Ultrastructural aspects of some life cycle stages of *V. mediterranica* n. sp. Fig. 8. Several MSV (*), each one showing different developmental stages of the life cycle: a, multinucleate sporont preparing for division; b, sporonts dividing by multiple fission; c, sporoblasts, some of them with episporontal tubular-like structures (arrows); d, spores without any episporontal tubular-like structures. Fig. 9. A MSV (*) showing some asynchronized stages of sporoblast maturation, some of which are in contact with several tubular-like structures (arrows).

At the onset of sporogony, the merogonial plasmodium receded from the surface dense coat (Figs. 4 and 5). After repeated nuclear division, each sporont had multiple unpaired nuclei and was enveloped by a thick amorphous and electron-dense outer coat (Figs. 4 and 5). Gradually, the plasmalemma of the parasite was detached from the dense outer coat and coalesced to form the continuous outer layer of the sporont. This process gave rise to the merontogenetic sporophorous vacuole, which contained several uninucleate sporonts. The episporontal space of the MSV was occupied by numerous episporontal amorphous substances distributed among the sporonts (Figs. 6 and 7).

Each sporont underwent repeated asynchronous nuclear divisions within the MSV followed by multiple division of the plasmodia, resulting in large numbers of sporonts (Figs. 6–8). At the final phase of this division, each MSV contained numerous sporoblasts (Figs. 8 and 9). The presence of up to 50 individual uninucleate sporoblasts, counted in serial ultrathin sections, were observed. The newly formed uninucleate sporoblasts took an irregular round shape (Fig. 9). In the same ultrathin section, it was possible to simultaneously observe some other developmental stages in the same MSV (Fig. 9). The internal organization possessed the classic primordial structure of the future spore organelles (Figs. 10 and 11). During this phase, the matrix of the MSV was partially occupied by episporontal granular secretory products (Fig. 10) that differentiated into episporontal tubular-like structures, while each sporoblast became gradually denser (Figs. 11 and 12). These tubular-like structures seemed continuous to the external portion of the sporoblast wall (Figs. 11 and 12), which disappeared during spore maturation (Fig. 13).

Mature spores were ellipsoid, 2.30 ± 0.22 μm in length, and 1.41 ± 0.21 μm wide ($n = 25$) (Figs. 11 and 13). The spore wall was 40 nm thick, with a distinct



FIGS. 10–13. Some ultrastructural aspects of spores of V. *mediterranica* n. sp. and its tubular-like structures. Fig. 10. A sporoblast surrounded by amorphous masses (*) showing the nucleus (N) and the primordium of the polar filament (PF). Fig. 11. Detail of a late sporoblast (Sb) and several tubular-like structures (TS). Some of them seem to be in close contact with the sporoblast wall (arrow) and surrounded by amorphous substances (*). Fig. 12. Details of several longitudinal and transverse sections of the tubular-like structures (TS) located among the sporoblasts (Sb) within the MSV (*). Fig. 13. A spore sectioned obliqually, showing the anchoring disc (arrow) and the polar filament (PF) sectioned at different plans. The MSV contains numerous amorphous masses (*).

plasmalemma, endospore, and exospore. The widest layer was the electron-translucent endospore. The exospore showed no ornamentation and was ~40 nm thick. The spore was uninucleate and possessed a typical microsporidian organization, constituted by an anisofilar polar tube with six to seven coils (rarely five coils). This structure was apically surrounded by the polaroplast (Fig. 13). When the spore attained full maturation, the sporoplasm was very dense and the internal arrangement was hardly seen (Figs. 8 and 13).

DISCUSSION

Our results demonstrate that the ultrastructure of the spores correspond to the phylum Microsporidia Balbiani, 1882 (Sprague and Becnel, 1998). The earliest stages take place in direct contact with the myofibrils of the host and the sporulation sequence that occurs inside a persistent intracellular sporocyst in the host cell (merontogenetic sporophorous vacuole) lead us to place the parasite within the family Pleistophoridae (Sprague *et al.*, 1992).

In a few genera, such as *Pleistophora, Vavraia,* and *Trachipleistophora,* the merontogenetic sporophorous vacuole is formed from the surface coat during merogony, as found in my observations. However, *Vavraia* and *Trachipleistophora* exhibit several morphological differences. In sporogony, *Vavraia* develops into a multinucleate sporogonial plasmodium within the MSV and then undergoes multiple fission while in *Trachipleistophora* the MSV encloses a uninucleate sporont that undergoes repeated binary fissions (Cheney *et al.*, 2000). My results, especially those with regard to the presence of a MSV and the division of the sporogonial plasmodium, are consistent with the morphology and ultrastructural data previously described for some species of the genus *Vavraia* and in particular for the type species *V. culicis* (Cheney *et al.*, 2000; Diarra and Toguebaye, 1991; Langdon, 1991). In addition, the presence of the persistent structures in which a substantial mass of external materials develops during the merogony forming the cyst wall (MSV) seems a strong argument to place the parasite here described in the genus *Vavraia* Weiser, 1977 (Canning and Hazard, 1982; Langdon, 1991; Larsson, 1986, 1999; Weiser, 1977). Finally, the genus *Vavraia* is the invertebrate parasitic equivalent to the vertebrate parasitic *Pleistophora* (Larsson, 1999).

Previously, I introduced the term vacuole (parasitophorous vacuole) (Azevedo, 1987) instead of vesicle, frequently used in this group by several authors (Canning and Hazard, 1982; Canning and Nicholas, 1980) to describe a more or less persistent sac-like structure containing a specific number of spores. In the present study, and in other described species, this microsporidian structure attains great diameters (sometimes 100 μm) and so, according to all classic ultrastuctural nomenclatures, the term used in this case and in other different descriptions of similar structures should be vacuole (Azevedo, 1987; Azevedo *et al.*, 2000; Darwish *et al.*, 1992; Sprague *et al.*, 1992).

The following features present the essential taxonomic characters used to identify this new species of the genus *Vavraia* that we named *Vavraia mediterranica* n. sp.

Taxonomic Summary

Vavraia mediterranica n. sp.

Type host: All life cycle stages developed in the skeletal muscle of the shrimp, *Crangon crangon* (Crustacea, Decapoda), within a merontogenetic sporophorous vacuole.

Type locality: Banyulus, French Mediterranean coast.

Pathogenic activity: Lysis and destruction of the parasitized tissue causing white discoloration of the tissue and the death of the host.

Diagnosis: With the characteristics of the genus. Merogony by binary fission with a sequence of unikaryotic cells having irregular sporogonial plasmodia with up to 50 nuclei that give rise to the same number of uninucleate spores. All developmental stages are surrounded by a thick and amorphous dense coat, external to the plasmalemma that gradually becomes the cyst wall (merontogenetic sporophorous vacuole). Matrix of merontogenetic sporophorous vacuole with granular masses that later become tubular-like structures attached to the spore wall. The mature spores are ellipsoidal to slightly pyriform and measure $2.30 \pm 0.22 \times 1.41 \pm 0.21$ μm. The polar filament is anisofilar and consists of a single coil with six to seven turns (rarely five).

Type material: Two slides containing some semithin sections of different life cycle stages of the holotype were deposited in the International Protozoan Type Slide Collection, National Museum of National History, Washington, DC 20560, U.S.A. (Accession No. 51557) and in the collection of the author.

Etymology: Specific name derived from the Mediterranean Sea.

ACKNOWLEDGMENTS

This study was partially supported by Engº A. Almeida Foundation (Porto, Portugal). I thank Professor C. P. Vivarès (Aubière, France) for providing the parasitized tissue and blocks. The excellent technical assistance given by Ms. Laura Corral and Mr. J. Carvalheiro and the computer assistance of Dr. Carla Silva are also gratefully acknowledged. I thank the anonymous reviewers for their most helpful comments and suggestions.

REFERENCES

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

Azevedo, C., Corral, L., and Vivarès, C. P. 2000. Ultrastructure of the microsporidian *Inodosporus octospora* (Thelohaniidae), a parasite of the shrimp *Palaemon serratus* (Crustacea, Decapoda). *Dis. Aquat. Org.* **41,** 151–158.

Canning, E. U., and Hazard, E. I. 1982. Genus *Pleistophora* Gurley, 1893: An assemblage of at least three genera. *J. Protozool.* **29,** 39–49.

Canning, E. U., Killick-Kendrick, R., and Killich-Kendrick, M. 1991. A new microsporidian parasite, *Flabelliforma montana* n. g., n. sp., infecting *Phlebotomus ariasi* (Diptera, Psychodidae) in France. *J. Invertebr. Pathol.* **57,** 71–81.

Canning, E. U., and Nicholas, J. P. 1980. Genus *Pleistophora* (Phylum Microspora): Redescription of the type species, *Pleistophora typicalis* Gurley, 1893 and ultrastructural characterization of the genus. *J. Fish Dis.* **3,** 317–338.

Cheney, S. A., Lafranchi-Tristem, N. J., and Canning, E. U. 2000. Phylogenetic relationships of *Pleistophora*-like Microsporidia based on small subunit ribosomal DNA sequences and implications for the source of *Trachipleistophora hominis* infections. *J. Eukaryot. Microbiol.* **47,** 280–287.

Darwish, A., Brooks, W. M., and Moore, C. B. 1992. Ultrastructural observations and taxonomic considerations of a microsporidium of the larch sawfly, *Pristiphora erichsonii:* Conspecificity with *Thelohamia pristiphorae* and *Pleistophora* sp. *J. Invertebr. Pathol.* **59,** 178–185.

Diarra, K., and Toguebaye, B. S. 1991. On the development cycle and ultrastructure of *Vavraia culicis* Weiser, 1947 (Microsporida, Pleistophoridae) with comments on the taxonomy of the genus *Vavraia* Weiser, 1977. *Eur. J. Protistol.* **27,** 134–140.

Langdon, J. S. 1991. Description of *Vavraia parastacida* sp. nov. (Microspora: Pleistophoridae) from marron, *Cherax tenuimanus* (Smith), (Decapoda: Parastacidae). *J. Fish Dis.* **14,** 619–629.

Larsson, J. I. R. 1999. Identification of Microsporidia. *Acta Protozool.* **38,** 161–197.

Larsson, J. I. R., and Voronin, V. N. 2000. Light and electron microscope study of *Agglomerata volgensae* n. sp. (Microspora: Dubosqiidae), a new microsporidian parasite of *Daphnia magna* (Crustacea, Daphnidae). *Eur. J. Protistol.* **36,** 89–99.

Larsson, R. 1986. Ultrastructural study and description of *Vavraia holocentropi* n. sp. (Microspora, Pleistophoridae), a parasite of lavae of the caddisfly *Holocentropus dubius* (Trichoptera, Polycentropididae). *Protistologica* **22,** 441–452.

Overstreet, R. M., and Weidner, E. 1974. Differentiation of microsporidian spore-tails in *Inodosporus spraguei* gen. et sp. n. *Z. Parasitenk.* **44,** 169–186.

Sprague, V. 1977. Annotated list of species of Microsporidea. *In* “Comparative Pathobiology” (L. A. Bulla, Jr. and Th. C. Cheng, Eds.), Vol. 2, pp. 31–334. Plenum, New York.

Sprague, V., and Becnel, J. J. 1998. Note on the name-author-date combination for the taxon Microsporidies Balbiani, 1882, when ranked as a phylum. *J. Invertebr. Pathol.* **71,** 91–94.

Sprague, V., Becnel, J. J., and Hazard, E. I. 1992. Taxonomy of phylum Microspora. *Crit. Rev. Microbiol.* **18,** 285–395.

Street, D. A., and Sprague, V. 1974. A new species of *Pleistophora* (Microsporida: Pleistophoridae) parasitic in the shrimp *Palaemonetes pugio. J. Invertebr. Pathol.* **23,** 153–156.

Vivarès, C. P. 1975. Étude comparative faite en microscopies photonique et électronique de trois espèces de microsporidies appartenant au genre *Thelohania* Henneguya, 1892, parasites des crustacés décapodes marins. *Ann. Sci. Nat. Zool.* **17,** 141–178.

Vivarès, C. P., and Azevedo, C. 1988. Ultrastructural observations of the life cycle stages of *Ameson atlanticus* sp. nov., a microsporidan parasitizing *Cancer pagurus* L. *J. Fish Dis.* **11,** 379–387.

Voronin, V. N., and Melnikova, O. Y. 1984. *Vavraia cyclocypris* sp. n., the first find of microsporidian parasite from Ostracoda (Crustacea). *Parazitologiya* **18,** 482–484.

Weiser, J. 1977. Contribution to the classification of Microsporidia. *Vestn. Cest. Spol. Zool.* **41,** 308–321.