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Nadelspora canceri N. G., N. sp., an Unusual Microsporidian Parasite of the Dungeness Crab, *Cancer Magister*

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ABSTRACT. The microsporidium *Nadelspora canceri* n. g., n. sp., is described from the striated musculature of the Dungeness crab (*Cancer magister*) in Oregon, USA. The needle-shaped spores were rounded anteriorly, tapered to a posterior point and measured 7.1–11.8 × 0.2–0.3 µm in fixed preparations. The extremely narrow spore diameter prevented observation of morphological details at the light microscopic level and ultrastructural details of mature spores were difficult to resolve. Meronts were not observed and the monokaryotic merozoites and sporonts were not contained within either parasitophorous or sporophorous vesicles. Sporonts were diploplastic and gave rise to monokaryotic sporoblasts that became narrow and elongate as they developed into immature spores with a developing polar filament. The nucleus was not clearly resolved in mature spores and may have been surrounded by the lamellar polaroplast. The polar filament was of nearly uniform diameter throughout most of its length and ended abruptly about three-fourths of the distance from the anterior end of the spore. Unusual spherical non-membrane bound granules surrounded the polar filament in a spiral arrangement. The new microsporidium resembles members of the family Mrazekiidae, but differs in lacking a diplokaryon at any stage. It is probably most closely related to *Baculea daphniae* from which it differs primarily by spore shape and size. The familial relationships of the genus *Baculea* have not been determined and it is proposed to include it with *Nadelspora* in the new family Nadelsporidae.

Supplementary key words. Crab muscle parasite, Microspora, ultrastructure.

THE Dungeness crab (*Cancer magister*) supports an important commercial and sport fishery on the Pacific coast of North America. The known disease agents affecting Dungeness crabs have been reviewed by Meyers et al. [12] and by Sparks [14] and include parasitic ciliates, trematodes, a fungal pathogen and a chlamydia-like organism. Microsporidian parasites have not been described. Dungeness crabs from coastal Washington, Oregon and California have been found to be infected by a microsporidian parasite with unusual needle-like spores. The structure of the parasite does not conform to that of any microsporidian described in the key to microsporidian genera by Larsson [7]. It replicates in the musculature of the crab host causing a whitish, opaque gross external appearance typical of other crustacean muscle-infecting microsporidians [13].

In this study, *Nadelspora canceri*, a new genus and species of microsporidian parasite is described based upon light and electron microscopic observations of infected Dungeness crab tissue.

Preliminary results of field studies in progress have shown that infection prevalence rates between 15 and 25% are common in some Oregon estuaries.

MATERIALS AND METHODS

Specimens for study were collected in Alsea Bay, Oregon either by raking in sandy tidepools at low tide or by crabbing with baited crab rings. Infected crabs were recognized by the whitish-

opaque gross appearance of muscle visible through the proximal periarticular membrane at the base of the thoracic periopods.

For light microscopy, smears of infected muscle tissue were examined in wet mounts under phase contrast optics, were fixed in methanol and stained with Giemsa, or were heat-fixed and stained by the Gram stain method. Giemsa and hematoxylin & eosin stained sections of infected muscle were prepared after fixation in Bouin's solution and processing for standard paraffin embedding and sectioning. Thick sections (2–3 µm) of tissue were also made on material embedded in plastic as described below and stained with aqueous toluidine blue.

For electron microscopy samples, cheliped meri were removed from infected crabs, opened sagittally, and the muscle removed. Myofibers were dissected into 2 mm cubes and placed into one of three fixatives: 1. 1.5% glutaraldehyde/1.5% paraformaldehyde in a 0.1 M cacodylate buffer, to which 5 mg% of calcium chloride, 6 mg% sucrose, and 3% sodium chloride were added; 2. the same fixative with the addition of 0.5% picric acid; 3. 1.7% glutaraldehyde in 0.1 M Millonig's phosphate buffer. The pH was adjusted to 7.2 in each fixative. Tissue was post-fixed in 2% osmium tetroxide and washed in buffer before dehydration. Some samples were dehydrated through a graded alcohol series, infiltrated in equal volumes of ethanol and LR White (London Resin Co.), before being placed in full strength LR White. After infiltration, specimens were placed in fresh resin in gelatin capsules and polymerized at 60° C overnight. Other samples were dehydrated through a graded acetone series and embedded in Spurr's low viscosity resin. Selected areas were chosen from thick-sectioned (0.5–1.0 µ) material, thin sectioned

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with a diamond knife, stained sequentially with ethanolic or aqueous uranyl acetate and lead citrate and examined with a Zeiss 10CA or a Philips CM12/STEM electron microscope at 60 kV.

RESULTS

Gross and light microscopic observations of infected crabs. Infected crabs were initially recognized when recreational crabbers complained that some crabs from Alsea Bay, Oregon had poor flavor. Examination of these crabs in the laboratory revealed crab muscle that was opaque with whitish streaks giving it a fibrous appearance. This contrasted with the homogenous, translucent appearance of unaffected crab muscle tissue. When viewed under a light microscope, affected muscle was found to contain a vast number of microsporidian spores that were of an unusual shape. Most microsporidian parasites of decapod crustaceans have spores that are approximately oval in shape [15]. In contrast, the gram-positive spores from Dungeness crabs were needle-like in appearance, long and very narrow, rounded at one end and tapering to a point at the other (Fig. 1). Fifty fixed, Giemsa-stained spores averaged 9.9 μm (SD = 1.3 μm) in length. Spore width could not be measured under the light microscope because of the narrow ($< 0.5 \mu\text{m}$) diameter. Very little of the internal structure of the spore was discernable at the light microscope level.

Striated muscle fibers throughout the entire crab were infected with the microsporidium (Fig. 2). Most crabs studied contained a preponderance of mature spores and developmental stages were difficult to detect in the light microscope. Infected muscle contained masses of the needle-shaped spores that were integral with the muscle bundles and were often difficult to differentiate from muscle fibers in wet mounts and in histological sections stained with Giemsa or hematoxylin and eosin. Spores were more easily seen in plastic embedded sections stained with toluidine blue (Fig. 3).

Electron microscope observations—developmental stages. Most crabs from which tissues were examined in the electron microscope contained predominantly mature spores. However, several crabs were examined that contained large numbers of parasite developmental stages. Meronts were not observed, but merozoites, sporonts, sporoblasts and immature spores were present and are described below.

The parasite caused disruption of infected muscle cells so that prespore stages and mature spores often occupied spaces within infected muscle fibers (Fig. 4–6). Neither parasitophorous vesicles of host origin nor sporophorous vesicles of parasite origin were present. The earliest parasite stages observed were free merozoites occupying striated muscle, often in close proximity to sporonts, sporoblasts, and immature and mature spores (Fig. 5, 6). Merozoites were irregularly rounded, measured approximately $3 \times 4 \mu\text{m}$, were bounded by a unit membrane, contained a single nucleus (monokaryon) and sparse ribosomes and endoplasmic reticulum. Merozoites sometimes occurred in rows or lines and appeared to mature into sporonts characterized by a shape similar to merozoites, but more elongate and with a thickened plasmalemma (Fig. 6, 7).

Sporogony followed sporont karyokinesis, development of an exospore layer and binary fission to form two sporoblasts (Fig.

8). Late sporonts were irregular in size and shape, but were generally elongated and contained a single nucleus bounded by a partially separated double nuclear membrane (Fig. 8) that was undulating in outline. The nucleus often displayed an invaginated nuclear membrane containing a distinctive centriolar plaque often with associated polar vesicles (Fig. 7, 8). Both the nucleoplasm and cytoplasm were quite electron lucent, especially the cytoplasm which contained membranes that probably represented rough endoplasmic reticulum, but ribosomes were sparse. The marked distention of some late sporonts, including those undergoing cytokinesis to form sporoblasts, was commonly observed (Fig. 9).

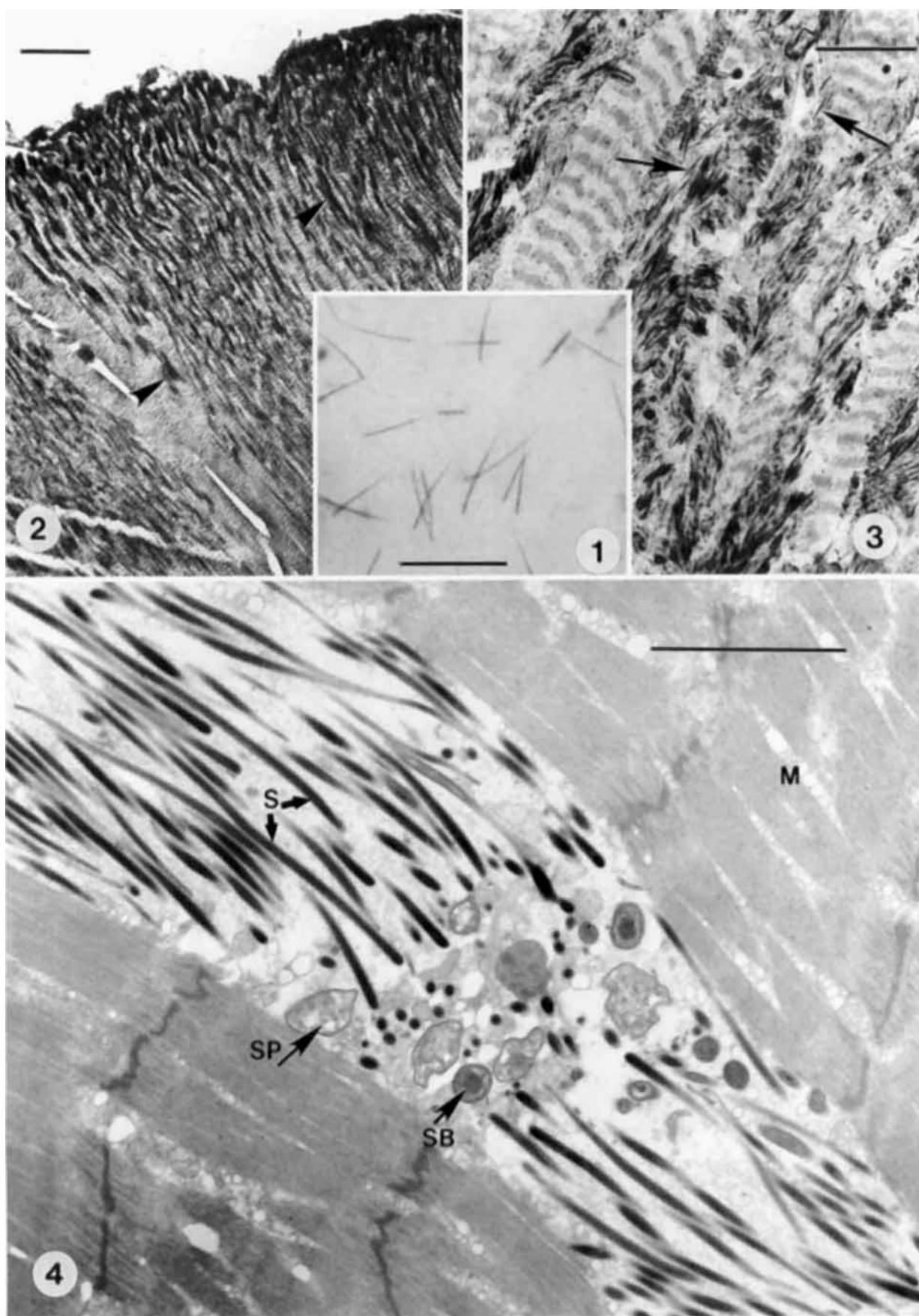
The development of sporoblasts into immature spores may involve a process in which sporoblasts condensed, became more elongate, narrower and more electron dense (Fig. 8, 9). Late sporoblasts and immature spores were characterized by a well established polarity, an elongate, electron dense nucleus (Fig. 8) and a developing polar filament that extended posteriorly from a button-shaped developing anchoring disc (Fig. 8, 10) and associated polar sac (Fig. 10). The exospore layer of the future spore wall was complete, but no endospore layer was present (Fig. 8, 10). No polaroplast was evident in immature spores. Maturation into mature spores apparently involved a continued progressive condensation of parasite cell contents with the cytoplasm and nucleus becoming increasingly electron dense and with the spores becoming greatly elongated and extremely narrow in diameter.

Electron microscope observations—spores. Due to the length and often slightly curved shape (Fig. 1, 4) of the long (9.9 μm) needle-like spores, entire spores were rarely observed in thin sections and spore lengths were measured in fixed smears under the light microscope. Conversely, the extremely narrow spore diameter dictated measurement in electron photomicrographs. Thirty spores measured in this way averaged 0.25 μm (SD = 0.028 μm) in diameter at the widest point near the anterior end.

The spore wall was composed of exospore and endospore regions (Fig. 11, 12). The exospore was layered, 38 nm in total thickness including an electron dense external double-layer that resembled a unit membrane and with the internal addition of layered granular material that was less electron dense. The external surface of the exospore seemed to consist of an accumulation of granular material to give an irregular, almost rough appearance. The endospore was narrow ($\sim 6 \text{ nm}$ thick), electron lucent, and adjacent to an indistinct plasma membrane.

The polar filament anchoring apparatus was directed to the side of the spore, was subterminal in location (Fig. 13, 14) and was enclosed in a short polar sac (Fig. 18). The polar filament was apparently isofilar with an average diameter of 73 nm although a slight narrowing of the diameter from a maximum of 81 nm to 37 nm was measured in cross sections as the filament occupied the middle portion of the spore where the spore began to taper toward the posterior tip. The filament appeared to end abruptly approximately three-fourths of the distance from the anterior end of the spore ($\sim 7.5 \mu\text{m}$) and to have no coiled portion. The distinctive bead-like material observed within the extreme posterior tip of numerous spores (Fig. 14) had a somewhat coiled appearance, but no connection to the polar filament could be detected.

Fig. 1–4. *Nadelspora canceri* n. g., n. sp. from muscle of *Cancer magister*. 1. Gram-stained smear of spores. Bar = 10 μm . 2. Low magnification of histological section showing intense concentrations of spores (arrowheads) in crab striated muscle. Bar = 100 μm . 3. Higher magnification of sectioned muscle showing individual spores (arrows). Bar = 10 μm . 4. Electron photomicrograph of infected muscle (M) showing spores (S) and cross sections of several sporonts (SP) and sporoblasts (SB). Bar = 0.5 μm .



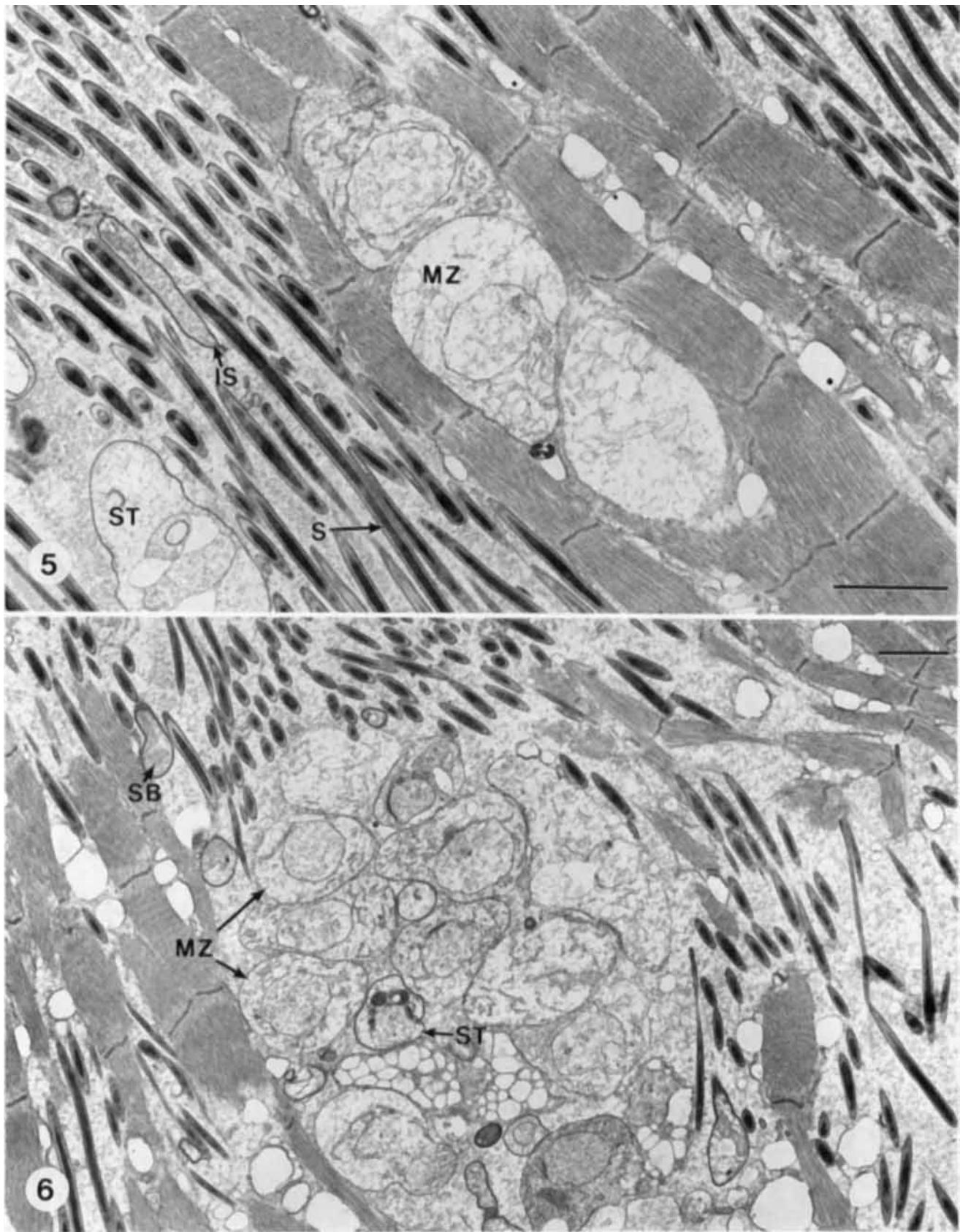


Fig. 5, 6. Developmental stages of *N. cancri*. 5. Merozoites (MZ) and sporont (ST) with lucent cytoplasm and nucleoplasm. Immature spore (IS) and numerous mature spores (S) in crab muscle. 6. Merozoites (MZ) arranged in rows, sporont (ST) and sporoblast (SB) with thickened plasmalemma. Bars = 2.0 μ m.

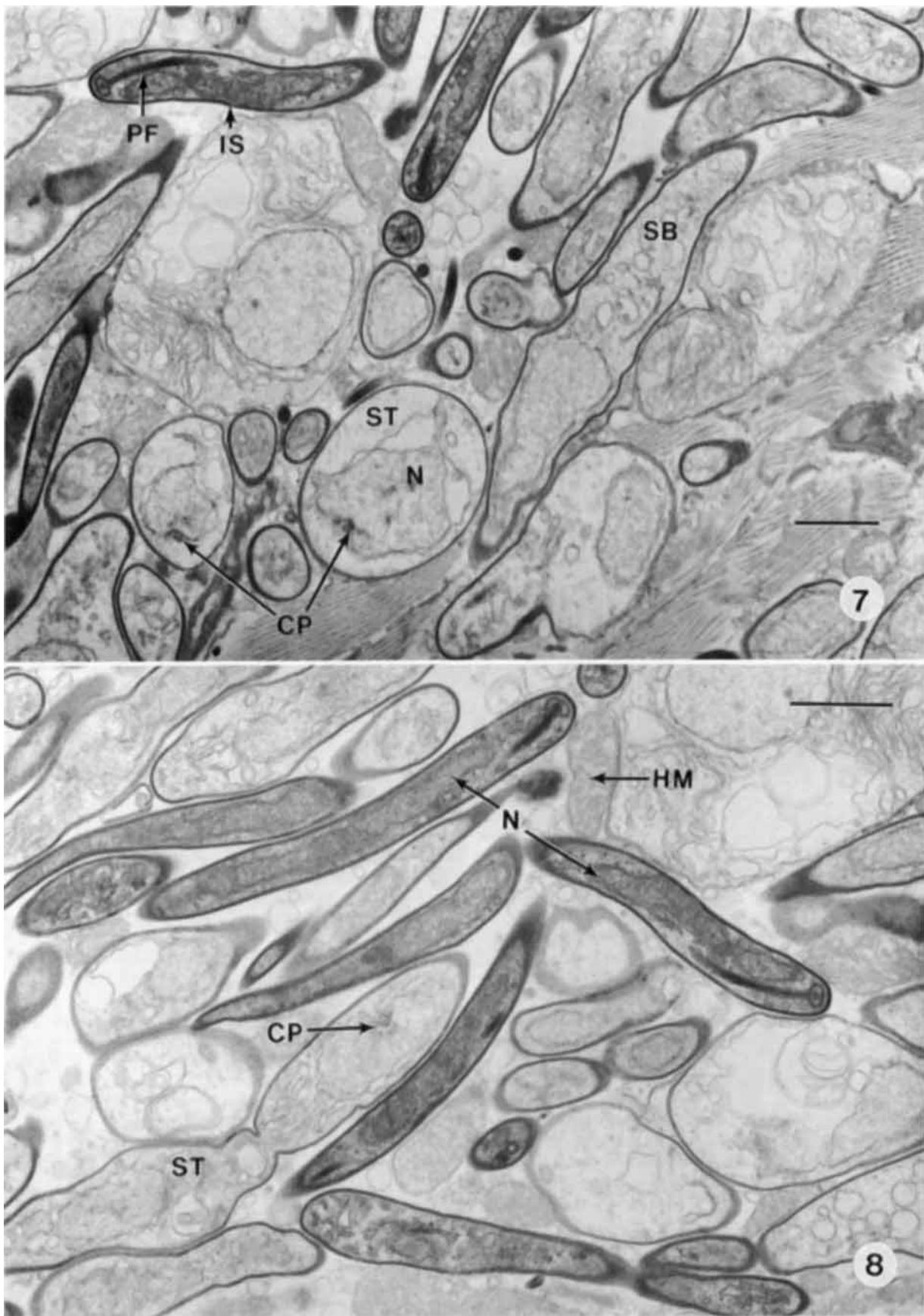


Fig. 7, 8. Developmental stages of *N. cancri*. 7. Sporonts (ST) showing rounded shape, thickened plasmalemma, and containing nuclei with distinctive nuclear plaques (CP) and associated polar vesicles. Elongate sporoblast (SB) and immature spores (IS) that contain a developing polar filament (PF). 8. Late sporont (ST) undergoing cytokinesis to form two sporoblasts and containing a nucleus with centriolar plaque (CP). Immature spores with electron dense nuclei (N) and developing polar filament. Host mitochondria (HM) present. Bars = 1.0 μm .

In cross section, the polar filament was layered and appeared to conform approximately to the typical layer pattern described by Larsson [5]. There was a relatively dense center with several possible sublayers, a less dense interspace layer, and an electron lucent layer next to an external unit membrane (Fig. 11, 12). In addition, an outside layer of granular material that circumscribed the polar filament in a distinctive spiral arrangement appeared to begin at the posterior border of the lamellar polaroplast and continued to about the posterior termination of the filament (Fig. 11, 12, 17). The spherical granules were uniform in size and measured approximately 20 nm in diameter.

The polaroplast was lamellar and extended posteriorly from the subterminal polar filament anchoring apparatus for about 1.7 μm (Fig. 18). The polaroplast was apparently not divided into anterior and posterior regions, rather it was uniformly lamellar throughout its length. However, lamellar material observed near the posterior limit of the spherical granules in one spore may represent a posterior polaroplast (Fig. 15). In cross sections, the polaroplast did not appear to completely surround the polar filament (Fig. 11). Although the nucleus was not unequivocally identified in mature spores, cross sectional views revealed a structure that could represent the nucleus (Fig. 16). This putative nucleus was adjacent to the polar filament and was surrounded by the polaroplast in a manner that could obscure the nucleus when viewed in longitudinal aspect. However, a structure that may have been a nucleus was also observed in association with the posterior lamellar material (Fig. 15).

In general, the internal morphology of mature spores was difficult to resolve in the specimens examined. This may have been due to the extremely narrow diameter of the spores leading to opaque, electron dense preparations.

DISCUSSION

Microsporidians are common parasites of crustaceans [14, 15] and a number have been reported to parasitize decapods in the Eastern North Pacific Ocean along the west coast of the United States [1, 13]. With the exception of the brief mention in an abstract of an undescribed microsporidium with typical oval spores (Morado, J. F. and Sparks, A. K., 1988. Infectious diseases of the Dungeness crab. Abstract, p. 16. American Fisheries Society International Fish Health Conference, Vancouver, Canada.), no microsporidian infections in *C. magister* have been reported.

The elongate, somewhat rod-shaped spore of *Nadelspora* superficially suggests relationships to other rod-shaped microsporidians. Of the 16 previously described genera that fall into the rod-shaped spore category [3, 5, 6, 9, 15], none belong to families that have characteristics that permit inclusion of *Nadelspora*. Six genera (*Cylindrospora*, *Helminchia*, *Ormieresia*, *Striatospora*, *Toxoglugea*, and *Resiomeria*) belong in the family Thelohanidae based upon the characteristics of being octosporoblastic with spores produced in sporophorous vesicles and of having diplokaryotic prespore stages, but monokaryotic spores [6]. Five genera (*Bacillidium*, *Hrabyeia*, *Jirovecia*, *Mrazekia*, and *Rectispora*) are in the family Mrazekidae characterized by a diplokaryotic state throughout the life cycle and the absence of a sporophorous vesicle [9]. The remaining five genera having

rod-shaped spores are: *Baculea*, *Desportesia*, *Cougaurella*, *Cystosporogenes*, and *Octosporea*. Of these, all but *Baculea* have spores contained within sporophorous vesicles and differ in other respects from *Nadelspora* [3, 4, 8, 15]. The genus *Baculea*, for which family relationships have not been determined [10], shares a substantial number of structural characteristics with *Nadelspora*.

Both *Nadelspora* and *Baculea*, as described for the only species *B. daphniae* [10], are monokaryotic throughout their life cycles. They have merozoites, sporonts and sporoblasts that contain relatively few ribosomes resulting in cytoplasm with a lucent appearance. Similarities between the immature spores are especially striking and include a developing polar filament with a laterally directed, subterminal anchoring apparatus and an elongate nucleus that nearly reaches the anchoring apparatus anteriorly. Both have elongate mature spores containing a straight isofilar polar filament that appears to end abruptly approximately three-fourths of the distance from the anterior end of the spore after narrowing very slightly and without forming coils. In both genera, the polaroplast is lamellar and probably does not completely surround the polar filament. In *Nadelspora*, there is no posterior polaroplast and none was described for *B. daphniae*.

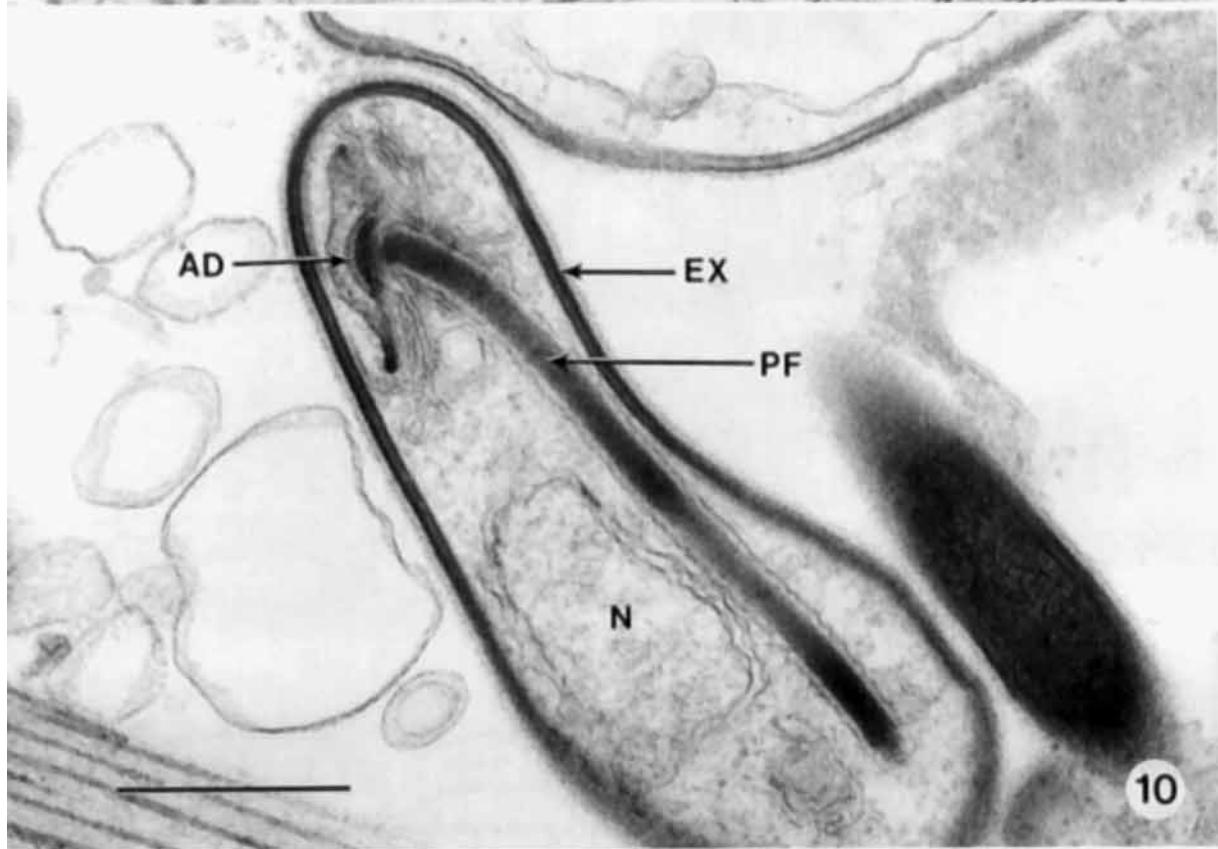
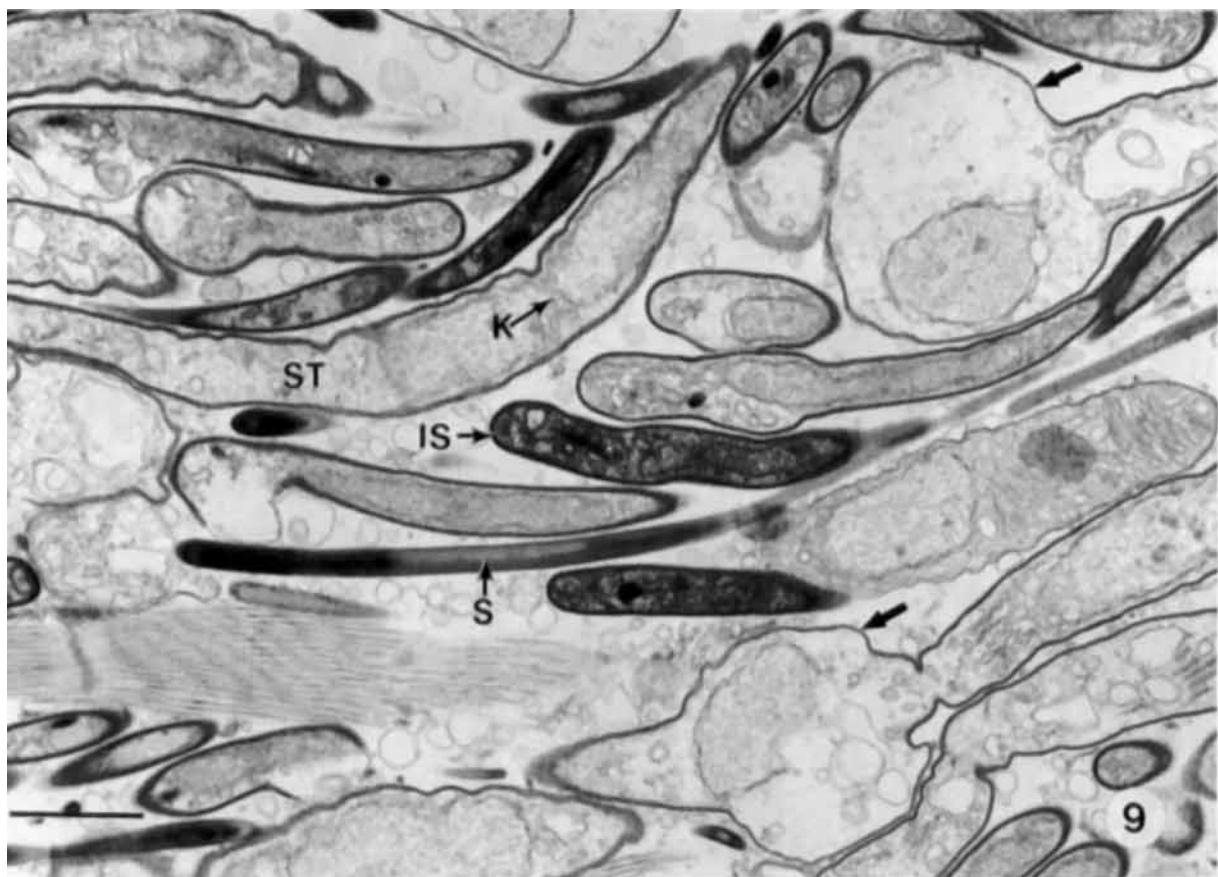
Among the differences between *Nadelspora* and *Baculea*, the most important is the major difference in spore shape. *Nadelspora cancri* is long (~10 μm) and needle-shaped, tapering to a posterior point while *B. daphniae* is very short (2.8–3 μm), bluntly rod shaped and of uniform diameter throughout its length. Both have very narrow spores, *N. cancri* measuring 0.25 μm and *B. daphniae*, 0.20 μm , which are among the narrowest spore diameters reported in the literature.

A significant feature of *N. cancri* is the presence of an organized, spirally arranged series of granules surrounding the polar filament and apparently reaching from the posterior margin of the polaroplast to the end of the filament. The granules are not membrane bound and are larger than ribosomes. Granules such as these have not previously been described as a component of a microsporidian spore (J. I. R. Larsson, pers. commun.). It is not known if the polar filament of *B. daphniae* is surrounded by a similar arrangement of granules.

Another difference between *N. cancri* and *B. daphniae* that could be considered important is the fact that at no point are the developmental stages or spores of *N. cancri* surrounded by a membrane, neither a sporophorous vesicle of parasite origin nor a parasitophorous vesicle of host origin. This contrasts with *B. daphniae* in which most developmental stages and all mature spores are contained within a membranous structure that according to Loubès & Akbarieh [10] is of host origin and therefore a parasitophorous vesicle. These vesicles are considered to be a part of the host response and therefore of little taxonomic value (J. I. R. Larsson, pers. commun.), [11] so they do not constitute a significant difference between *Nadelspora* and *Baculea*. Canning [2] considers the parasitophorous vesicle of *Baculea* to be a sporophorous vesicle and places *Baculea* in the family Glugeidae. In light of the above conclusions [11], assignment to the family Glugeidae is not valid.

Since *N. cancri* and *B. daphniae* have many similarities that

Fig. 9, 10. Developmental stages of *N. cancri*. 9. Elongate sporont (ST) undergoing karyokinesis (K). Late sporonts with distended plasmalemma to give a ballooning appearance (heavy arrows). Bar = 1.0 μm . 10. Late sporoblast with well developed exospore (EX), electron lucent nucleus (N), developing polar filament (PF) and an anchoring disc (AD) within a developing polar sac. Bar = 0.5 μm .



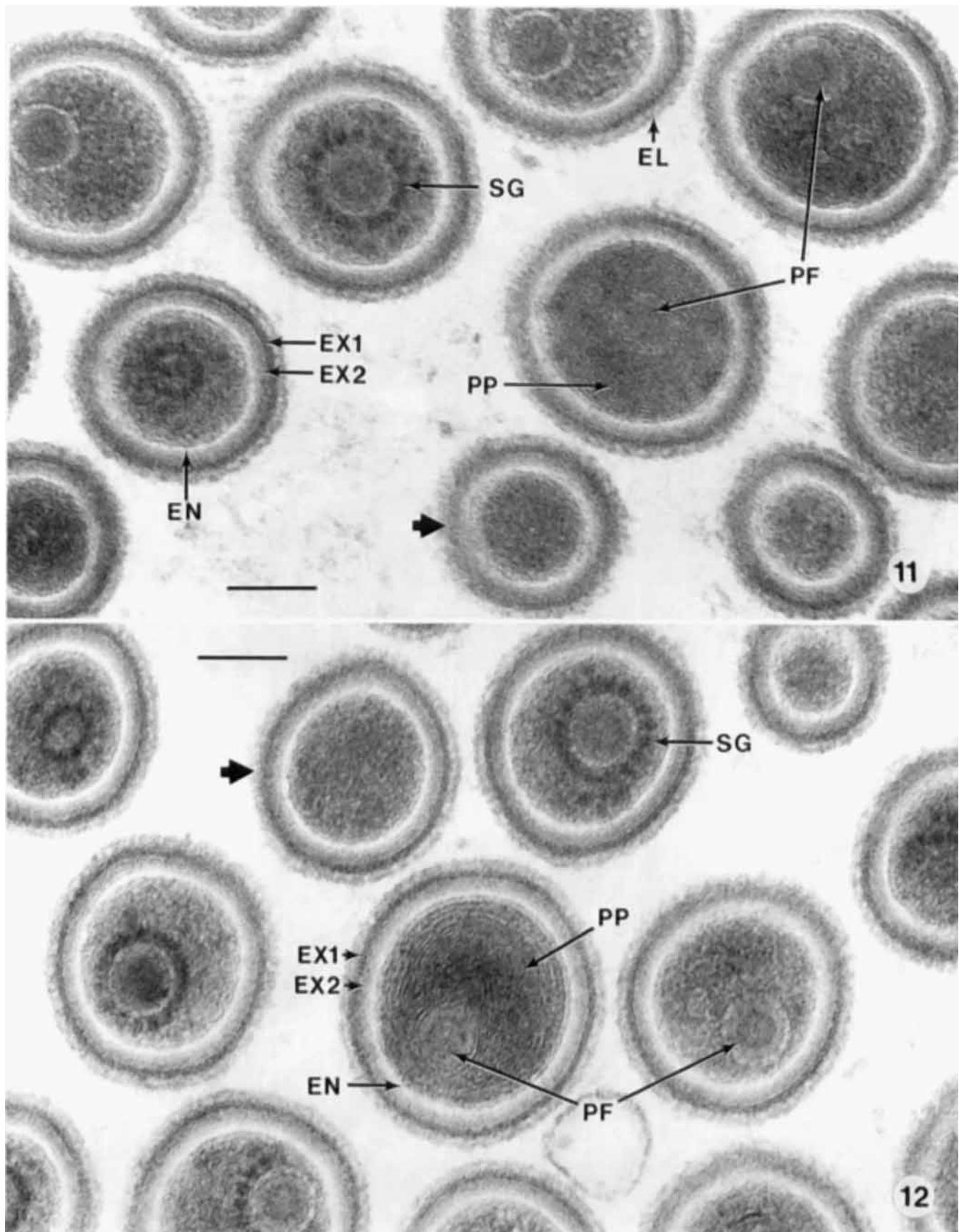
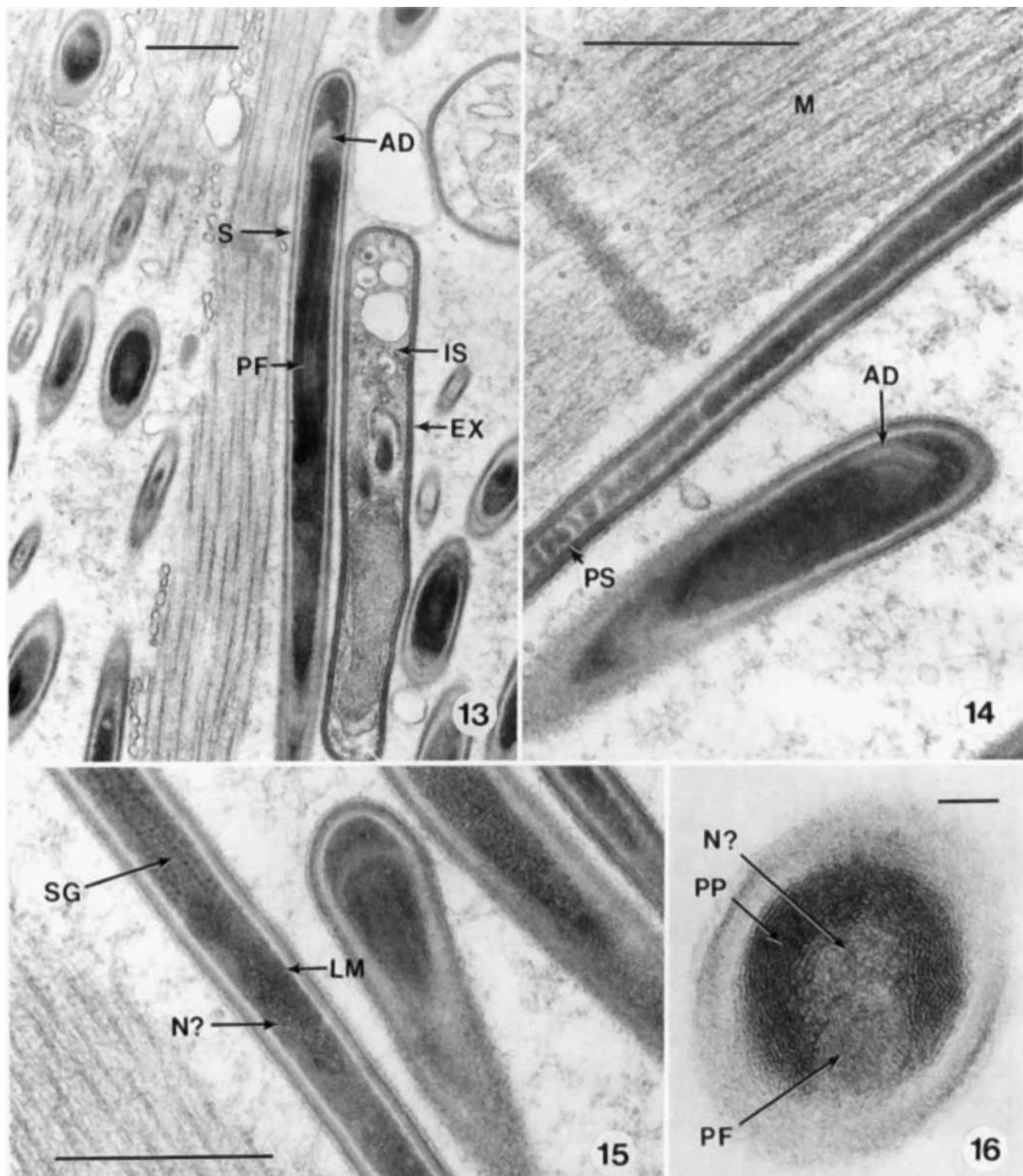


Fig. 11, 12. Mature spore structure. Cross sections of mature spores at several levels from anterior to posterior. Spores with an amorphous external layer (EL) covering a layered exospore with unit membrane (EX1) and internal granular layer (EX2) and a narrow endospore (EN). The lamellar polaroplast (PP) nearly surrounds the layered polar filament (PF). Posterior to the polaroplast level, spherical granules (SG) surround the polar filament. The polar filament is absent from posterior portions of the spore (heavy arrows). Bars = 0.01 μm .



13–16. Mature spore structure. 13. Mature spores (S) showing subterminal, laterally directed polar filament (PF) anchoring disc (AD). Immature spores (IS) with spore wall of exospore (EX) only. Bar = 1.0 μm . 14. Mature spores showing anchoring disc (AD) and bead-like structures in posterior spore tip (PS). Crab muscle (M). Bar = 0.5 μm . 15. Mature spore showing spherical granules (SG) surrounding polar filament. lamellar structure (LM) (posterior polaroplast?) and possible nuclear material (N?). Bar = 0.5 μm . 16. Cross section of mature spore with polaroplast (PP), polar filament (PF) and possible nuclear material (N?). Bar = 0.05 μm .

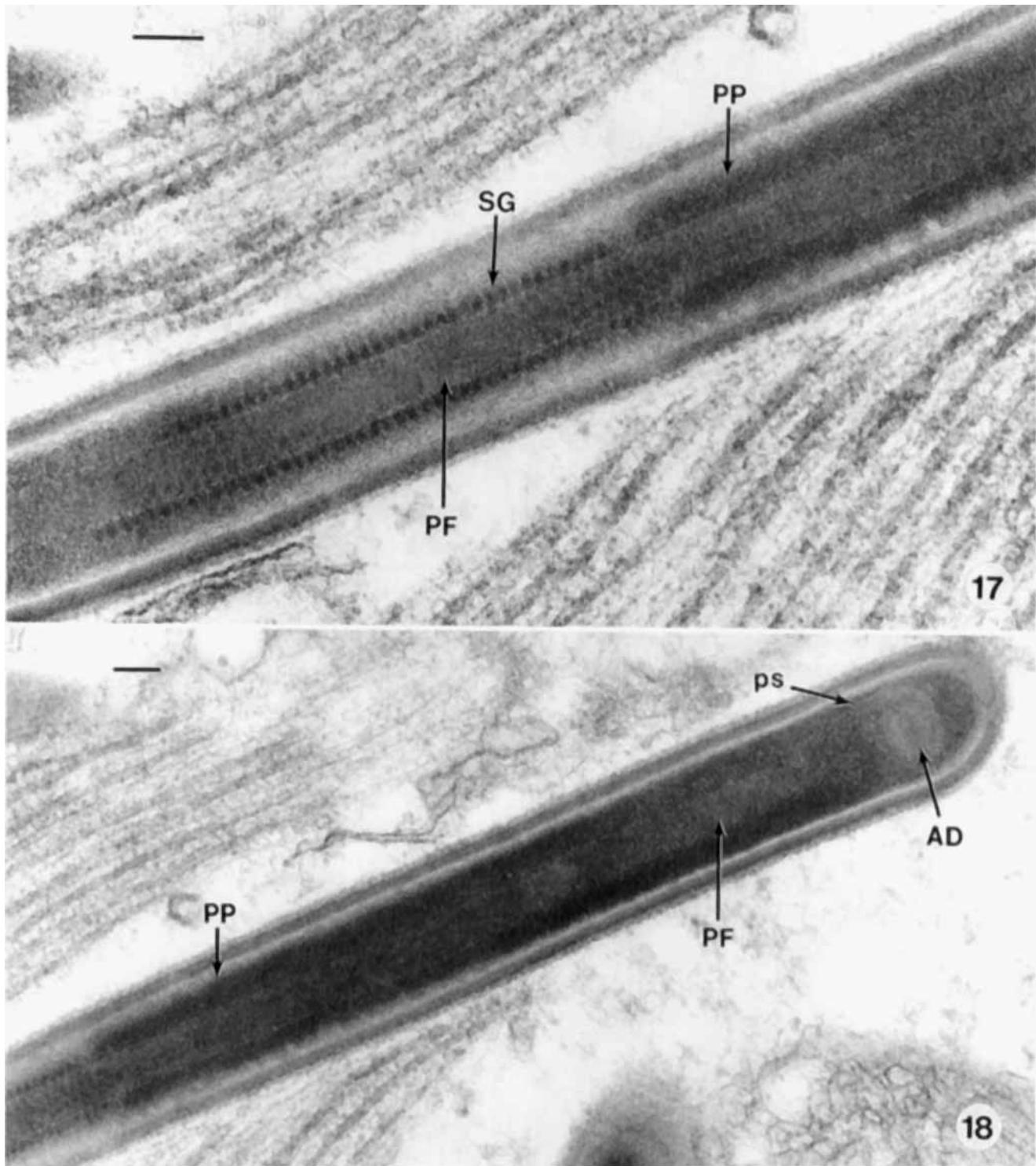


Fig. 17, 18. Mature spore structure. 17. Spore showing polaroplast (PP) and spherical granules (SG) in spiral arrangement around polar filament (PF). 18. Spore showing anchoring disc (AD), polar sac (ps), polar filament (PF) and posterior extent of polaroplast (PP). Bars = 0.1 μm .

suggest probable taxonomic relationships and since no family of Microspora exists that will accommodate either of them, a new family, Nadelsporidae, is proposed.

Nadelsporidae n. fam.

Diagnosis. Microspora, monokaryotic throughout all stages of the life cycle and lacking sporophorous vesicle of parasite origin. Spores elongate, either needle-shaped and tapering to a narrow tip or rod-shaped with blunt ends. Polar filament isofilar, ending abruptly without coils before reaching posterior end of spore. Polaroplast lamellar. Genera *Nadelspora* (this report) and *Baculea* Loubès and Akbarieh, 1978.

Nadelspora n. g.

Diagnosis. Merogony not observed. Merozoites and sporonts with sparse ribosomes and endoplasmic reticulum and monokaryotic. Sporonts diplosporoblastic, sporoblasts elongate and somewhat sausage-shaped, becoming further elongated and electron dense as immature spores develop. Immature spores contain a developing polar filament, a laterally directed anchoring disc, complete exospore, but no endospore layer and no polaroplast. Spores elongate, cylindrical, extremely narrow and needle-shaped tapering to a posterior tip. Polar filament isofilar, uncoiled and not reaching posterior portion of spore. Spherical granules not bound by a membrane and 20 nm in diameter occur in distinctive spiral arrangement around the polar filament from the posterior border of the polaroplast to the posterior end of the filament. Polaroplast lamellar and not divided into anterior and posterior portions.

Etymology. From the German *Nadel* or needle referring to the spore shaped like a small needle or *minuten Nadeln*.

N. canceri n. sp.

Merogony. Merozoites usually in rows within host muscle cells, not within sporophorous or parasitophorous vesicles. Bouts of merogony unknown.

Sporogony. As for the genus. Nucleus of sporoblasts bounded by undulating nuclear membrane. Sporoblasts often apparently distended to give a ballooning appearance.

Spores. Immature and mature spores as for the genus. Mature spore dimensions: 7.1–11.8 (fixed and stained) × 0.20–0.30 µm (from electron photomicrographs). Spore wall 44 nm with 38 nm thick exospore. Polar filament ending about three-fourths of the distance from the anterior end of the spore. Average diameter 73 nm although narrowing to 37 nm as the spore narrows posteriorly. Polaroplast surrounding, but not completely encircling polar filament, ending about 1.7 µm from anterior end of spore. Nucleus not clearly observed, but probably encircled by polaroplast in anterior portion of spore.

Host tissue infected. All striated muscle.

Type host. *Cancer magister* Dana (Crustacea, Decapoda).

Type locality. Alsea Bay, Lincoln County, Oregon, USA.

Types. Syntypes on slides No. 5440 and 102344.

Deposition of types. In the International Protozoan Type Slide Collection at Smithsonian Institution, Washington, DC (USNM #43203 and 43204) and in the collection of the authors.

Etymology. Alluding to the genus of the host crab.

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