

Description of *Vavraia parastacida* sp. nov. (Microspora: Pleistophoridae) from marron, *Cherax tenuimanus* (Smith), (Decapoda: Parastacidae)

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Abstract. A pleistophorid microsporidian is described from marron, *Cherax tenuimanus* (Smith). The parasite is polysporous, forming eight, 16, 32 or 64 ovoid spores in persistent, two-layered merontogenetic sporophorous vesicles. Nuclei remain isolated throughout merogony and sporogony. The sporophorous vesicle forms from the amorphous coat surrounding meronts, which thickens and condenses into two layers during sporogony. The parasite is placed in *Vavraia* Weiser, 1977, and named *Vavraia parastacida* sp. nov.

Introduction

The pathology of a recently recognized microsporidiosis of marron, *Cherax tenuimanus* (Smith), was described in a previous paper (Langdon 1991). The marron is a freshwater crayfish supporting a valuable recreational fishery and a developmental culture industry. This paper presents a species description of the causative pleistophorid, based principally on ultrastructural criteria. There are currently 11 genera available for polysporous microsporidians which produce a sporophorous vesicle; many have been described since 1980 and generic determination requires electron microscope studies (Canning, Killick-Kendrick & Killick-Kendrick 1991).

Materials and methods

Type specimens were collected from a farm dam near Perth, Western Australia (116°E, 32°S), by seine-netting marron and examining them for gross signs of microsporidiosis. Wet smears were prepared from the lesions in tail and thoracic muscle, and small cubes of affected tissues were fixed in 10% (v/v) buffered formalin for light microscopy or 2·5% (v/v) glutaraldehyde in phosphate buffer at pH 7·3 for electron microscopy. Histological sections cut at 4 µm were stained with haematoxylin and eosin (H&E), Giemsa's stain, or Ziehl-Neelsen's acid-fast stain with Loeffler's methylene blue as counterstain (ZN-L). Air-dried smears were stained with Wright's stain. Ultra-thin sections in Spurr's resin were stained with 2·7% Reynold's lead citrate and 2% aqueous uranyl acetate for electron microscopy. Semi-thin resin sections were stained with 1% toluidine blue and examined in the light microscope. Wet spore preparations were photographed with an objective micrometer under the microscope, and the pictures projected at a total magnification of ×10 000 to enable accurate spore measurements.

Results

The expanding spore masses occupied the sarcoplasm without xenoma formation and without host response until the sarcolemma was breached. Vegetative and sporogonial stages occurred amongst vesicles of spores within single host cells (Figs 1a & 2).

Meronts

Meronts were identifiable in histological sections as variably shaped, strongly basophilic in H&E sections, apparently uni- or multinucleate forms in various stages of division (Fig. 1a). At the ultrastructural level the meronts were bounded by a fine unit membrane with an outer amorphous coat (Figs 2 & 3). The cytoplasm was rich in ribosomes; up to three isolated nuclei were observed in ultra-thin sections. The meronts were rounded or irregular in shape but never found in linear multinucleate configurations. No meiotic forms or diplokarya were observed. Merogony was presumably by asexual fission but was not observed. Strands of the amorphous coat persisting between adjacent meronts, or meronts and sporonts, suggested that plasmotomy had occurred and included the coat (Fig. 3).

Sporophorous vesicle formation

Sporogenesis was signalled by retraction of the plasmodial unit membrane from the amorphous coat and thickening of the amorphous coat (Fig. 3). This resulted in a multinucleate sporogonial plasmodium separated from the vesicle wall, newly formed from the amorphous coat, by a narrow space (Figs 2 & 3). An irregular electron-lucent line divided the vesicle wall into two layers even at the stage of early retraction of the sporogonial plasmodium (Fig. 3). These two layers became more distinct as sporogenesis continued (see below), resulting in a two-layered sporophorous vesicle around the sporoblasts and spores. The vesicle layers condensed into two finer layers during spore maturation (Figs 7 & 8). The vesicle persisted around the mature spores and was visible in wet smears or semi-thin resin sections under the light microscope (Fig. 1a, b).

Sporonts and sporogony

Retraction of the multinucleate sporogonial plasmodium from the developing vesicle wall was followed by fission to yield uninucleate sporoblasts (Figs 1 & 4). The fission appeared simultaneous in most cases (Fig. 4), but occasionally part of the plasmodium was observed to be undergoing fission after the remainder had separated into uninucleate sporoblasts. No rosette formations as described by Canning & Hazard (1982) were detected in the electron microscope, but uninucleate sporoblasts arranged in rings could be seen in histological sections (Fig. 1a). The sporoblasts were initially irregular in shape and tightly apposed along the fission lines (Fig. 4), but retracted to more rounded forms (Fig. 5). During this rounding stage, the sporont wall was thickened by irregular patches of amorphous material deposited on the unit membrane (Fig. 5), which fused to form a regular electron-dense exospore layer (Fig. 6). Tubular and fibrillar elements remained in the vesicle (Figs 5 & 6).

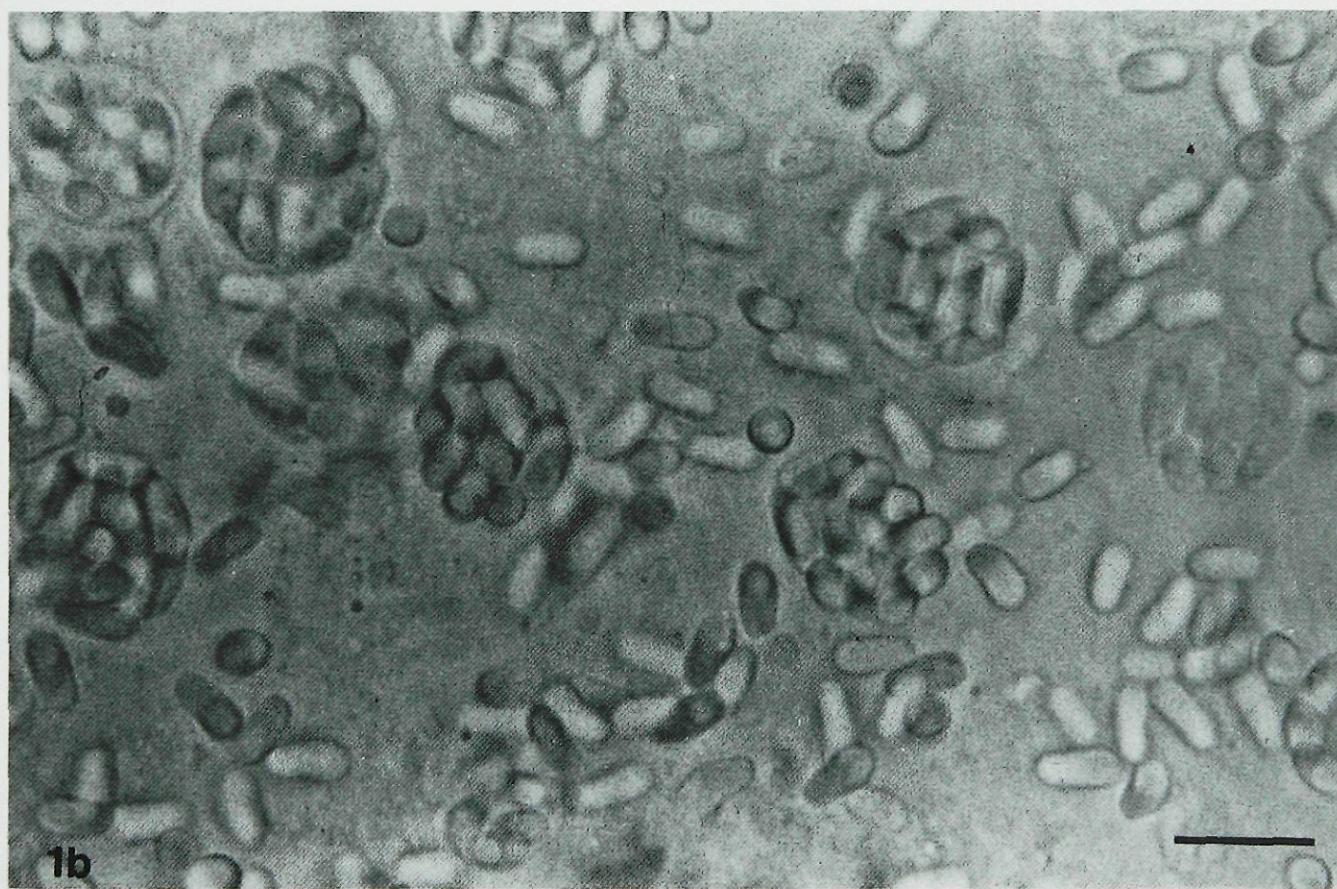
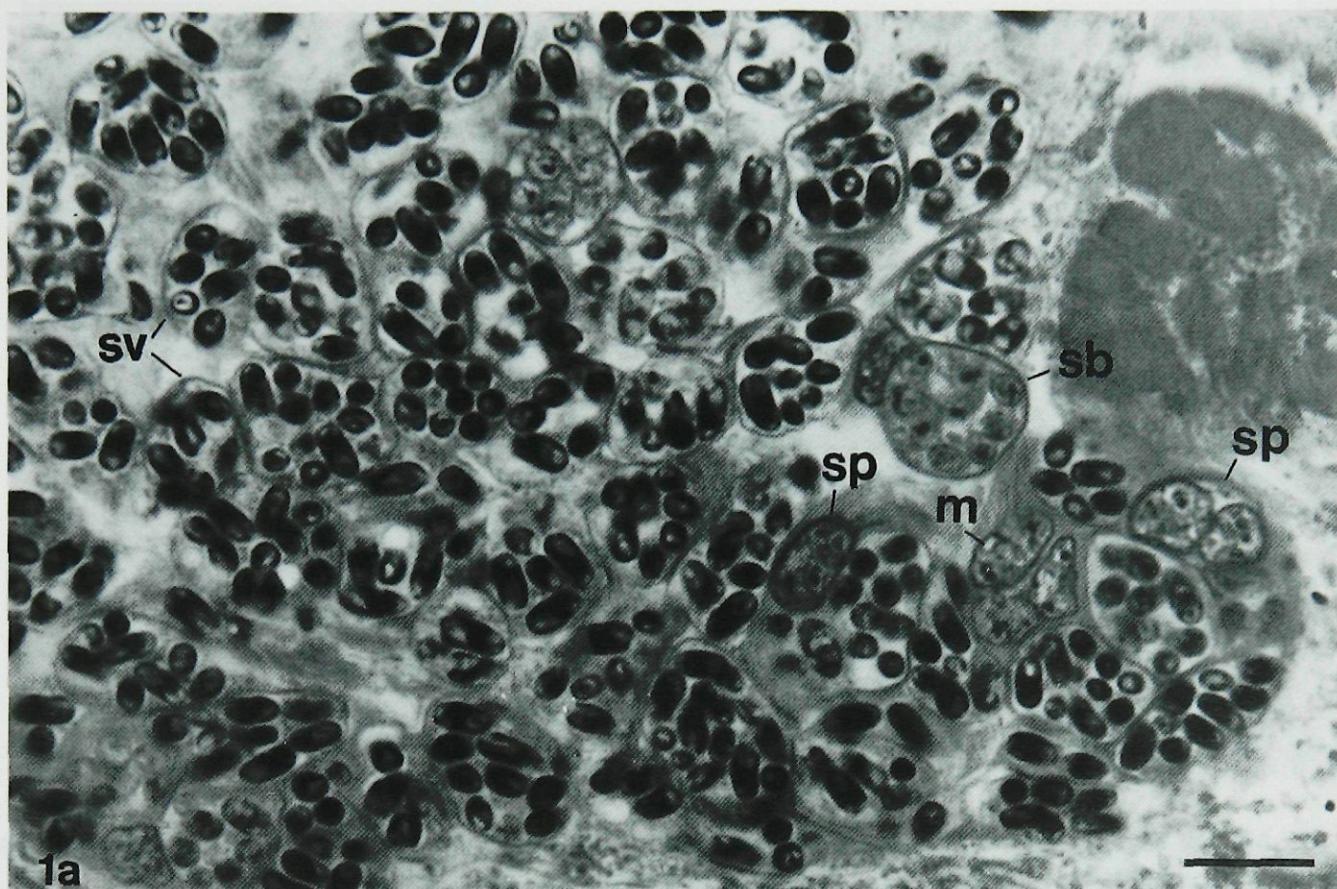


Figure 1. Light micrographs of infected muscle. (a) Resin section showing developmental sequence within a single myofibre: m, irregularly shaped vegetative stages, probably meronts, in various stages of division; sp, multinucleate sporogonial plasmodia with thickened vesicle wall, some undergoing fission; sb, uninucleate sporoblasts following fission of plasmodia, some in ring arrangements; sv, sporophorous vesicles containing spores showing posterior vacuole (toluidine blue, bar = 10 µm). (b) Wet preparation showing freed spores with posterior vacuole, and eight or 16 spores within vesicles (from Langdon 1991, bar = 10 µm).

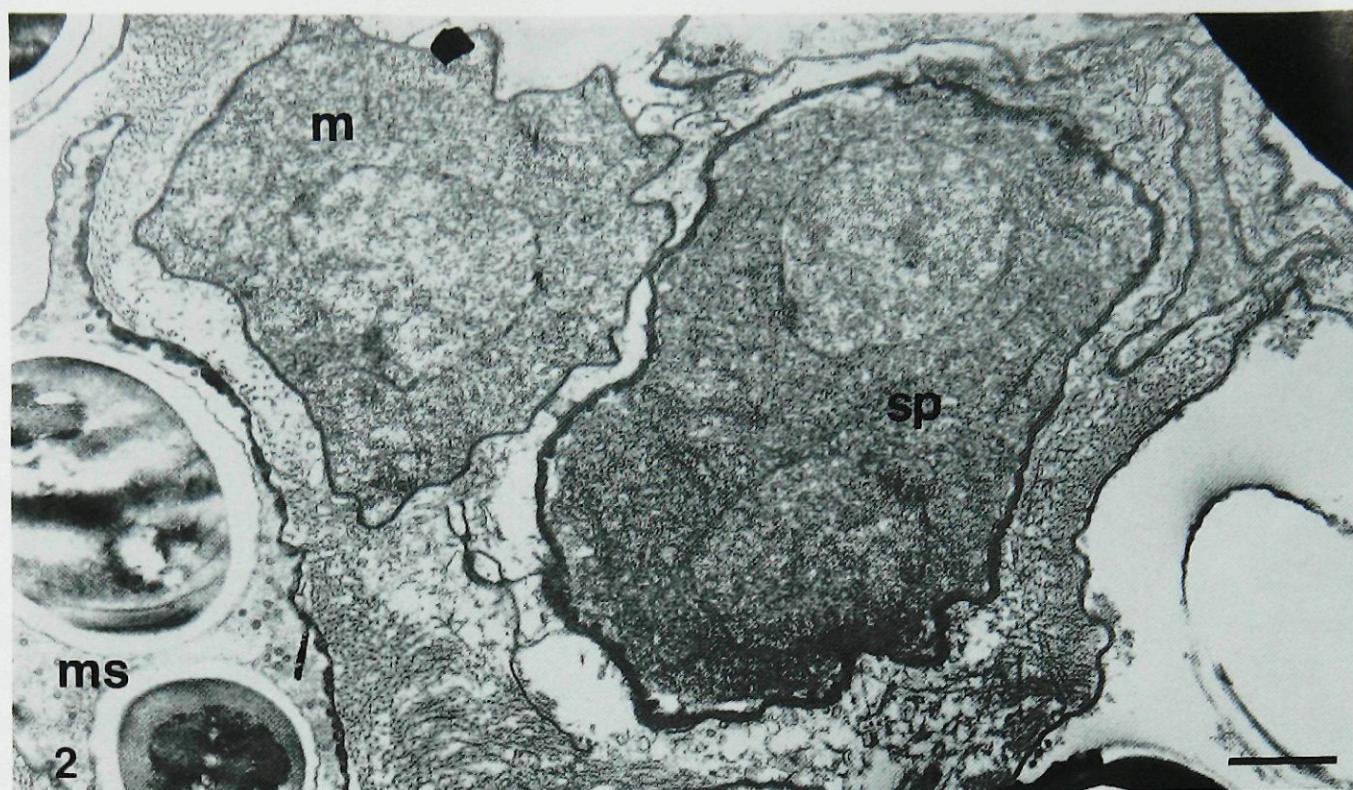


Figure 2. Meront (m), multinucleate sporogonial plasmodium (sp) and mature spores (ms) within the same host myofibre. Note strands of amorphous coat between meront and sporont (bar = 1 μm).

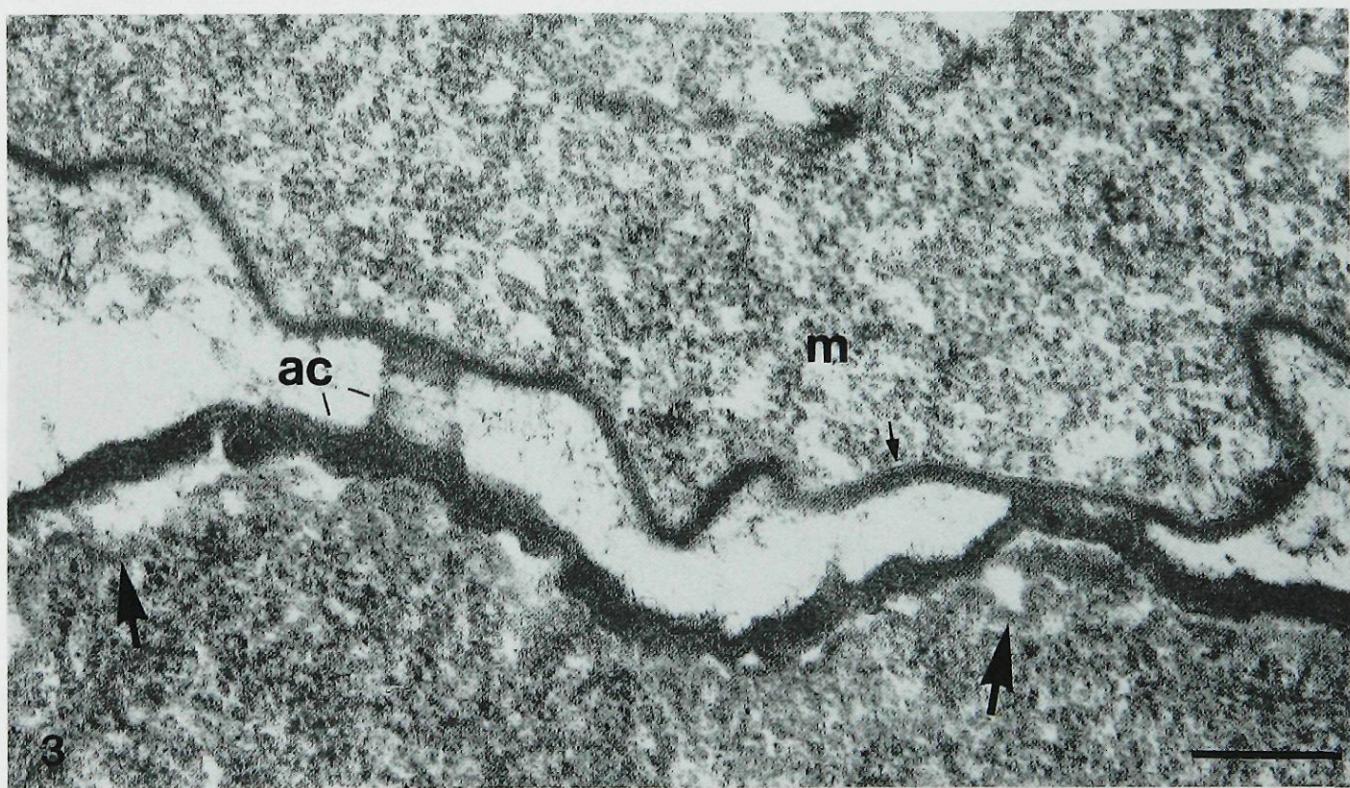


Figure 3. Enlargement of Fig. 2. Note unit membrane (small arrow) of meront (m) underlying amorphous coat. Retraction of sporogonial plasmodium is seen as separation of unit membrane (large arrows) from thickened amorphous coat (ac) which appears two-layered in some areas (bar = 0.5 μm).

Sporogenesis

Sporogenesis was signalled by elongation of the sporoblasts and irregular crenation of their unit membrane and exospore (Fig. 6). An electron-lucent endospore layer was then deposited, separating the crenated exospore from the unit membrane (Fig. 7). At this stage, the isofilar

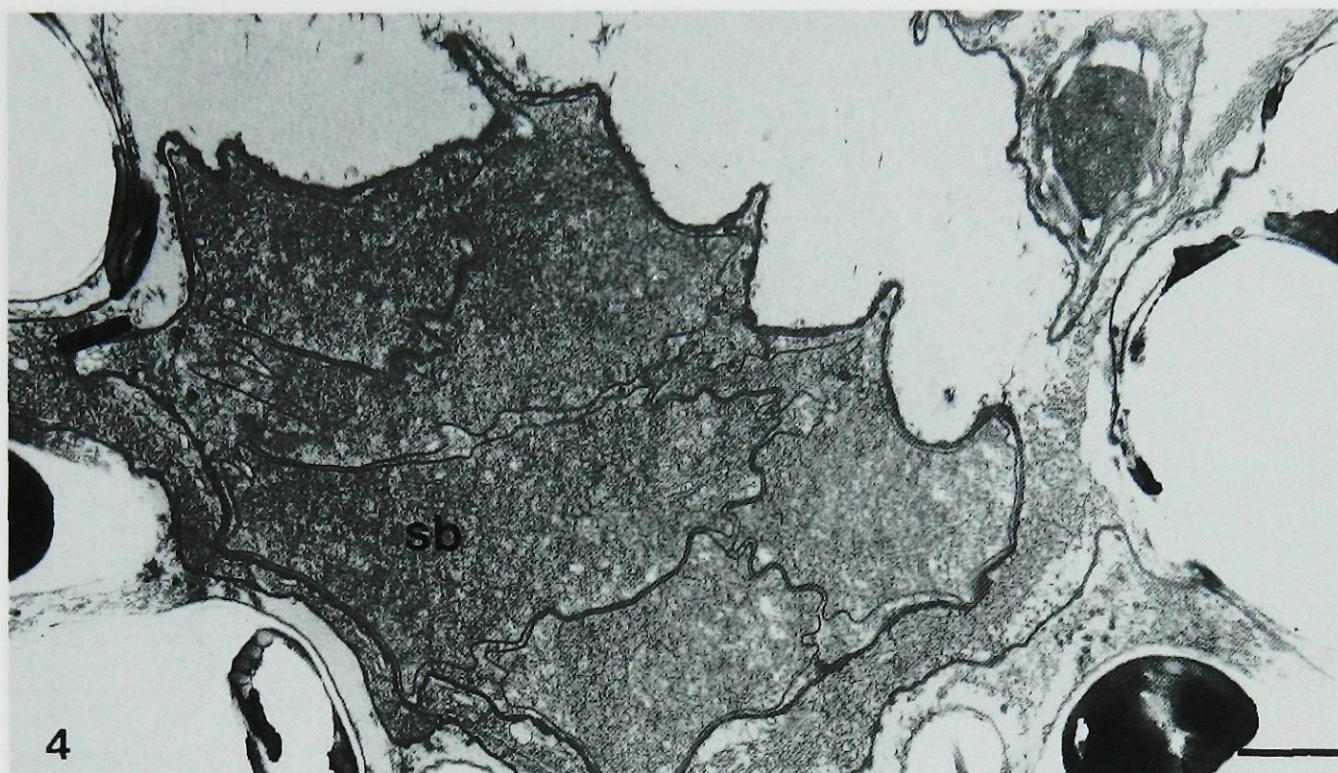


Figure 4. Fission of sporogonial plasmodium yielding irregularly shaped young sporoblasts (sb) (bar = 1 μm).

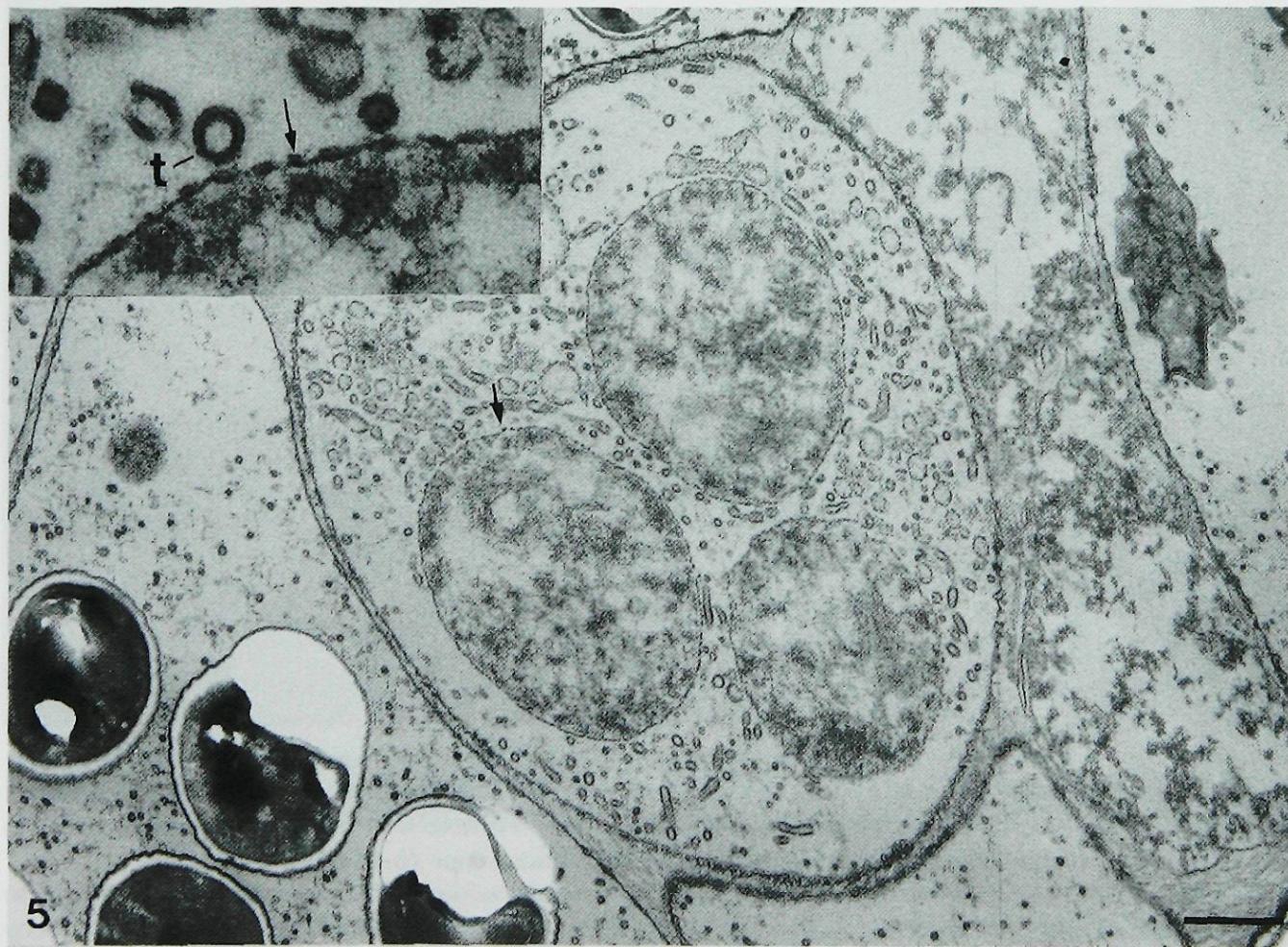


Figure 5. Rounded sporoblasts with irregular thickening deposits on their unit membrane (arrows). Inset is magnified 10 times compared to main figure. Tubular remnants (t) are visible in the sporophorous vesicle (bar = 1 μm).

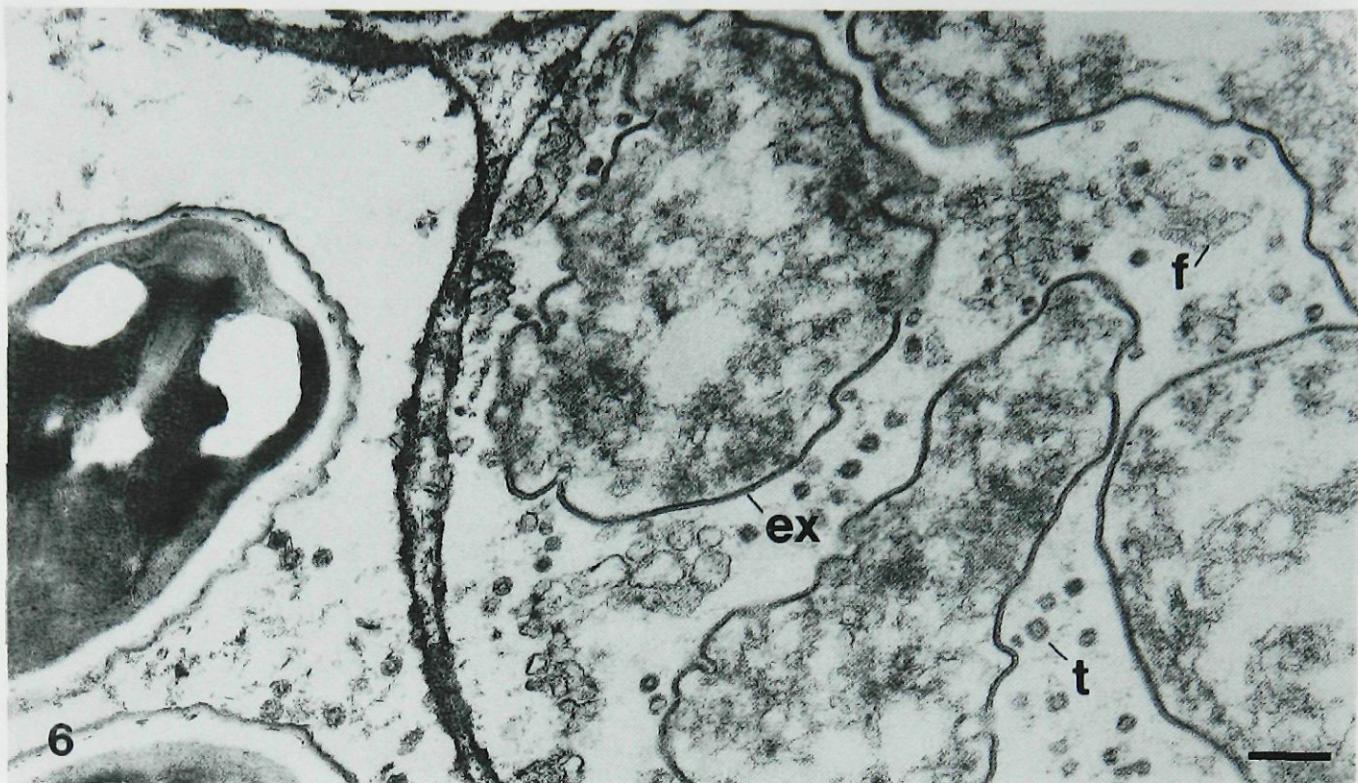


Figure 6. Elongated sporoblasts entering sporogenesis. The thickening deposits on the unit membrane have fused to form the future exospore layer (ex). Note the persistent tubular (t) and fibrillar (f) remnants in the sporophorous vesicle (bar = 0.5 μ m).

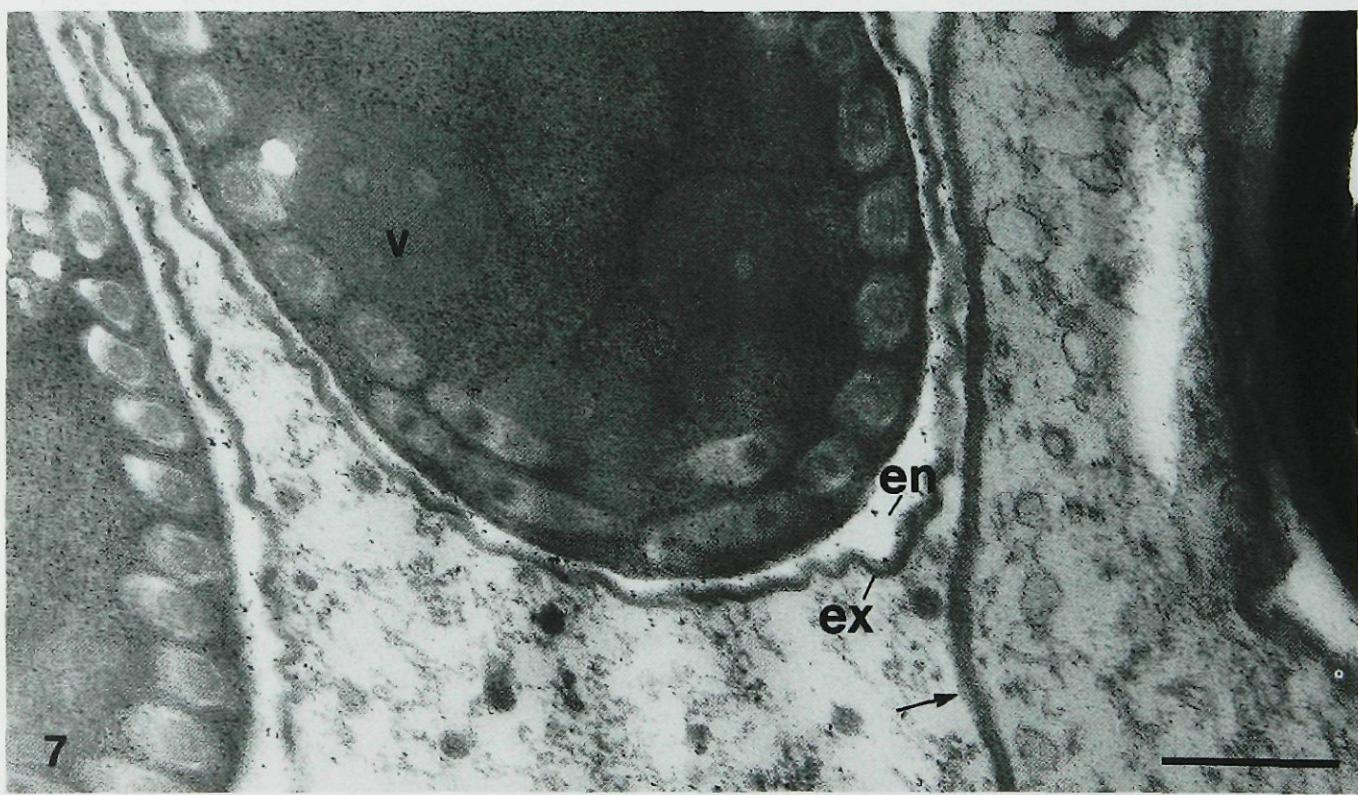
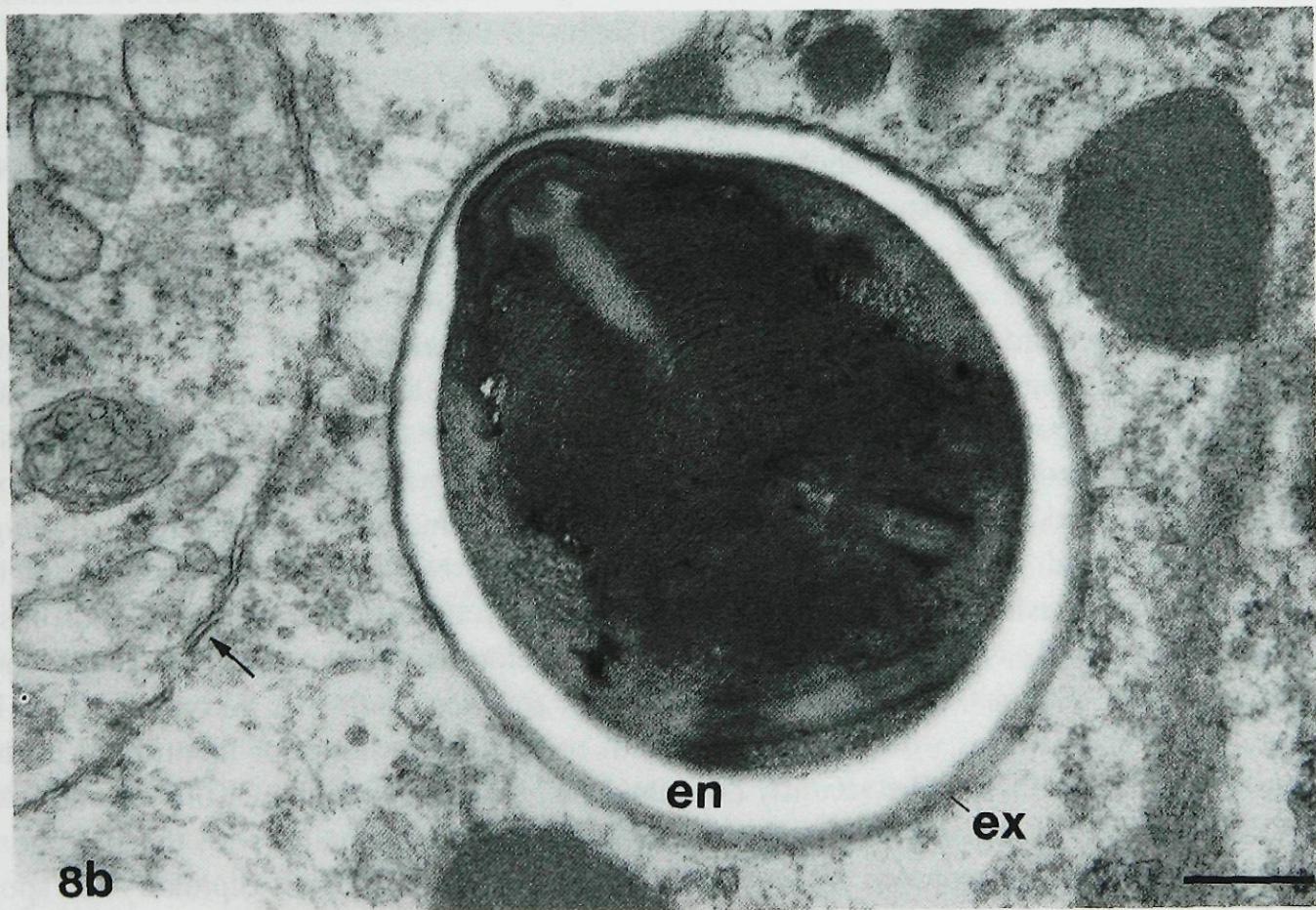


Figure 7. Immature spores showing coils of isofilar polar filament in section, crenated exospore (ex) and expanded endospore (en) layer, within two-layered sporophorous vesicle wall (arrow). Several vesicles (v) are evident in the area visible as a posterior vacuole in the light microscope (bar = 0.5 μ m).

polar filament had formed in nine to 11 coils (Figs 7 & 8). The endospore continued to thicken and the crenations in the exospore largely disappeared as it was stretched away from the spore body, resulting in the mature spore (Fig. 8a, b).



8a



8b

Figure 8. Mature spores. Note isofilar polar filament (f) coiled in posterior of spore in (a). The vacuole (v) is artifactual tearing. Manubroid and lamellar parts of the polaroplast are visible in (b). The exospore (ex) and endospore (en) layers are fully developed in (b). The sporophorous vesicle wall is resolved into two fine layers (arrow) (the rupturing is artifact) (bar = 0.5 µm).

Spores

The ovoid spores occurred in vesicle groups of 8, 16, 32 and occasionally larger numbers, presumably 64, and were often slightly curved when packed in the vesicle or released from vesicles burst on the microscope slide (Fig. 1a, b). The 0.2- μm -thick isofilar polar filament formed nine to 11 visible coils in the posterior part of the spore, around an area containing several vesicles (Fig. 7) that was also visible as a vacuole in the light microscope (Fig. 1a, b). The spore wall was slightly bulbous around the vacuole and slightly tapered anteriorly. The anterior of the spore was occupied by the manubroid part of the polaroplast, its anterior or lamellar portion and posterior cystic portion (Fig. 8). Overall spore dimensions were ($x \pm \text{S.D.}$, range): length 5.49 ± 0.36 , 5.0–6.1; width 2.59 ± 0.17 , 2.2–2.8 ($n = 20$).

Discussion

The microsporidian is an undescribed species and, being polysporous in persistent sporophorous vesicles, is clearly a pleistophorid. The species conforms to the genus *Vavraia* Weiser, 1977, as redefined and distinguished from *Pleistophora* Gurley, 1893, by Canning & Hazard (1982), in the following characteristics:

- (1) Nuclei were isolated in merogonial and sporogonial stages, not diplokaryotic.
- (2) Meronts were surrounded by an amorphous coat applied to the unit membrane.
- (3) A sporophorous vesicle was formed by thickening of the amorphous coat and retraction of the sporogonial plasmodium, and re-organization of the coat into two distinct layers.
- (4) Sporogonial fission was usually synchronous and multiple, yielding uninucleate sporoblasts.
- (5) Eight, 16, 32 or 64 monomorphometric, ovoid spores with a distinct posterior vacuole in the light microscope, isofilar polar filament and two-part lamellar polaroplast were contained in the persistent sporophorous vesicle.

The species lacks characteristics of, and so is excluded from, other available polysporous genera (cf. Canning *et al.* 1991) as follows. *Ovavesicula* Andreadis & Hanula, 1987 has diplokaryotic nuclei and 32 spores per vesicle (Andreadis & Hanula 1987). The young sporonts of *Polydispyrenia* Canning & Hazard, 1982, are diplokaryotic, and the sporophorous vesicle is membranoid and sporontogenetic in origin. *Cystosporogenes* Canning, Barker, Nicholas & Page, 1985, has merogonial and sporogonial stages in a fine membranous vesicle of unknown origin (Canning, Barker, Nicholas & Page 1985). Xenoma formation is characteristic of *Glugea* Thelohan, 1891, and, as redescribed by Canning, Lom & Nicholas (1982), meronts of this genus develop in a cisterna of host smooth endoplasmic reticulum. Meronts and sporonts of *Endoreticulatus* Brooks, Becnel & Kennedy, 1988, develop in a cisterna of host rough endoplasmic reticulum (Brooks, Becnel & Kennedy 1988). *Baculea* Loubes & Akbarieh, 1978, has elongate rod-shaped spores with the polar filament reduced to its anterior manubroid portion (Loubes & Akbarieh 1978). The recently described *Flabelliforma* Canning, Killick-Kendrick & Killick-Kendrick, 1991, has a fine, membranous vesicle which invaginates with the sporogonial wall to form fan-shaped sporonts. *Pseudopleistophora* Sprague, 1977 has diplokaryotic sporoblasts, and *Microsporidium* is a collective group in which several species have been placed temporarily (Canning *et al.* 1991).

The above genera, *Microsporidium* aside, thus have positive characters not shared by the pleistophorid from marron, which is closer to *Vavraia* Weiser, 1977 and *Pleistophora* Gurley, 1893 (cf. Canning & Hazard 1982). *Pleistophora* is characterized by channels traversing an electron-lucent layer of the amorphous coat of the future sporophorous vesicle during merogony

(Canning & Nicholas 1980), which were not observed in the present parasite. Canning & Nicholas (1980) also considered the occurrence of a second octosporous macrosporulation sequence to characterize *Pleistophora*, although species lacking macrospores have also been placed in the genus without revision (Faye, Toguebaye & Bouix 1990). The present species from marron displayed a single sporulation sequence leading to monomorphometric spores, within a two-layered sporophorous vesicle derived from an amorphous coat of merontogenetic origin, as occurs in *Vavraia* (Canning & Hazard 1982). Other features consistent with this genus (cf. Canning & Hazard 1982) were the mode of exospore deposition on the sporoblast wall, the persistence of amorphous coat strands between recently divided vegetative stages, and the occurrence of 8, 16, 32 or more spores per sporophorous vesicle.

However, certain characteristics of *Vavraia* were not shown to be present in the marron parasite. The mode of merogonial fission was not positively determined, and a rosette stage in fission of the sporogonial plasmodium was not observed. Canning & Hazard (1982) defined sporogonial fission in *Vavraia* as occurring via a rosette stage before separation of the individual sporoblasts. Rosette formations could occur in the present species, just prior to the stage shown in Figs 1 and 4, but these were not detected ultrastructurally. Pre-spore stages were rare and difficult to locate in the present material, so rosette and other stages may have remained undetected. Characteristics of those stages which were detected and examined ultrastructurally do exclude the marron parasite from all the polysporous genera, as discussed above, except *Vavraia*. Thus, the marron parasite is placed in this genus and named *Vavraia parastacida* sp. nov. in recognition of its pathogenicity for parastacid crayfish.

Pleistophorids with indistinguishable spore characteristics have also been found in wild marron from the Warren River, south-west Western Australia, in gilgies, *Cherax quinquecarinatus* (Gray), from the type locality, and in cultured redclaw crayfish, *Cherax quadricarinatus* (Von Mortens), imported into quarantine from south-east Queensland (Langdon 1991).

Vavraia parastacida sp. nov.

Type host:	<i>Cherax tenuimanus</i> .
Other hosts:	<i>Cherax quinquecarinatus</i> and <i>Cherax quadricarinatus</i> .
Site of infection:	Locomotor muscle, cardiac muscle, gastro-intestinal muscle and adjacent serosa, gills, hepatopancreas, antennal gland, eye, connective tissues.
Locality:	South-west Western Australia (<i>C. tenuimanus</i> , <i>C. quinquecarinatus</i>), south-east Queensland (<i>C. quadricarinatus</i>).
Meronts:	Isolated nuclei, uni- and multinucleate forms with amorphous coat overlying unit membrane. Fission process not determined but probably by plasmotomy.
Sporonts:	Multinucleate sporogonial plasmodium retracts from thickened amorphous coat to form sporophorous vesicle. Uninucleate sporoblasts formed by simultaneous fission; presence of rosette formations not determined. Progressive fission yielding sporoblasts asynchronously also observed. Sporoblast wall thickened by irregular deposits of amorphous material which fuse to form exospore.
Sporophorous vesicle:	Thin two-layered wall around eight, 16 or 32 (rarely, 64) spores or sporoblasts, derived from a merontogenetic amorphous coat which is thickened around sporogonial plasmodium and resolved into two

- layers during sporogonial retraction and fission. Contains tubular and fibrillar remnants following fission.
- Spores:** Oval and slightly conoid anteriorly, large prominent posterior vacuole visible in light microscope, monomorphometric, length 5.5 ± 0.4 , $5.0-6.1$; width 2.6 ± 0.2 , $2.2-2.8 \mu\text{m}$ ($x \pm \text{S.D.}$, range). Finely lamellated polaroplast with anterior lamellar and posterior cystic portions, polar filament thick ($0.2 \mu\text{m}$), isofilar, and arranged in nine to 11 coils.
- Classification:** Phylum Microspora Sprague, 1977; Order Microsporida Balbiani, 1882; Suborder Pansporoblastina Tuzet *et al.*, 1971; Family Pleistophoridae Stempell, 1909; Genus *Vavraia* Weiser, 1977.
- Reference specimens:** Zoological Reference Collection, National University of Singapore, Kent Ridge, Singapore. Specimen No. ZRC. 1991. 1001 (type specimen). National Registry of Veterinary Pathology, Taronga Zoo, Mosman, New South Wales, Australia. Specimen No. V0010.

No *Vavraia*, *Pleistophora* or other pleistophorid species have been reported previously from Australian parastacid crayfish, although a *Pleistophora*-like parasite was recently observed in association with metacercaria in yabbies, *Cherax destructor* Clark, from South Australia (O'Donoghue, Beveridge & Phillips 1990). Other microsporidians noted in Australian crayfish appear to be *Thelohania*-like species (cf. Langdon 1991). *Vavraia parastacida* sp. nov. appears to be the first *Vavraia* species described from decapod crustaceans. Studies of its life cycle and transmission are in progress.

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