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Cell Structure of Shellfish Pathogens and Hyperparasites in the Genera *Minchinia, Urosporidium, Haplosporidium,* and *Marteilia*–Taxonomic Implications

FRANK O. PERKINS

Introduction

Light microscope studies of species in the genera *Minchinia* Labbé, 1896, *Haplosporidium* Caullery and Mesnil, 1899, and *Urosporidium* Caullery and Mesnil, 1905, showed that they are related and belong in the order Balanosporida (Caullery and Mesnil, 1899) Sprague, 1979, formerly termed the Haplosporida and herein referred to as the balanosporidans. The judgment was based primarily on spore structure (Caullery, 1953; Sprague, 1963) and has, since then, been confirmed by studies of fine structure (Ormières and de Puytorac, 1968; Ormières et al., 1973; Perkins, 1968, 1969, 1971, 1975a; Perkins et al., 1975, 1977; Rosenfield et al., 1969). Also related to the Balanosporida are the oyster pathogens, *Marteilia refringens* Grizel, Comps, Bonami, Cousserans, Duthoit, and Le Pennec, 1974, and *Marteilia sydneyi* Perkins and Wolf, 1976. The available structural information on species of the four genera is reviewed herein and arguments presented for considering them to be interrelated. *Marteilia* spp. have been placed in a separate order, Occlusosporida Perkins, 1975, but in the same class, Stellatosporea (Caullery, 1953) Sprague, 1979.

ABSTRACT–The ultrastructure of shellfish pathogens and hyperparasites in the genera Minchinia, Urosporidium, Haplosporidium, *and* Marteilia *is reviewed and new structural information provided. Emphasis is placed on the variations in size and structure of haplosporosomes, a unique organelle common to all species in the group. Arguments for allying* Marteilia *spp. with the other species are presented based on observations of haplosporosomes and internal cleavage during sporulation.*

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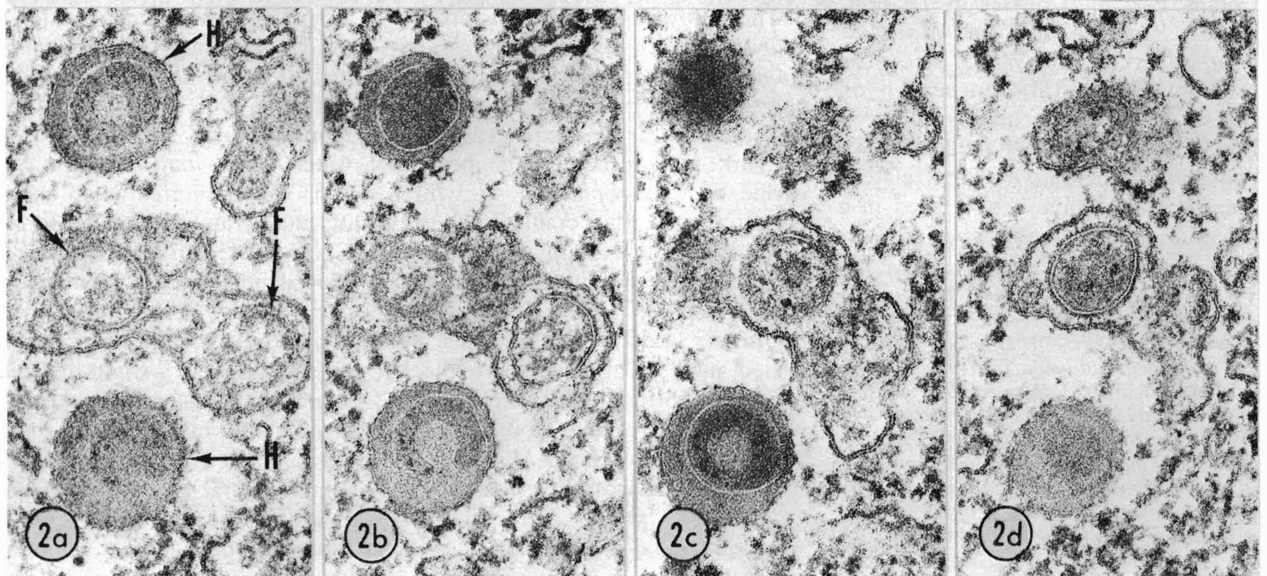
It is believed that considerations of the kind presented herein are of interest beyond phylogeny and taxonomy. If the various pathogens and parasites under discussion are established to be closely related, it should be recognized, because information gained from studies of the life history, ecology, physiology, disease control, etc. of one species may then be expected to yield insight into the biology of any of the other species.

The following comparisons of *Urosporidium, Minchinia, Haplosporidium,* and *Marteilia* species are made considering organelle systems, then sporulation. *Urosporidium crescens* De Turk, 1940 is found in the metacercariae of *Carneophallus* sp. which parasitizes the blue crab, *Callinectes sapidus,* of the eastern and southern U.S. estuaries causing the syndrome called "pepper crab disease" by workers in the seafood industry. The encysted metacercariae become black, thus resembling small peppercorns when the hyperparasite sporulates. This blackening also occurs in *Microphallus* sp. metacercariae from grass shrimp, *Palaemonetes pugio,* of the southern Atlantic Coast of the United States, infected with *Urosporidium* sp. Likewise in the surf clam, *Spisula solidissima,* from along the eastern continental shelf of the United States, a species of immature anisakid worm (possibly *Paranisakiopsis pectinis* Cobb, 1930; see Lichtenfels et al., 1977) becomes black when the hyperparasite, *Urosporidium spisuli* Perkins, Zwerner, and Dias, 1975, sporulates. This causes consternation in the seafood industry since the worms become highly visible against the light-colored clam tissues. The clam, *Abra ovata,* from the Rhone delta in France is parasitized by the trematode, *Gymnophallus nereicola,* which is hyperparasitized by *Urosporidium jiroveci* Ormières, Sprague, and Bartoli, 1973. The trematode also becomes black when spores of the balanosporidan are formed.

Minchinia nelsoni Haskin, Stauber, and Mackin, 1966 is a serious pathogen of oysters (*Crassostrea virginica*) along the eastern mid-Atlantic Coast of the United States and *Minchinia costalis* (Wood and Andrews, 1962) Sprague, 1963 causes severe localized mortalities of *C. virginica* in Virginia waters. *Minchinia louisiana* Sprague, 1963 causes mortalities in one species of mud crab, *Panopeus herbstii,* in Gulf of Mexico and Atlantic Ocean coastal populations. *Minchinia* sp. as described by Perkins (1975a) is now considered to be *M. louisiana* since the only difference, a slight differential in spore length, is not considered to be significant. Final proof that the species are identical will depend upon results of ultrastructural studies to be done on the Louisiana species. There is not enough information to judge whether *Minchinia* sp. as described by Rosenfield et al. (1969) is the same as *M. louisiana.*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Minchinia armoricana van Banning, 1977 parasitizes the European flat oyster, *Ostrea edulis*, from Dutch and French waters. *Haplosporidium ascidiarum* Doboscq and Harrant, 1923 has been found in three species of tunicates in European coastal waters (Ormières and de Puytorac, 1968).

Marteilia refringens, the lethal agent of Aber disease in European flat oysters, *O. edulis*, is well described in this symposium (Alderman, 1979; Balouet, 1979; Cahour, 1979; Grizel, 1979). Its closely related counterpart, *M. sydneyi* Perkins and Wolf, 1976, in Australian east coast waters causes severe mortalities of *Crassostrea commercialis*.

As opposed to *Urosporidium* spp., sporulation of the above-mentioned species of *Minchinia* and *Marteilia* does not result in blackening of the host tissues. Color change may or may not occur in the host tissues. If it does, the tissues become slightly yellow or green.

Figure 1.—Plasmodium of *Minchinia nelsoni* in oyster hepatopancreas. Nucleus (N); mitochondrion (M); multivesicular body (MV) where haplosporosomes are presumably formed; pseudopodium (?) (P); mature haplosporosome (H). Golgi apparatus (G). 15,000×.

Figures 2a-d.—Serial sections through mature (H) and forming (F) haplosporosomes in *Minchinia nelsoni* plasmodium. The forming organelles are components of a multivesicular body. Note cup-like configuration of internal membrane and spherical shape of lower, free haplosporosome 84,000×.

Materials and Methods

Techniques used in specimen preparation may be found in the relevant papers reviewed herein. Unpublished data on *Urosporidium* sp. in metacercariae of *Microphallus* sp. found in *P. pugio* were derived from specimens collected under Folly Bridge in the Charleston, S.C. area. They were fixed using the glutaraldehyde and osmium tetroxide techniques described in Perkins (1975a).

Results

Haplosporosomes

The most striking and most consistent similarity among the species examined is the presence of organelles found in the plasmodia (Fig. 1) which either disappear from the protoplast (*Minchinia* spp., *Urosporidium* spp.) or from that part of the protoplast which differentiates into spores (*Marteilia* spp.) during sporulation. They reappear in developing spores and become prominent in mature spores. The organelles, termed haplosporosomes, consist of spheroidal, vermiform, cuneiform, or club-shaped units in the fully differentiated state (Fig. 1-13), 29 to 249 nm in the shortest axis and up to 650 nm in the longest axis (Table 1). Spherical or spheroidal (Fig. 2a-d, 4), vermiform or club-shaped (Fig. 3, 6), and pyriform (Fig. 5) configurations are found in the plasmodia, whereas spheroidal (Fig. 11), pyriform (Fig. 10), vermiform or club, and truncated club or cuneiform (Fig. 12, 13) types are found in the spores. During differentiation the organelles may be highly polymorphoric. Despite their varied shapes and sizes, when mature their substructure is similar, consisting of a delimiting unit membrane and a continuous internal membrane which separates the organelle into a cortex and medulla both of high electron density (Fig. 2a-d, 3-6, 10-13). The interface membrane may assume a pyriform, cup, or spherical shape (Fig. 2a-d, 14) in spherical or spheroidal haplosporosomes or may simply follow the profile of the organelle equidistant from the delimiting organelle membrane (Fig. 3, 6, 12).

Plasmodial haplosporosomes appear to be formed from multivesicular bodies (MVB) (Fig. 1, 7, 8, 14). I have now seen such formative regions in plasmodia of *M. refringens, M. sydneyi, Minchinia nelsoni,* and *U. crescens,* but not *M. costalis, M. louisiana, U. spisuli,* and *Urosporidium* sp. They were also not reported from *U. jiroveci*

Figures 3-6.—Plasmodial haplosporosomes of *Minchinia louisiana* (Fig. 3) *M. nelsoni* (Fig. 4), *M. costalis* (Fig. 5), and *Marteilia refringens* (Fig. 6) Note internal membrane between cortex and medulla and variations in shape: Club-like (Fig. 3), spherical (Fig. 4), pyriform (Fig. 5), and vermiform (Fig 6) 120,000×, 116,000×, 77,000×, and 175,000×, respectively.

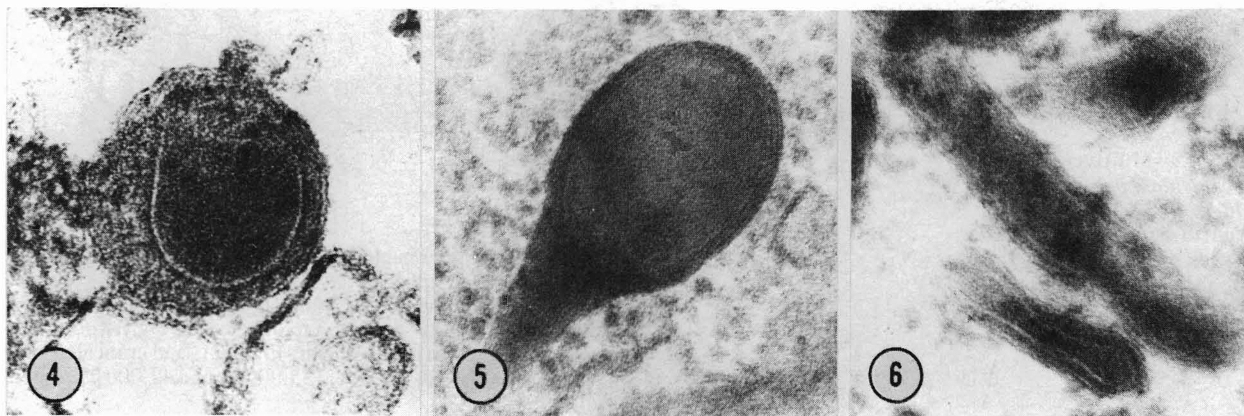
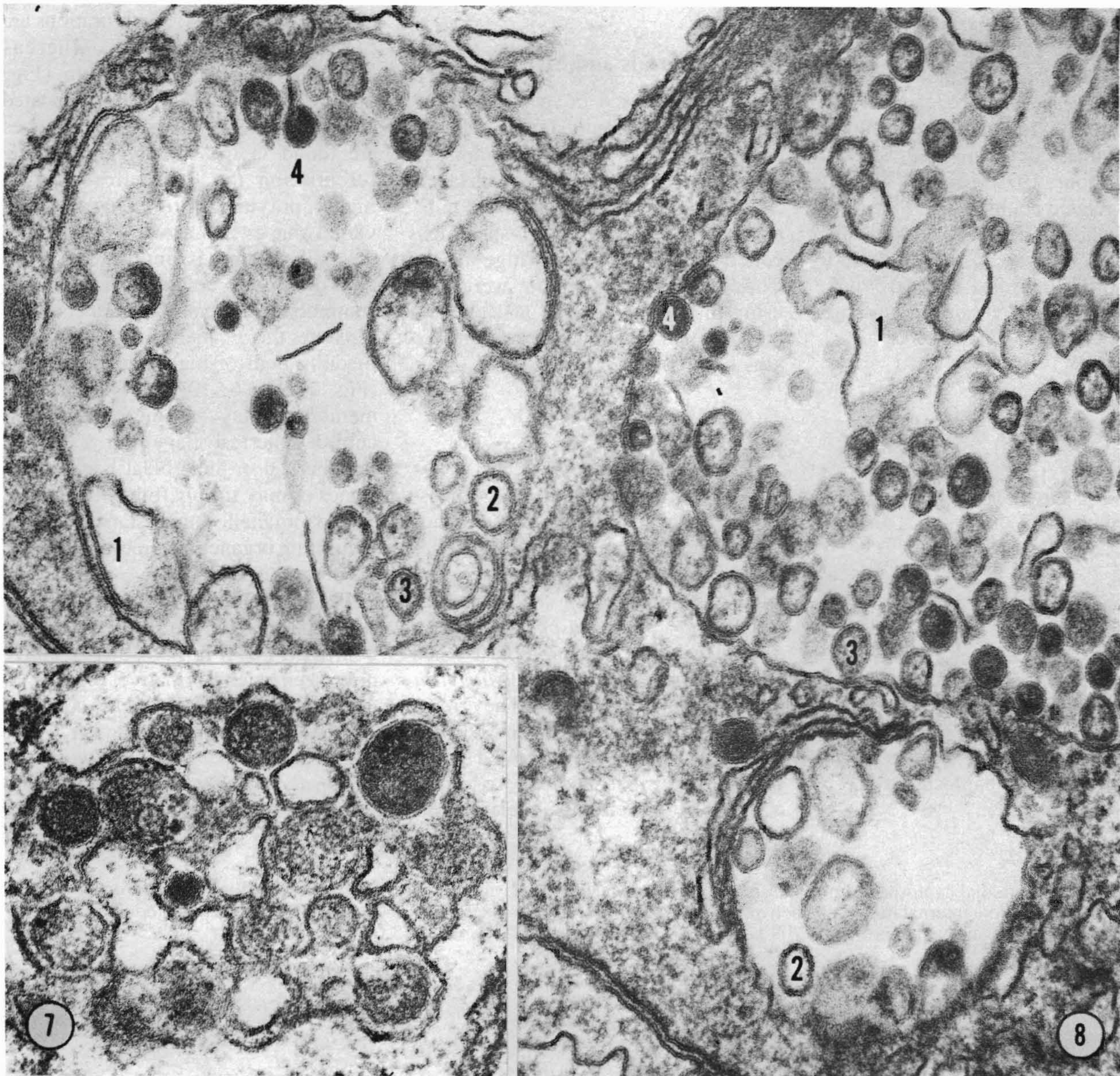
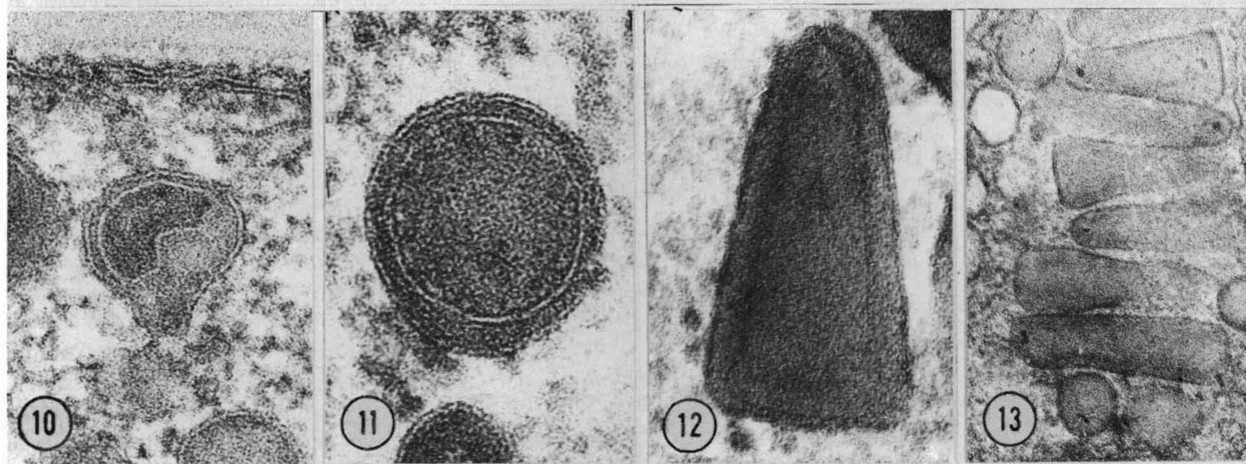


Figure 9.—Nearly mature spore of *Marteilia refringens*. Nucleus (N_2) of intermediate sporoplasm; spore wall (W); haplosporosome (H) in outermost sporoplasm; double membrane-limited vesicles (V). 47,000×.



Figures 7, 8.—Multivesicular bodies of *Minchinia nelsoni* (Fig. 7) and *Marteilia refringens* (Fig. 8) believed to be organelles for synthesis of haplosporosomes. The probable maturation sequence is indicated by 1→4. See Figure 14 also. Figure 7, 85,000×; Figure 8, 108,000×.

Figures 10-13.—Spore haplosporosomes of *Urosporidium spisuli* (Fig. 10), *Marteilia sydneyi* (Fig. 11), *Minchinia costalis* (Fig. 12, 13). Note delimiting membrane and membrane between cortex and medulla. Terminology used in text to denote shape: Pyriform (Fig. 10), spherical (Fig. 11), cuneiform (Fig. 12), and truncated club (Fig. 13). 123,000×, 215,000×, 135,000×, and 42,000×, respectively.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(Ormières et al., 1973), *M. armoricana* (van Banning, 1977, 1979), and the blue crab balanosporidan (Newman et al., 1976). However, I suspect that the formative regions will eventually be demonstrated in the other species, because in all studies, except those of Newman et al. (1976), sporulation was occurring in the specimens being observed. Possibly the plasmodia observed by the latter workers had ceased to synthesize haplosporosomes in preparation for spore formation.

Plasmodia of *Minchinia* spp. and *Urosporidium* spp., which are converting to sporonts, form a delimiting thin wall (ca. 20 nm thick in most species, but up to 131 nm in *U. crescens*) around the protoplast which persists through sporocyst maturation (Perkins, 1969, 1971, 1975a; van Banning, 1979). Thus, initiation of sporulation can be detected; however, loss of the haplosporosome formative areas (MVB's) may occur before then. In *Marteilia* spp., delimiting walls are only formed around those parts of the protoplast which form spores. Haplosporosomes and their formative regions (Fig. 8) are found only in the portion of the protoplast lying outside the walls and persist through sporulation. As with *Urosporidium* spp. and *Minchinia* spp., the cytoplasm within the wall does not acquire haplosporosomes until they appear in spores (Perkins, 1976; Perkins and Wolf, 1976).

Vesicles or haplosporosome primordia within the plasmodial MVB's appear to bud from the periphery of the MVB's thereby forming free units (Fig. 14). The delimiting membrane of haplosporosomes is thus derived from the delimiting membrane of the MVB, and the unit membrane which lies between the cortex and medulla is the membrane of the former vesicle. The inner membrane appears only as an electron light zone in glutaraldehyde fixed preparations but can be resolved in $KMnO_4$-fixed cells (figure 5 of Perkins, 1975a). Generally, the medulla acquires material of high electron density first during development followed by the cortex.

Individual vesicles within the MVB's vary greatly in size and shape (Fig. 14). Presumably, subdivisions and enlargements occur to yield units of a narrow size range prior to being incorporated into the haplosporosome which is budded from the MVB periphery. Fibrillar substructure can be seen in the medulla of immature haplosporosomes (see fig. 13 e, f—Perkins, 1968). Their identity is not known, but may be related to the fact that MVB's of *Minchinia nelsoni* plasmodia are Feulgen positive. Such staining characteristics have not been noted in other stellatosporeans, possibly because the organelle densities and mass have not been great enough to detect the stain.

Two basic mechanisms may be utilized for haplosporosome formation in spores, one represented by *Minchinia* spp. and *Urosporidium* spp. and the other by *Marteilia* spp. In *Minchinia louisiana* spores, haplosporosomes appear to arise from MVB's in much the same way as in *M. nelsoni* plasmodia. The MVB's are derived from a Golgi apparatus-like organelle ("spherule" of classical literature) at the anterior end of the spore (Perkins, 1975a). Haplosporosome origins in *M. nelsoni* and *M. costalis* spores are less well known, but appear to arise directly from the Golgi apparatus-like cisternae as evidenced by accumulation of electron dense material (Perkins, unpublished data). In *Urosporidium* sp. and *U. crescens* evidence for the "spherule" being a Golgi apparatus and the site of haplosporosome formation is strongest since haplosporosomes were found in the cisternae (Perkins, 1971; unpublished data). In *U. spisuli* a similar sequence was suggested, although cisternae were not organized into an anastomosing network like a

Table 1.—Sizes of mature haplosporosomes in different species of balanosporidans[1].

Species	Cell type	Shape	Diameter or Length (nm)	Width (nm)	Citation
Minchinia nelsoni	Plasmodium	Spherical	137-217 ($\bar{x}$=175) (N=20)		New data
		Oblate spheroid	151-288 ($\bar{x}$=201) (N=30)	130-249 ($\bar{x}$=168) (N=30)	New data
	Spore	Oblate spheroid	162-239 ($\bar{x}$=214) (N=25)	130-217 ($\bar{x}$=156) (N=25)	New data
		Vermiform or club	214-391 ($\bar{x}$=267) (N=25)	66-174 ($\bar{x}$=134) (N=25)	New data
M. costalis	Plasmodium	Oblate spheroid	214-272 ($\bar{x}$=235) (N=20)	162-235 ($\bar{x}$=201) (N=20)	Perkins, 1969 and new data
		Pyriform	218-336 ($\bar{x}$=289) (N=30)	154-215 ($\bar{x}$=181) (N=30)	"
	Spore	Truncated club or cuneiform	350-650 ($\bar{x}$=480) (N=20)	140-220 ($\bar{x}$=180) (N=20)	"
M. louisiana	Plasmodium	Club	300-586 ($\bar{x}$=456) (N=7)	129-186 ($\bar{x}$=155) (N=11)	Perkins, 1975a
	Spore	Pyriform	133-200 ($\bar{x}$=174) (N=15)	104-151 ($\bar{x}$=124) (N=20)	Perkins, 1975a and new data
M. sp.	Plasmodium	Spherical	150-200 ($\bar{x}$=175) (N=?)	— —	Newman et al., 1976
Urosporidium crescens	Plasmodium	Spherical	123-159 ($\bar{x}$=139) (N=10)	— —	Perkins, 1971
		Oblate spheroid	133-200 ($\bar{x}$=158) (N=10)	110-133 ($\bar{x}$=119) (N=10)	New data
	Spore	Pyriform	172-218 ($\bar{x}$=199) (N=15)	126-178 ($\bar{x}$=152) (N=15)	New data
U. spisuli	Spore	Oblate spheroid	97-164 ($\bar{x}$=121) (N=25)	83-149 ($\bar{x}$=101) (N=75)	Perkins et al., 1975 and new data
		Pyriform	114-190 ($\bar{x}$=153) (N=10)	86-139 ($\bar{x}$=117) (N=10)	New data
U. sp. (from *Microphallus* sp. in *Palaemonetes pugio*)	Spore	Spherical	69-115 ($\bar{x}$=88) (N=45)	— —	New data
Marteilia refringens	Plasmodium	Vermiform or club	130-490 ($\bar{x}$=240) (N=30)	43-130 ($\bar{x}$=60) (N=40)	Perkins, 1976 and new data
	Spore	Oblate spheroid	175-203 ($\bar{x}$=189) (N=16)	71-158 ($\bar{x}$=111) (N=34)	Perkins, 1976
		Spherical	98-196 ($\bar{x}$=113) (N=18)	— —	New data
M. sydneyi	Plasmodium	Vermiform or oblate spheroid	146-603 ($\bar{x}$=312) (N=28)	29-65 ($\bar{x}$=52) (N=28)	Perkins and Wolf, 1976 and new data
	Spore	Vermiform or oblate spheroid	148-288 ($\bar{x}$=187) (N=30)	44-163 ($\bar{x}$=96) (N=30)	Perkins and Wolf, 1976 and new data

[1]Widths of club-shaped and pyriform haplosporosomes were measured through the most enlarged portion of the organelle.

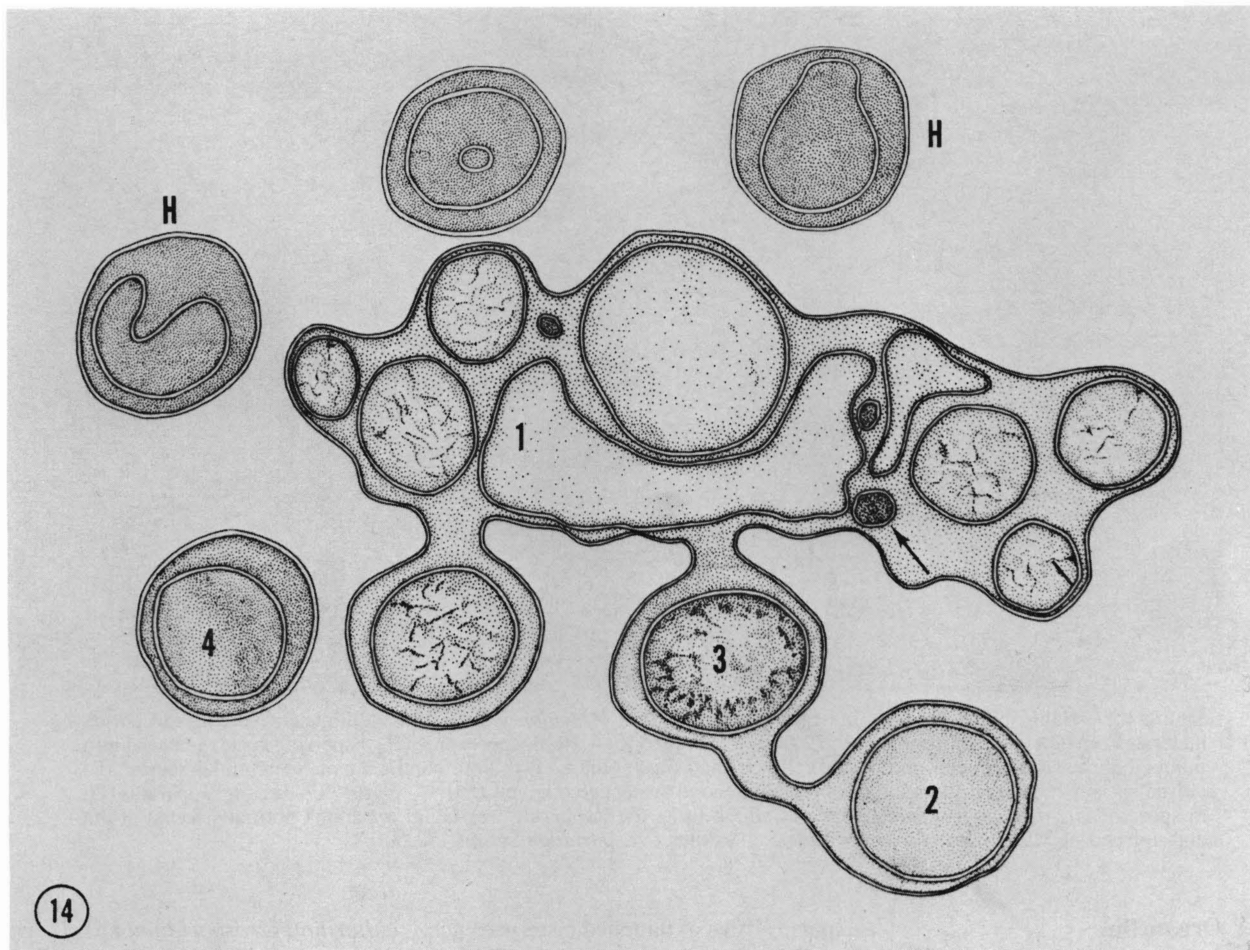

Figure 14.—Diagrammatic representation of haplosporosome (H) formation from multivesicular body (MVB) as seen in *Minchinia nelsoni* plasmodia. Within the MVB large polymorphic vesicles (1) pinch off spherical vesicles (2) both with low density contents. Fibrillar and granular material is added to the interior of the vesicles (3), they migrate to the MVB periphery, and pinch off the MVB periphery thereby acquiring an additional, delimiting membrane (4). Additional electron-dense material is subsequently added to the cortex. Small, dense bodies (arrow) in the MVB may enlarge to participate in haplosporosome formation.

Golgi apparatus (Perkins et al., 1975). Although it was not mentioned in Ormîères et al. (1973), *U. juroveci* may also form haplosporosomes in cisternae of the Golgi apparatus-like organelle as is suspected from examination of Figure 13 where at least one haplosporosome-like structure can be seen in a cisterna. *Minchinia armoricana* spores have a ''spherule'' and truncated, club-shaped haplosporosomes which resemble those of *M. costalis* (Perkins, 1969; van Banning, 1977); however, no evidence for formation of haplosporosomes in the cisternae of the European parasite were presented. *Haplosporidium ascidiarum* spores have a ''spherule,'' but no involvement in haplosporosome formation was mentioned (Ormîères and de Puytorac, 1968).

In *Marteilia refringens* and *M. sydneyi* spores there are no anastomosing cisternae resembling Golgi apparati nor are there MVB's which could give rise to haplosporosomes. They appear to arise individually in the outermost sporoplasm (Perkins and Wolf, 1976) and are never found in the middle or inner sporoplasms (Fig. 9).

Haplosporosomes are known to be liberated from plasmodia of *Minchinia nelsoni* and enter oyster cells intact (Fig. 15) or to be emptied into the space between the plasmodium and host cell (Fig. 16). In each case the cortex material appears to decrease first in electron density indicating loss of or chemical change in the cortical material. Haplosporosomes in which cortex and medulla had become less dense were not recognized. The organelles may also be deposited between the host cell and early sporont or plasmodium in a population of sporulating cells of *U. crescens* (Perkins, 1971). Since sporulation is associated with extensive host cell damage in most species it is suggested that haplosporosome release and dispersion may be related to host cell lysis.

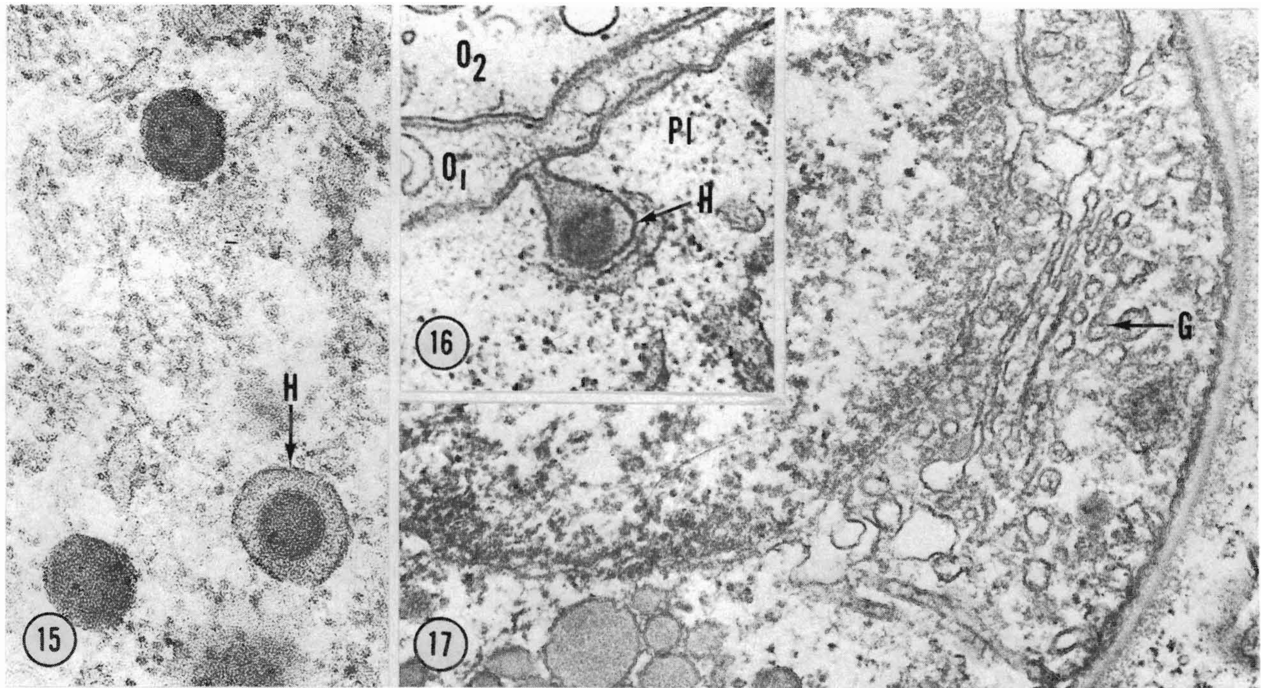

Figure 15.—Haplosporosomes (H) in hepatopancreas cell of *Minchinia nelsoni*-infected oyster. Note loss of cortex material from labeled haplosporosome. 72,000 ×. Figure 16.—Haplosporosome (H) dispersing cortex material into intercellular region between one oyster cell (O_1) and plasmodium (Pl). Note continuity of intercellular region and cul-de-sac in which haplosporosome is situated. Second oyster cell (O_2). 54,000 ×. Figure 17.—Golgi apparatus (G) in spore of *Urosporidium spisuli*, a species which lacks the "spherule" or Golgi apparatus normally found at the anterior end of *Minchinia*, *Haplosporidium*, and other *Urosporidium* spores. 58,000 ×.

Other Organelles

Mitochondria of the Stellatosporea are either tubulo-vesicular in substructure as in *Minchinia* spp. and *Urosporidium* spp. (Fig. 1) (Perkins 1969, 1975a) or are vesicular with shelf-like cristae as in *Marteilia* spp. (Perkins, 1976; Perkins and Wolf, 1976). Cristae were numerous and easily visualized in *Minchinia* spp., less so in *Urosporidium* spp., and difficult to find in *Marteilia* spp. A paucity of cristae is typical of many parasitic Protozoa (Tandler and Hoppel, 1972). In all cases mitochondria are easily located because the electron light areas of the vesicular mitochondria reveal the DNA nucleoid which distinguishes the organelle from cytoplasmic vesicles (Perkins, 1969, 1976; Perkins and Wolf, 1976).

Although questioned in previous papers (Perkins, 1968, 1975a), Golgi apparati are now known to be present in *Minchinia nelsoni, M. louisiana,* and *U. spisuli* plasmodia. They appear as sparse arrays of flattened cisternae each of which has an anastomosing substructure typical of Golgi apparati. Budding of vesicles from the nuclear envelope and fusion with the proximal face of the organelle are observed (Fig. 17, 18). On the distal face of *M. nelsoni* Golgi apparati, cisternae curl into nearly circular profiles (Fig. 19). On the inner face of the curve electron dense material is deposited. Whether these structures become spherical and then metamorphose into haplosporosomes has not been determined. If so, it is not known how they might interact with the multivesicular bodies suspected to be the haplosporosome formative regions (see previous "Haplosporosome" section). Golgi apparati of the other balanosporidan plasmodia have not been observed if they exist.

The "spherule" or mass of anastomosing cisternae appears in the anterior end of the sporoplasm of developing spores of *M. nelsoni, M. costalis, M. louisiana, M. armoricana, H. ascidiarium, Urosporidium* sp., *U. crescens,* and *U. jiroveci*. They appear to be Golgi apparati in that anastomosing cisternae comprise the substructure and haplosporosomes have been observed to be formed therein; however, the typical stacked layers of flattened vesicles are never visualized. It is interesting to note that *U. spisuli* spores lack a "spherule," but contain a typical Golgi apparatus (Fig. 17). Neither Golgi apparati nor "spherules" have been observed in *Marteilia* spp.

Only in *Minchinia nelsoni* plasmodia have nuclear structure and mitosis been observed in detail. Nuclei are typically found in pairs with a concavity in the surface of each nucleus where they face each other (figure 10 in Perkins, 1975b). There is a persistent mitotic apparatus, found during interphase and in mitotic nuclei, which consists of two spindle pole bodies free in the nucleoplasm and not attached to the nuclear envelope with a bundle of 33-53 microtubules between them (Perkins,

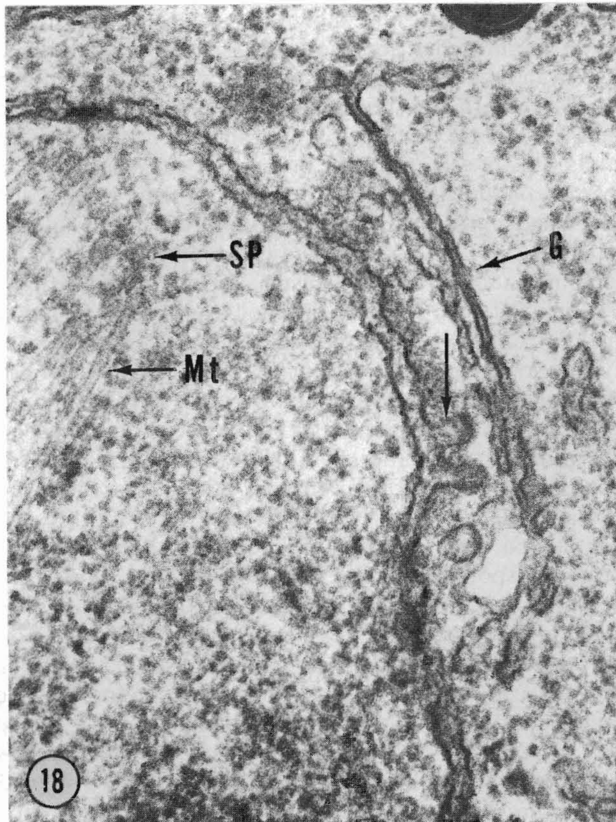

Figure 18.—Golgi apparatus (G) in *Minchinia nelsoni* plasmodium. Note budding (arrow) of nuclear envelope toward proximal face of apparatus and paucity of appratus cisternae. Spindle pole body (SP), mitotic apparatus microtubules (Mt). 65,000 ×.

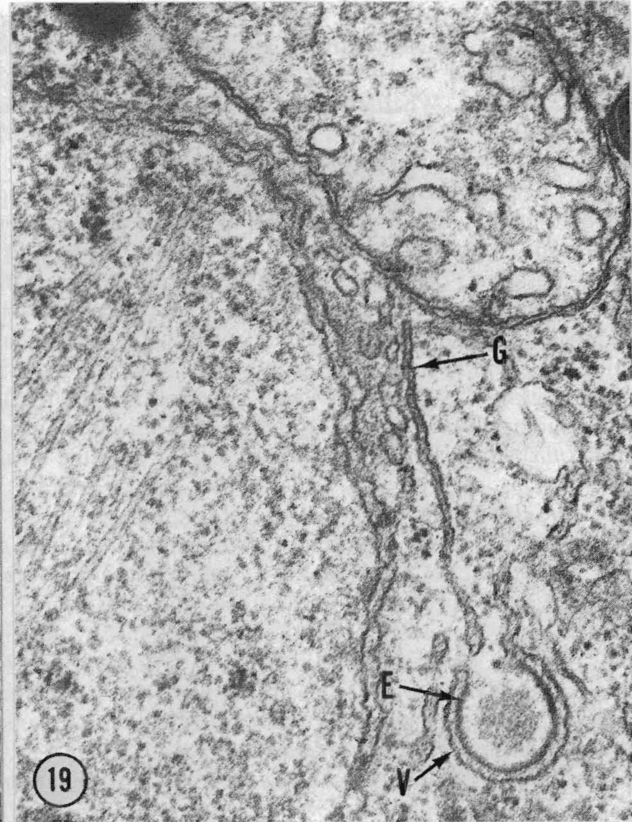

Figure 19.—Golgi apparatus (G) of *Minchinia nelsoni* plasmodium showing development of vesicle (V) from recurved apparatus cisterna. Electron-dense material (E) is added to inner face and ultimately fills the medulla region. Despite the resemblance to developing haplosporosomes, such structures are believed to be unrelated to haplosporosomes. 50,000 ×.

1975b). When mitosis occurs the nuclear envelope remains intact and all mitotic microtubules are contained within the envelope. The nuclear medial profile goes from circular at interphase to a spindle shape at metaphase then a dumbbell shape at telophase. The nucleolus remains peripherally located throughout and appears to pull apart during division. In *M. louisiana* and *M. costalis,* nuclear division occurs in the same manner, but whether the interphase nucleus retains the mitotic apparatus has not been determined. In *Marteilia refringens* and *M. sydneyi,* mitosis was not observed nor were centrioles or spindle pole bodies seen. Ginsburger-Vogel and Desportes (1979) have seen centrioles consisting of a singlet ring of microtubules in *Marteilia* sp. from amphipods; thus a reexamination of the oyster pathogens for centrioles is warranted.

Sporulation

Spore formation in *Minchinia* spp. and *Urosporidium* spp. appears to consist of enlargement of plasmodia, formation of a wall around the cells, increase in numbers of nuclei, then condensation of cytoplasm around each nucleus to yield uninucleate sporoblasts. However, nuclear fusion, followed by meiosis, may occur in the sequence as evidenced by studies of *M. louisiana* (Perkins, 1975a) where pairing of small (ca. 3.0 μm diameter) nuclei and large (>4 μm) nuclei were observed in sporonts. Sporoblast nuclei were about 3.8 μm diameter. Further evidence for meiosis lies in the observation of synaptonemal complex-like and polycomplex-like structures in sporont nuclei. Polycomplex-like structures have also been seen in immature spore nuclei of *Marteilia refringens*.

There are two proposals to explain spore differentiation from sporoblasts. From studies of *U. crescens,* Perkins (1971) suggested that invagination of the sporoblast periphery carved out the sporoplasm thus yielding the anucleate extraspore cytoplasm and the uninucleate sporoplasm. Ormières et al. (1973) suggested that in *U. jiroveci* a binucleate sporoblast formed the mature spore as a result of one half partially engulfing the other half, followed by degeneration of the nucleus of the

outermost protoplast. Separation of the innermost protoplast then occurred to form the sporoplasm, free within the extraspore cytoplasm. Whether both or one of the mechanisms occurs in *Minchinia* spp. and *Urosporidium* spp. remains to be determined.

After delimitation of the sporoplasm the spore wall is formed in the extraspore cytoplasm and consists of a cup with the anterior end occluded by a tongue of wall material, termed the lingua, in *Urosporidium* spp. (Perkins et al., 1977; Perkins, 1971; Ormières et al., 1973) and by a cap of wall material in *Minchinia* spp. (Perkins, 1968, 1969, 1975a).

The above-described sequence for sporoblast formation predominates; however, at least in *M. louisiana,* internal cleavage of sporoblasts occurs within the sporont protoplast without cytoplasmic condensation (Perkins, 1975a). The mechanism of sporoplasm delimination within the sporoblast was not determined; however, fully mature spores are known to be formed as a result of this type of sporoblast formation.

Internal cleavage also occurs in *Marteilia refringens, Marteilia* sp., and *M. sydneyi* during formation of sporangia and spores (Ginsburger-Vogel and Desportes, 1979; Perkins, 1976; Perkins and Wolf, 1976), but condensation of cytoplasm to form sporoblasts does not occur. The earliest cell type observed in newly infected hosts consists of a uni- or binucleate cell (Grizel et al., 1974). I observed no less than two nuclei per cell in *M. refringens* and *M. sydneyi.* Because the cells were without walls and had more than one nucleus, they were termed plasmodia. Whether they always consist of an uninucleate cell within an uninucleate cell (see figure 5 of Perkins and Wolf, 1976) from the earliest stage of infection or may consist of a binucleate cell is problematical. Cells which we interpreted (Perkins, 1976; Perkins and Wolf, 1976) to be simple binucleated ones could have been endogenously separated. Nevertheless, the term plasmodium has been used in protozoology for multinucleate cells with endogenous subdivisions (Poisson, 1953).

From the binucleate, endogenously cleaved stage, sporulation is initiated by enlargement of the cells and multiplication of the internal cells which then serve as sporangia. Thus the complex becomes a sporangiosorus (i.e., a cell containing several sporangia). Spores are formed in the sporangia and consist of three uninucleate sporoplasms, an intermediate one containing an inner sporoplasm, all of which are contained in an outer sporoplasm (Fig. 20). As they approach maturity, the spores are fully delimited by a thin wall which lacks any lingua or cap. Grizel et al. (1974) used the terms "primary cell" for sporangiosorus, "secondary cell" for sporangia, and "tertiary cell" for the spores. Internal delimitation of all nucleated units (sporangia, spores, sporoplasms) during sporulation is accomplished by vesicle fusion (Perkins, 1976; Perkins and Wolf, 1976). After spore maturation the protoplasm, not included within the spore wall, degenerates.

Wall ornamentation around spores of *Minchinia* spp. and *Urosporidium* spp. is formed in the extraspore cytoplasm which then disperses in the case of *Minchinia* spp. leaving the ornaments which are threads (Fig. 21) (Perkins, 1968, 1969, 1975a) or ribbons (Perkins, 1969). In *Urosporidium* spp., ribbons are formed in *U. crescens* (Perkins, 1971) and *U. jiroveci* (Ormières et al., 1973) and a labyrinthine complex in *U. spisuli* (Perkins et al., 1977) and *Urosporidium* sp. (Perkins, unpublished data). The extraspore cytoplasm probably disperses revealing the ornaments, but this has not yet been observed. With the possible exception of *U. crescens* and *U. jiroveci,* substructure of the ornaments appears to be species specific. *Marteilia* spp. form no ornaments around the spores. Only membrane whorls resulting from degeneration of extraspore cytoplasm in the sporangium are found wrapped around the wall.

Discussion

In attempting to establish the taxonomic affinities of *Marteilia* spp., I have suggested that they are related to the haplosporidans (Perkins, 1976), now known as the balanosporidans, and Sprague (1979) has erected the family Marteiliidae in the order Occlusosporida to accommodate them. It appears reasonable to ally *Marteilia* spp. with the balanosporidans, because haplosporosomes, with their unique substructure, are found in all species studied and not in other species of microorganisms. The organelles are found only in plasmodia and spores, not in the intermediate cell stages leading to spore formation. The suspected mode of haplosporosome formation from multivesicular bodies occurs in at least one indisputable balanosporidan, *Minchinia nelsoni,* as well as *Marteilia* spp. Internal cleavage during spore formation is found in at least one established balanosporidan, *Minchinia louisiana,* as well as *Marteilia* spp. One problem in accepting balanosporidan affinities for *Marteilia* spp. lies in the multicellular sporoplasm. Whether the extraspore cytoplasm has a nucleus during differentiation which is later lost as suggested by Ormières et al. (1973) remains to be proven. If so, those spores could also be called multicellular in origin (Sprague, 1979), particularly since the ornaments formed in the extraspore cytoplasm are an integral part of the spore.

Another problem lies in the general multicellularity of *Marteilia* spp. with cells engaged in sporulation (i.e., sporangia within a sporangiosorus and spores within sporangia). In balanosporidans there are only spores within a sporont, not an intermediate cell type. Whether one should consider such a difference of enough importance to warrant placement of *Marteilia* spp. in a class separate from the balanosporidans should await further ultrastructural studies of other species resembling the *Marteilia* spp. already studied.

The centrioles found in *Marteilia* sp. by Ginsburger-Vogel et al. (1976) and Ginsburger-Vogel and Desportes (1979) are of potential significance in efforts to determine the taxonomic affinities of *Marteilia* spp. since presence or absence of microtubular centrioles is considered by many workers as a marker of phylogenic significance (Pickett-Heaps, 1969; Fulton, 1971).

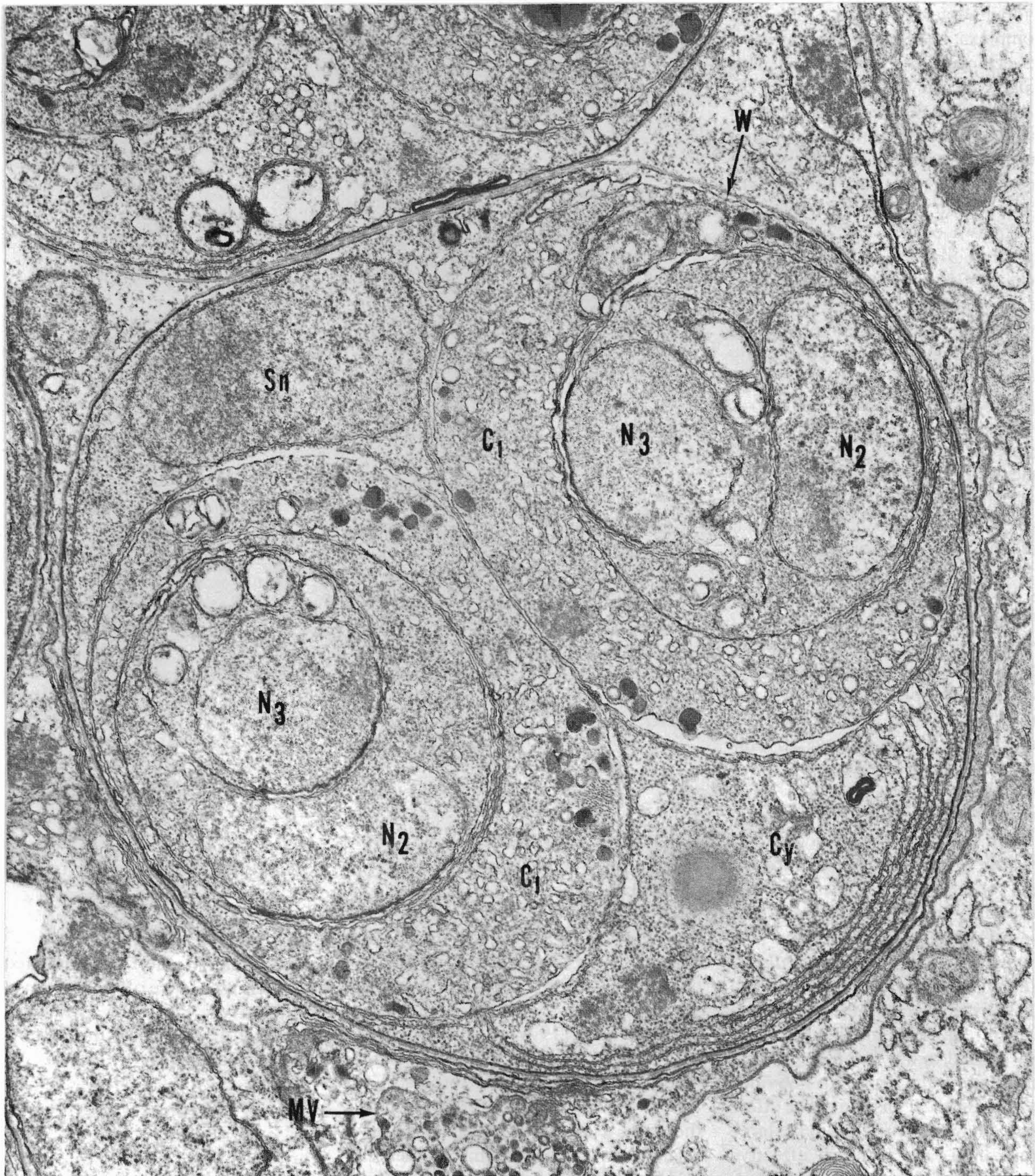

Figure 20.—Two developing *Minchinia refringens* spores in sporangium. Sporangial nucleus (Sn) and cytoplasm (Cy_2) which is not incorporated into spores; intermediate sporoplasm nuclei (N_2), inner sporoplasm nucleus (N_3), cytoplasm of outer sporoplasm (C_1), sporangial wall (W), multivesicular body (MV) of sporangiosorus (Cy_1) which is not incorporated into sporangia. 9,000×.

Coccidian Protozoa of the subphylum Apicomplexa have microtubular centrioles arranged in a singlet ring with ninefold symmetry (Dubremetz, 1973) as was found in *Marteilia* sp.

However, the Apicomplexa also include species which form spindle pole bodies (SPB's) (no microtubular substructure) as for example, *Plasmodium* spp. (Aikawa et al., 1972). Thus the existence of SPB's in balanosporidans (Perkins, 1975b) and microtubular centrioles in *Marteilia* sp. does not necessarily serve as evidence that the two are not closely related. It will be interesting to determine which organelle type is found in *M. refringens* and *M. sydneyi*.

Since numerous biochemicals are available today for control of protozoan diseases of humans and farm animals, considerations of ultrastructure and phylogenetic affinities have particular significance. For example, it is known that the antimalarial drug, pyrimethamine, has an inhibitory effect on nuclear division in *Plasmodium berghei nigeriensis* (Peters, 1974). Since the mitotic apparati of *Plasmodium* spp. and *Minchinia* spp. are similar, one might expect the drug to inhibit nuclear division in the oyster pathogens. This hypothesis needs to be tested for pyrimethamine as well as for other chemotherapeutic agents which inhibit mitosis in species of the Apicomplexa where both spindle pole bodies and centrioles consisting of singlet rings of microtubules are found.

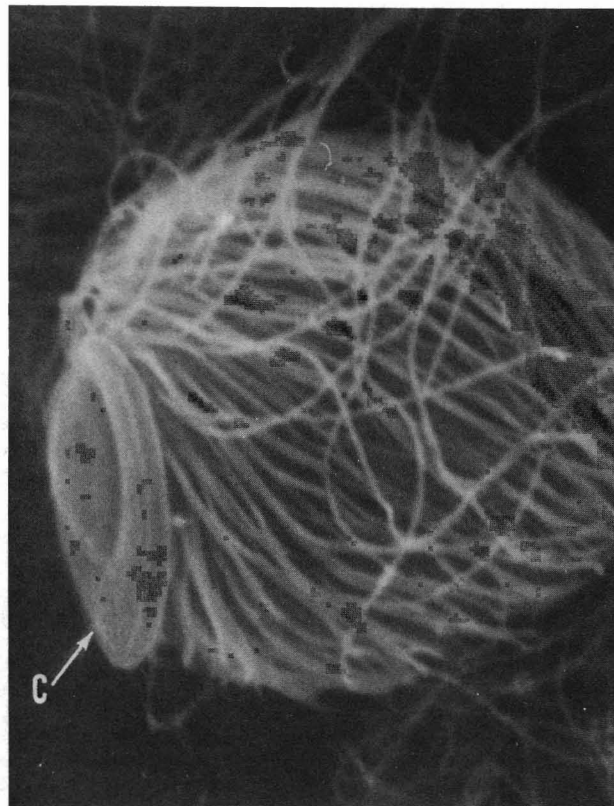

Figure 21.—Scanning electron micrograph of *Minchinia louisiana* spore showing spore wall cap (c) and thread-like spore wall ornaments. 10,000×.

Even when the mode of action of a drug is not known, the drug should be considered as a possible control for a shellfish disease when the shellfish disease agent can be demonstrated to be closely related to the species known to be inhibited by the drug. It is obvious that estuaries or oceans cannot be effectively treated with drugs due to the large volumes; however, if drugs effective against shellfish diseases can be found, they could be used under holding tank or aquaculture conditions where a limited volume of seawater would be involved for selected time periods. If the shellfish acquired immunity after being "cured" then subsequent addition to the estuary or ocean would not result in reinfection. Such an approach needs to be explored.

[Note added in proof. Two publications have appeared since this paper was presented which have information relevant to the taxonomic position of *Marteilia* spp. Desportes and Ginsburger-Vogel (1977) have suggested that *Marteilia* spp. should be considered as members of a new order, Marteiliida, in the Cnidosporidia, because they have a pluricellular structure. Current and Janovy (1977) have observed inclusions in the sporoplasm of *Henneguya exilis*, one of the Myxosporidia, which resemble haplosporosomes; however, the resolution was not adequate to make definitive judgements. Therefore, affinities of *Marteilia* spp. with the balanosporidans and the uniqueness of haplosporosomes for the Stellatosporea must be reconsidered.]

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