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A New Microsporidium, *Nosema cristatellae* n. sp. in the Bryozoan *Cristatella mucedo* (Bryozoa, Phylactolaemata)

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A microsporidian infecting cells of the body wall of the phylactolaemate bryozoan *Cristatella mucedo* is described. All stages of the parasite are diplokaryotic and lie in direct contact with the host cell cytoplasm. Sporogony is probably disporoblastic. Spores measure 7.5 × 5.1 μm and have 22–32 coils of the polar tube arranged in several rows and a bell-like polaroplast of compact membranes. The parasite is assigned to the genus *Nosema* as a new species, *Nosema cristatellae*. It is differentiated from the previously described parasites of *Alcyonella* (=*Plumatella*) *fungosa* (Bryozoa), named *Myxosporidium bryozoides* and *Nosema bryozoides*, by spore characters and tissue specificity. Although it was found in a different species of bryozoan, it is not known whether *N. cristatellae* is infective to *P. fungosa*.

Key Words: *Nosema cristatellae;* microsporidia; *Cristatella mucedo;* Bryozoa; Phylactolaemata; parasitic; body wall; ultrastructure.

INTRODUCTION

Korotneff (1892) described a parasite which was first seen in association with the testicular funiculus and then became free in the body cavity of the bryozoan *Alcyonella* (=*Plumatella*) *fungosa*. The parasite was identified as belonging to the "Myxosporidien" and named *Myxosporidium bryozoides*. Thélohan (1895) assumed that the parasite belonged to the microsporidia and transferred it to the microsporidian genus *Glugea* as *Glugea bryozoides*. The microsporidian nature was accepted by Labbé (1899) but he transferred it to the genus *Nosema*. We have confirmed that both myxozoan and microsporidian species occur in Bryozoa by finding both in specimens of *Cristatella mucedo* (Bryozoa, Phylactolaemata), in some cases as a double infection (Fig. 1). The myxozoan parasite of *C. mucedo*, which has four polar capsules, has been described and named *Tetracapsula bryozoides* by Canning *et al.* (1996).

From Korotneff's illustrations and description it is unclear whether or not his assignment of the parasite to the group now known as the Myxozoa was correct. It is possible that the large multinucleate plasmodia, which floated free in the body cavity of *P. fungosa* correspond to the "sacs" full of proliferating cells and sporogonic stages of *T. bryozoides*. If so, the structures interpreted as nuclei by Korotneff could correspond to the small proliferative cells in the sacs and the "cytoplasm" would be the matrix of the sacs, in which the presporogonic and sporogonic stages are free floating. Korotneff did not give any measurements for the spores but a rough estimate of 8.0–9.0 μm can be obtained from his illustration (Fig. 11 in Korotneff, 1892). His description of the spores like melon seeds (Melonensamen) with two vacuoles matches the spores of the microsporidian species that we have found in *C. mucedo* rather than those of the myxozoan *T. bryozoides* which are spherical (Canning *et al.*, 1996). Unfortunately the nature of the parasites seen by Korotneff remains unresolved.

Braem (1911) and Schröder (1914) also found parasites in *P. fungosa*, infecting the testicular cells. The infected cells broke away from the testicular funiculus and became free floating in the coelomic cavity. Their descriptions, particularly that of Schröder, suggest that these parasites were indeed microsporidia. Braem (1911) recorded that the spores and schizonts had two semicircular nuclei, an observation that could be interpreted as two nuclei in diplokaryotic arrangement. Schröder (1914) described a single polar filament extruded from some spores and illustrated typical diplokaryotic microsporidian spores. These observations are in accord with the genus *Nosema*. Unfortunately both Braem and Schröder used the name *Nosema bryozoides* for their microsporidia, a name that Labbé had used for Korotneff's parasite. If Korotneff's parasite was a species of Myxozoa, then *N. bryozoides* becomes a synonym of *M. bryozoides* and cannot be used for a microsporidium.

The microsporidium from *C. mucedo* was studied by

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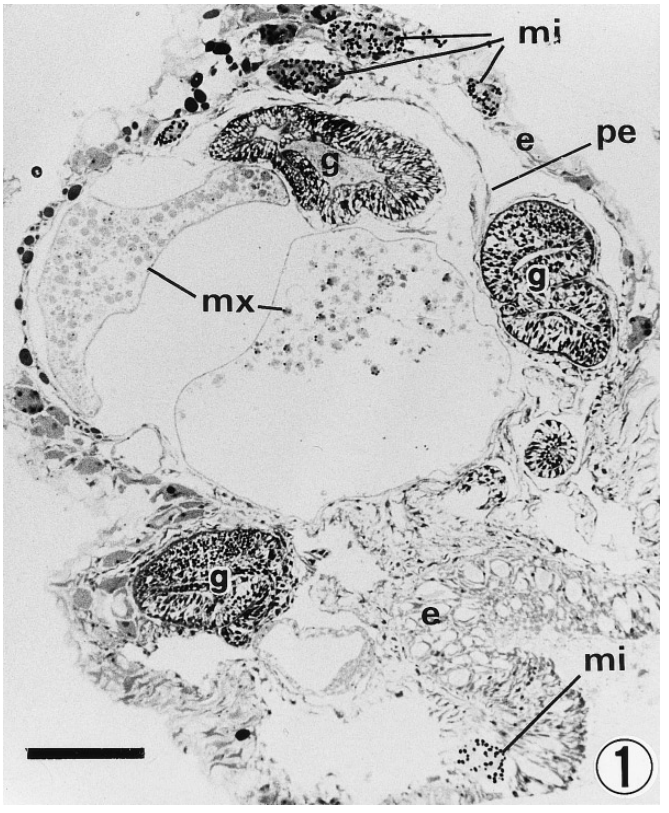
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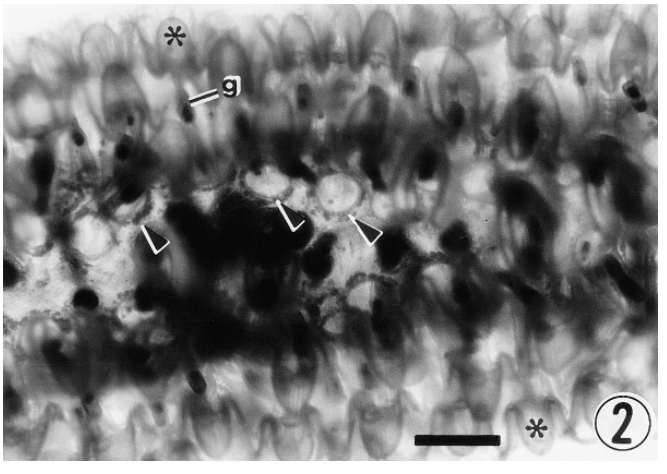
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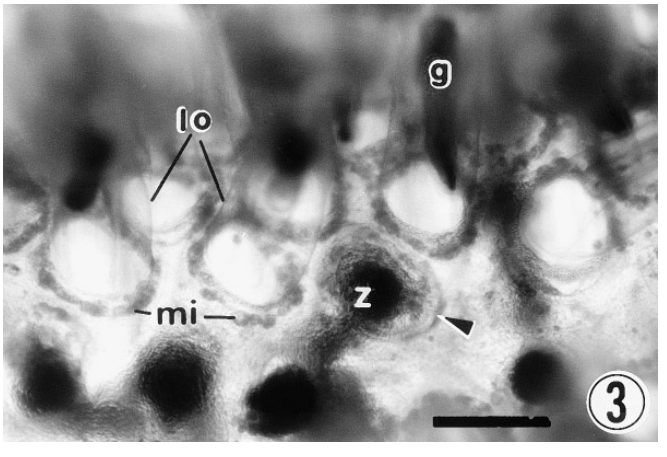
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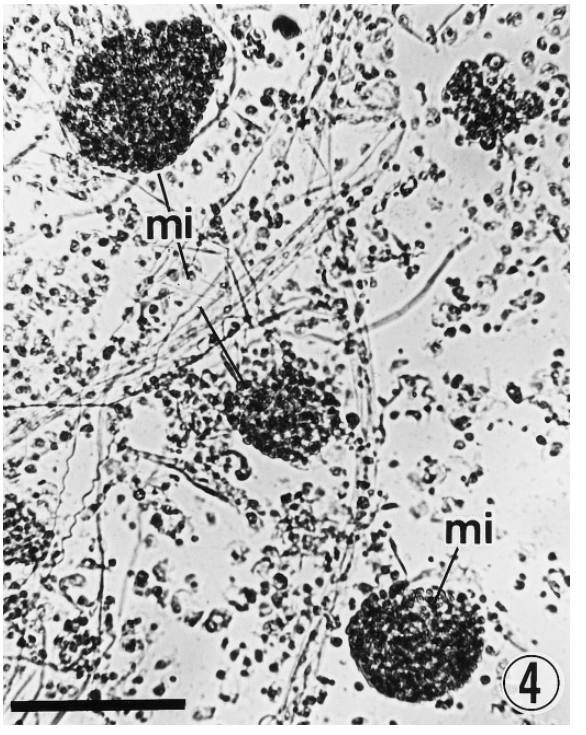
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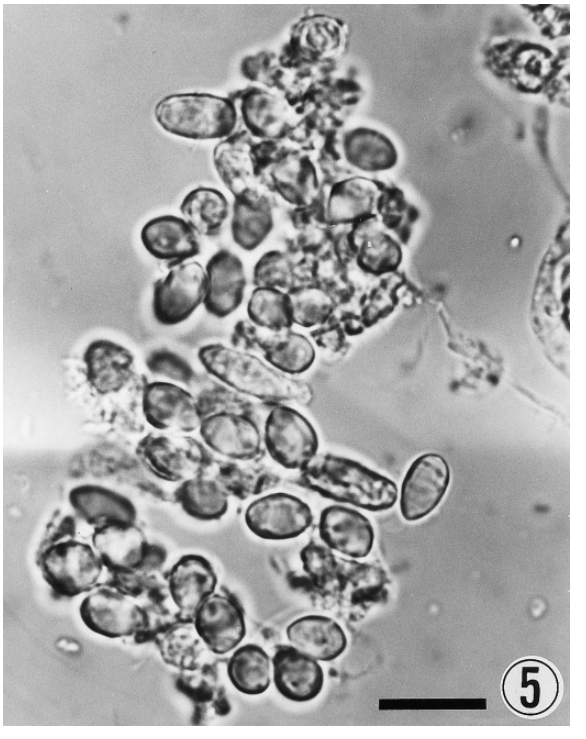
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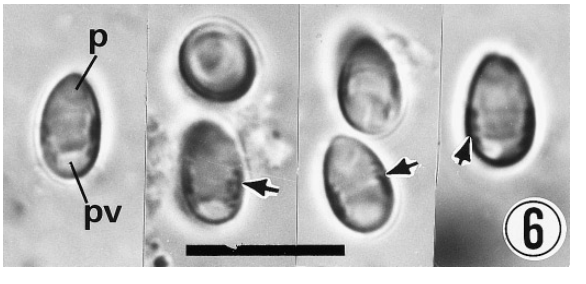
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electron microscopy and spore measurements were compared with those given by Braem (1911) and Schröder (1914) for the parasites in *P. fungosa*. We concluded that the species in *C. mucedo* is new and propose the name *Nosema cristatellae* n.sp. for it.

MATERIALS AND METHODS

Colonies of *C. mucedo* were collected from several small lakes in the county of Berkshire, England. Beale Bird Park Lake is designated as the type locality for *N. cristatellae*, the national grid reference being SU619781. *C. mucedo* colonies were photographed fresh under a dissecting microscope. Pieces of degenerating colonies were smeared onto glass slides and were examined without fixation under a Leitz Dialux microscope. Spores were examined fresh and their measurements calculated as means and standard errors. For electron microscopy pieces of infected colonies were fixed in Karnovsky's fixative in 0.1 M cacodylate buffer, pH 6.5, postfixed in 1% (w/v) OsO_4 in cacodylate buffer, washed in buffer, dehydrated in a graded series of ethanol, and embedded in Agar 100 resin (Agar Scientific). Semithin sections (1.0 µm) were mounted on glass slides and stained with toluidene blue for orientation, identification, and photography of infected tissues. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with an AEI EM 801 electron microscope.

RESULTS

Light Microscopy

The infected cells were identified as epithelial cells of the body wall (Fig. 1), particularly those at the base of individual lophophores but also at the growing edge of the colony. Heavily infected colonies were recognized at low magnification by their granular or "blotchy" appearance and partial or complete degeneration of some zooids. During normal degeneration of zooids, retracted lophophores are resorbed smoothly below the colony surface. In infected colonies, degeneration results in distinct localized swelling at the base of the lophophore stalk, the swelling being due to hypertrophy of infected cells. This swelling appeared as a ring or "torus" made up of a series of granules each representing an infected cell (Figs. 2 and 3). Other infected cells were scattered on the colony surface.

In disrupted preparations of the bryozoan, the spores remained as dense clusters (Fig. 4) held together by a considerable amount of cell debris (Fig. 5) and possibly by the persisting cell membrane. These clusters remained intact even after several months storage of dead bryozoan colonies at 4°C. The cell debris made it difficult to obtain pure spores in large numbers. The smallest spore-containing cells observed measured 16 × 12 µm. Among the largest were two which measured 216 × 74 and 132 × 116 µm and there was a complete range in between. Spores measured 7.32 ± 0.12 × 5.14 ± 0.07 ($n = 50$). They were pyriform with a rounded posterior end and pointed anterior end (Figs. 5 and 6). The polaroplast and posterior vacuoles were clearly visible, as circumscribed anterior and posterior pale areas and the coils of the polar tube were seen as a series of dense lateral spots and as faint lines crossing the spore in the posterior half.

Electron Microscopy

Infected cells were greatly hypertrophied according to the number and stage of the parasites they harbored (Figs. 7 and 8). The enlarged cells became detached from their adjacent cells, but remained partly connected to them by tenuous filopodial processes (Fig. 8). Some appeared to have been internalized from the body wall to occupy spaces close to the peritoneal cells lining the coelomic cavities (Fig. 1). Infected cells contained multiple nuclei also enlarged relative to those of uninfected cells each with a large dense nucleolus in nucleoplasm of moderate density (Fig. 8). Cells containing mainly meronts were electron dense with normal cytoplasm except that mitochondria were aligned to the contours of the parasites, so that meronts were almost encircled by mitochondria (Fig. 9). As the number of parasites increased the host cell cytoplasm assumed a

FIG. 1. Slightly oblique section through part of a colony of *Cristatella mucedo* showing epithelial cells infected with *N. cristatellae* (mi) and large sacs representing a myxozoan species *Tetracapsula bryozoides* (mx) in the coelomic cavity. e, epithelium; pe, peritoneum; g, gut. Bar, 100 µm.

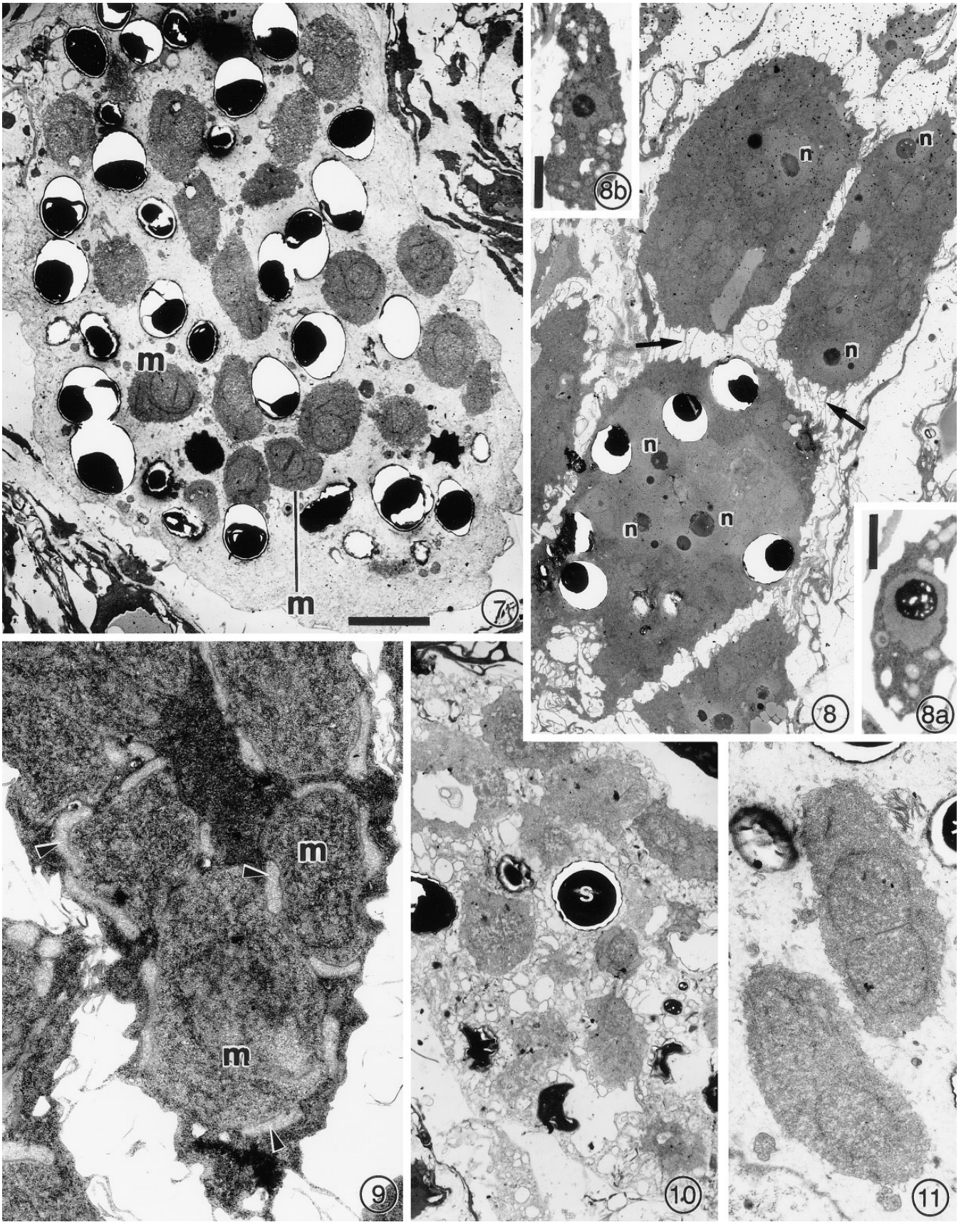
FIG. 2. Part of a living colony of *C. mucedo*. The lophophores (*) are at the end of stalks through which passes the gut (g). The circular bases of the lophophore stalks (arrowhead) are accentuated by the presence of infected cells which appear as dense granules. Bar, 800 µm.

FIG. 3. Enlargement of part of a colony as in Fig. 2, showing rings of infected cells (mi) at the bases of the extended lophophores. The gut (g) runs through the transparent lophophore stalks (lo). A degenerate zooid (z) is also encircled by an almost complete ring of infection (arrowhead). Bar, 600 µm.

FIG. 4. Fresh squash preparation of degenerating *C. mucedo*. The infected hypertrophied epithelial cells (mi) remain virtually intact. Bar, 130 µm.

FIG. 5. Fresh squash preparation after application of further pressure to the *C. mucedo* (as in Fig. 4). The spores of *N. cristatellae* are more dispersed but remain aggregated within residual epithelial cell debris. Bar, 10 µm.

FIG 6. Fresh spores of *N. cristatellae* showing polaroplast (p), posterior vacuole (pv), and polar tube coils (arrows). Bar, 10 µm.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highly vacuolated, foamy appearance, with loss of ribosomes and mitochondria (Fig. 10). Host cells containing many spores were so degenerate that it is unlikely that they could support growth and completion of sporulation by the remaining sporonts.

All stages of the microsporidium had diplokaryotic nuclei and lay in direct contact with the host cell cytoplasm. Meronts, bounded by a simple plasma membrane, were rounded (Figs. 7, 9, and 10) or elongate (Fig. 11), with irregular margins. The diplokaryon occupied about two-thirds of the width of the organisms. Stages with more than one diplokaryon were not observed, suggesting that division was rapid and occurred by binary fission. Sporonts were not unequivocally recognized but one stage in a degenerating host cell appeared to have a thickened membrane and to divide by binary fission (Fig. 12). The absence of multinucleate stages and the presence of pairs of crenated sporoblasts (Fig. 13) also suggested that the species is disporoblastic. Sporoblasts showed early development of the endospore, a single diplokaryon and abundant ribosomes, encircling the nuclei (Fig. 14).

The spore wall was composed of a 40-nm-thick exospore and endospore of thickness varying between 30 nm over the anchoring disk and up to 225 nm elsewhere (Figs. 15–17). The varying thickness of the endospore caused the wall to have a wavy outline giving some spores a rugose outline (Fig. 15). This may have been an artifact as some spores had an endospore of more or less uniform thickness except at the anterior tip (Fig. 16). The diplokaryotic nuclei occupied the center of the spore and around these the polar tube was coiled 22–32 times in several rows (Figs. 15 and 17). The anchoring disk of the polar tube was embedded in a polar sac which had unusually short lateral extensions and the polaroplast was composed of very compact membranes in bell-like formation, running from the polar sac to the region where the polar tube first began to coil (Fig. 16). More widely spaced, less distinct polaroplast membranes occupied the region around the straight part of the polar tube. Ribosomes in parallel arrays were prominent around the nuclei. In section, the polar tube showed seven concentric dark and light bands around a central lucent core (Fig. 18).

DISCUSSION

Although the description of *N. cristatellae* is incomplete in some details, especially of sporogony, this study and that of Canning *et al.* (1996) confirm that Bryozoa act as hosts to both microsporidia (Phylum Microspora) and myxozoans (Phylum Myxozoa).

Development of *N. cristatellae* in *C. mucedo* is in direct contact with host cell cytoplasm, the nuclei are in diplokaryotic arrangement, and sporogony is probably disporoblastic. These characters are in accord with those of the genus *Nosema* as currently defined. Spores of *N. cristatellae* show an unusually large range in the numbers of coils of the polar tube, this being unrelated to spore maturity. The spores of *N. cristatellae*, measuring 7.5 × 5.1 µm, are of similar size to those of *N. bryozoides*, the microsporidium described from *P. fungosa*, i.e., 7.8 × 5–6 µm (Braem, 1911) and 7 × 4 µm, rarely 10 × 5 µm (Schröder, 1914). *P. fungosa* occupies the same habitats as *C. mucedo* but experimental transmissions are not possible because the bryozoa do not survive long when removed from their habitats. However, *N. cristatellae* infects epithelial cells of the body wall of *C. mucedo*. Also, although the infected cells may separate from adjacent cells, they remain external to the peritoneal lining of the coelomic cavity. *N. bryozoides* infected the testicular cells of *P. fungosa*, which themselves became free floating within the coelomic cavity.

Infected cells of *C. mucedo* packed with spores of *N. cristatellae* remained intact even after several months storage at 4°C in totally degenerate bryozoan colonies, in which no other cells could be identified (Fig. 4). This implies that significant changes are induced in the host cell plasma membrane and/or cytoplasm to enable the cell to keep its integrity around bundles of spores. However, no special strengthening of the cell surface was evident, in contrast to the hypertrophic cells caused by *Glugea anomala*, where multiple layers of surface coat are secreted (Canning *et al.*, 1982).

Sprague (1978) proposed that the species currently included in the genus *Nosema* are a heterogeneous group, many of which will have to be transferred to other genera when satisfactory criteria are established for a new generic assignment. Unfortunately the few

FIG. 7. Hypertrophied epithelial cell of *C. mucedo* detached from normal epithelial cells and containing spores and diplokaryotic stages, probably meronts (m). Bar, 6.0 µm. Bar also relates to main picture of Figs. 8–11.

FIG. 8. Several hypertrophied infected epithelial cells with multiple nuclei (n) attached to each other by filose connections (arrows) Bar, 7.5 µm. Insets (a) and (b) are normal epithelial cells. Bar, 2.9 µm (a) and 1.8 µm (b).

FIG. 9. Meronts (m) surrounded by mitochondria (arrowheads) in host cells with dense cytoplasm. Note filose connections between host cells. Bar, 1.25 µm.

FIG. 10. Heavily infected cell containing meronts and spores (s). Note "foamy" vacuolated cytoplasm of degenerate host cell. Bar, 4.5 µm.

FIG. 11. Diplokaryotic meronts in degenerate host cell. Bar, 2.5 µm.

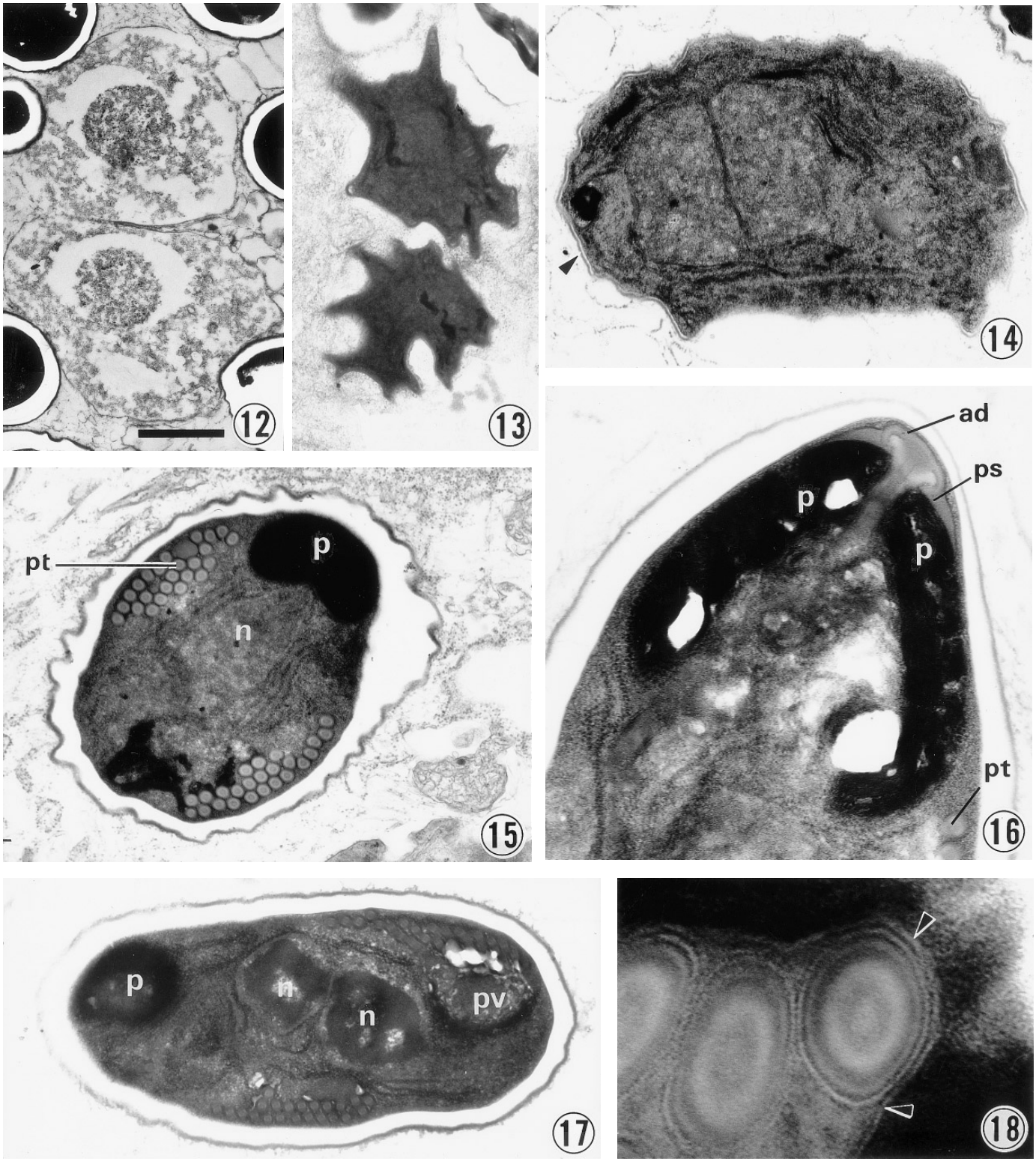

FIG. 12. Degenerate stage with patchy surface coat, possibly a dividing sporont. Only one nucleus visible in each half. Bar, 2.3 μm, relates to Figs. 12–18.

FIG. 13. Pair of crenated sporoblasts. Bar, 1.5 μm.

FIG. 14. Diplokaryotic sporoblast at an early stage in formation of the endospore (arrowhead). Bar, 0.65 μm.

FIG. 15. Mature spore showing dense polaroplast (p), one nucleus (n) of the diplokaryon surrounded by ribosomes, and 24 coils of the polar tube (pt) arranged in several ranks. Note varying thickness of endospore and rugose outline. Bar, 0.64 μm.

FIG. 16. Anterior end of spore. The base of the polar tube is inserted into the anchoring disk (ad) within a polar sac (ps) with short lateral extensions overlying the anterior margin of the densely packed membranes constituting the outer "bell" of the polaroplast (p). The spore has a smooth outline. pt, polar tube. Bar, 0.39 μm.

defining characters of the genus *Nosema* based on morphology, i.e., development in direct contact with host cell cytoplasm (no sporophorous vesicles), diplokaryotic nuclei, and disporoblastic sporogony, allow inclusion in the genus of many species which are probably not related. Baker *et al.* (1994) compared a number of species attributed to the genera *Nosema* and *Vairimorpha* using sequence data for a 350-nucleotide-region of the large (23S) subunit rRNA gene. Although the species investigated were all from insects, it was clear that not all the species were closely related. The *Vairimorpha* species (from Lepidoptera) fell into two groups and the *Nosema* species fell into a lepidopteran group, including the type species *N. bombycis*, with the others being as unrelated to each other as each was to the lepidopteran group. Thus molecular data have revealed the potential for new taxa even among so-called *Nosema* species infecting insects and the potential must be greater for the species which infect other phyla. The current host range for *Nosema* spp. spans from various protists right through to the vertebrates, including man. Although it is important to understand the relationships of these species, there will be little point in establishing a multitude of new genera based on complete or partial gene sequences without "user-friendly" morphological criteria to back up the new taxa.

Taxonomic summary of Nosema cristatellae *n. sp.*

Type host. *Cristatella mucedo* Cuvier, 1798 (Bryozoa, Phylactolaemata).

Type locality. Beale Bird Park Lake in Berkshire, England: Ref. SU619781.

Site of infection. Epithelium, particularly at the base of lophophores, where hypertrophy of infected cells appears as a ring of granules.

Interface. All stages in host cell cytoplasm. Terminally infected cells are degenerate but the cell membrane persists around aggregates of spores.

Merogony and sporogony. Division apparently by binary fission in both phases. Meronts elongate or rounded, the diplokaryon occupying two-thirds of the width. Sporonts and early sporoblasts did not fix well, as all showed degenerate cytoplasm and nuclei. Late sporoblasts were crenated or irregular but preservation of cytology was good.

Spores. Diplokaryotic. Pyriform, $7.3 \pm 0.12 \times 5.1 \pm 0.07$ (fresh, mean $\pm$ SE, $n = 50$). Polar tube isofilar with 20–32 coils in several rows. Polaroplast with prominent compact membranes in bell-like formation outside an inner zone of inconspicuous membranes.

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FIG. 17. Spore showing the two nuclei (n) of the diplokaryon, traces of the polaroplast (p), and posterior vacuole (pv) and 22 coils of the polar tube. Bar, 0.71 μm.

FIG. 18. Cross-section of polar tube coils showing seven concentric layers around an electron lucent core. Several coils appear to be enveloped by another complex of dark–light–dark layers (arrowheads). Bar, 0.058 μm.