Structure and Biology of *Marteilia* sp. in the Amphipod, *Orchestia gammarellus*

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Introduction

A review of knowledge about the structure and biology of a parasitic protist observed in the amphipod crustacean, *O. gammarellus,* is presented. In this species, different sex-ratio abnormalities were studied (Ginsburger-Vogel, 1973, 1974, 1975). In peculiar populations, some females, called thelygenous females, produce broods with a majority of females (mean of 80 percent) and the rest, predominantly, are intersex males. "Micro-cells" were observed in thelygenous females and intersex males. These cells are seen in the general body cavity under the epidermis and in some organs, such as gonads (Fig. 1).

Results

Structure of *Marteilia* sp.

Electron microscope studies of these cells (Ginsburger-Vogel, et al., 1976) revealed some features similar to the structures described for *M. refringens* (Grizel et al., 1974; Perkins, 1976) and *M. sydneyi* (Perkins and Wolf, 1976) and also some differences. In most cases, the parasite is observed in the form of primary cells, containing 1-12 secondary cells (Fig. 2). These cells, in their turn, can hold one or two successive generations. The primary cells can be found in the host's cells (Fig. 3) and also in the surrounding fluid. Their size is 5-45 μm according to the number of secondary cells they contain.

Characteristic inclusions are found in their cytoplasm. Multivesicular bodies, consisting of spherical vacuoles (0.7-1.5 μm), are delimited by a single membrane and contain numerous vesicles with more or less electron-dense contents. Small stick-shaped, osmiophilic inclusions, very similar to the haplosporosomes described by Perkins (1971), are distributed throughout the cytoplasm or localized in some areas, particularly at the periphery of the cell. In addition, electron-light vesicles, a few mitochondria, smooth ergastoplasm, and ribosomes are found in the cytoplasm of primary cells. In all the stages observed, multinucleate primary cells were never found. The striated inclusions, characteristic structures in the cytoplasm of *M. refringens,* but absent in *M. sydneyi,* are not found in the cytoplasm of *Marteilia* sp.

The secondary cells are unlike the primary cells in that they lack haplosporosomes and multivesicular bodies. Mitochondria are relatively numerous (from three to five by cell section) and are characterized by a light matrix with a network of fibrils (perhaps DNA) and a paucity of cristae (Fig. 5). They are often seen near the nuclear membrane. Ribosome density is always greater than in the primary cells thus giving them a darker appearance.

Two problems were investigated in order to solve the taxonomic position of this species: The structure of the kinetic center and the mechanism of spore formation. In *Marteilia* sp., the kinetic center consists of centrioles associated with a differentiation of the nuclear membrane (Desportes and Ginsburger-Vogel, 1977). In longitudinal sections, centrioles appear as short cylinders (200-240 nm long) positioned above a thickening of the inner nuclear membrane (Fig. 4). The centrioles are perpendicular to each other. In transverse sections, they appear as a ring of nine singlets of microtubules surrounding a less visible central one (Fig. 5). Each singlet gives birth to a lateral arm. The diameter of the ring is 180 nm. Bundles of extended cytoplasmic microtubules are associated with the centrioles.

This unusual nine-plus-one type of centriole is a structure found in some Protists. It has been observed mainly in Coccidia (Roberts et al., 1970a, b) and Gregarinida (Molon-Noblot, 1977), but also in *Tetrahymena pyriformis* (Allen, 1969) and *Chlamydomonas reinhardi* (Johnson and Porter, 1968). The relationships between the centriole and

ABSTRACT–A new species of Marteilia *was observed in the amphipod* Orchestia gammarellus. *Electron microscope studies show affinities with* Marteilia refringens *and* Marteilia sydneyi. *Parasites are observed in the form of primary cells containing 1-12 secondary cells. The primary cells contain characteristic inclusions: Multivesicular bodies and haplosporosomes. The kinetic center consists of centrioles associated with a thickening of the inner nuclear membrane. Centrioles consist of a ring of nine singlets of microtubules. Presporulation stages appear as secondary cells or sporangia containing one or two spore primordia, each of them containing one or two cells produced by endogeneous budding. Relations between the presence of the parasite and some sexual abnormalities observed in estuarine populations of the host are discussed on the basis of experimental results and field observations.*

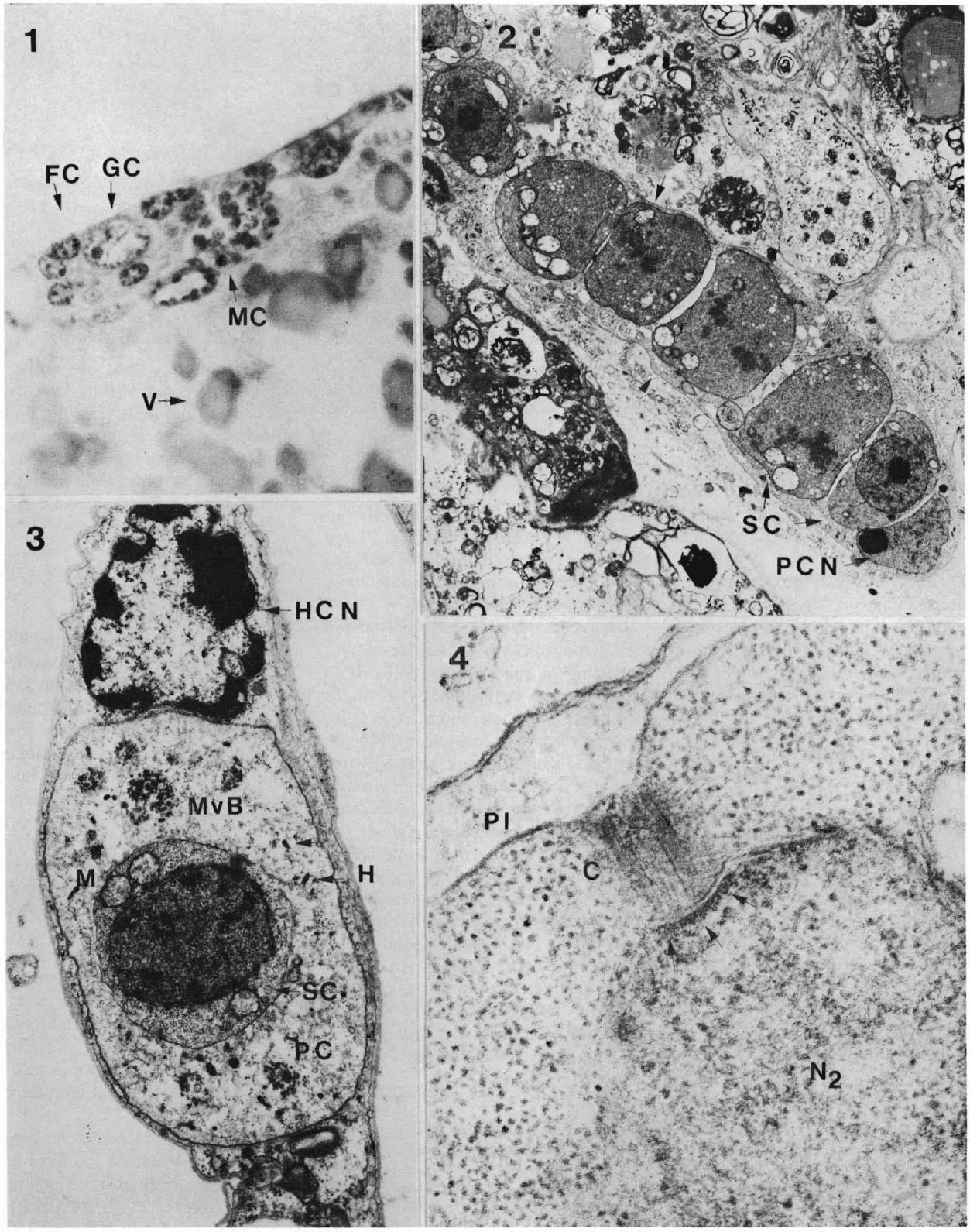

Figure 1.—Micrograph of "micro-cells" observed near the germinative zone of an ovary. Follicular cell (FC); germinal cell (GC); microcell (MC); vitelline platelet (V). 2,300×. Figure 2.—*Marteilia* sp. primary cell containing secondary cells (SC). Arrows indicate the plasmalemma of the primary cell. Primary cell nucleus (PCN). 4,000×. Figure 3.—Intracellular stage of *Marteilia* sp. Host cell nucleus (HCN); multivesicular bodies (MvB); haplosporosomes (H); mitochondria (M); secondary cell (SC); primary cell (PC). 11,000×. Figure 4.—Longitudinal section of centriole (C) of *Marteilia* sp. Nucleus of secondary cell (N_2); plasmalemma (Pl). Arrows indicate the thickening of the inner nuclear membrane associated with the centriole. 100,000×.

the nuclear membrane and the mode of spindle differentiation recall what was observed in the chytrid, *Phlyctochytrium* (McNitt, 1974). In this species, the spindle is organized as in *Marteilia* sp. from a diplosome. One of both centrioles is applied near a thickening of the nuclear membrane.

These observations help to distinguish *Marteilia* sp. from typical Haplosporida where no centriolar structure has been described. Moreover, Perkins (1975) showed that schizogonial mitosis observed in plasmodia of different haplosporidian species (as *Minchinia* or *Urosporidium*) are characterized by spindles issued from intranuclear spindle pole bodies. As centrioles were not described in previous works done on *Marteilia refringens* and *M. sydneyi,* the main question is to know if they are present or not in these species. A nuclear membrane thickening, very similar to that observed in *Marteilia* sp., was found in *M. refringens* (Fig. 6), but the surrounding structure is not clearly a centriole. Nevertheless, these pictures show likenesses between the kinetic centers of both forms.

The knowledge of the shape and structure of the spore is certainly very important in order to judge the systematic position and affinities of the studied species. Numerous pictures of different stages of sporogenesis were observed, but never typical spores. In these stages, cytoplasm of the primary cell appears more or less reduced, with an enlargement of the vacuolar space including secondary cells. In comparison with *M. refringens,* secondary cells can be considered as sporangia containing one or two spore primordia, each of them containing one or two cells arising from endogeneous budding (Fig. 7). The cytoplasm and nucleus of the sporangia show degenerative forms. A thin fibrillar material is deposited in the vacuolar space surrounding spore primordia. The cytoplasm of the spore primordium contains elongate or circular electron-dense bodies.

On the basis of cytological characters, it can be concluded that the presently studied species or form shows many features in common with *M. refringens*.

Table 1.—Results of grafts of various organs of thelygenous females and intersex males (♂i) in normal females: Analysis of the progeny.

Nature of the grafted organ	Ovary of thelygenous female	Muscle of thelygenous female	Blood of thelygenous female	Testis of intersex male
No. of positive cases/No. of total cases (percentages)	17/17 (100%)	12/17 (70%)	9/11 (80%)	9/15 (60%)
Negative results (Masculinity rate)	—	105 ♂, 91 ♀ (53.5%)	32 ♂, 36 ♀ (47.1%)	223 ♂, 251 ♀ (47.1%)
Positive results	52 ♂, 17♂i, 803 ♀	19 ♂, 103 ♂i, 598 ♀	37 ♂, 81 ♂i, 706 ♀	82 ♂, 69 ♂i, 871 ♀
(Masculinity rate)	(7.9%)	(14.5%)	(16.7%)	(12.5%)
(Intersexuality rate)	(24.6%)	(84.5%)	(68.6%)	(45.6%)
Control experiments (grafts of homologous organs of normal individuals) (Masculinity rate)	86 ♂, 95 ♀ (47.5%)	33 ♂, 32 ♀ (51.5%)	35 ♂, 83 ♀ (50.6%)	105 ♂, 97 ♀ (51.5%)

Biology of *Marteilia* sp.

Cells of the parasite may be found in various organs or tissues, in particular testes and ovaries, spermiducts, the androgenic gland, under epidermal adipose tissue, pericardium, and hemolymph. They seem able to move in and out of the organs, digesting the basal membrane (Fig. 8). This movement is accompanied by a structural change in the cytoplasm of primary cells. The location of the parasite cells is less restricted than is observed for *M. refringens,* but they were never found in the digestive tract and hepatic caeca.

Although *Marteilia* sp. was called a parasite, it seems not to have any pathogenic effect, in the common sense of this term, on its hosts. Indeed, animals seem affected neither in their longevity nor in their growth or reproductive abilities. However, in the oldest animals, numerous "chitinous nodules" develop under the epidermis. These nodules appear in Crustacea in response to various parasitic infestations.

Animals bearing *Marteilia* sp. cells are affected by some sexual abnormalities extensively studied elsewhere (Ginsburger-Vogel, 1974, 1975). In order to explain these abnormalities (thelygeny of the female and intersexuality of the male), the existence of a feminizing factor able to transform a part of genetic males into females or intersex males was postulated. The question is whether *Marteilia* sp. is the feminizing factor. At present, there is only indirect evidence that such is the case. Indeed, *Marteilia* sp. is always found in thelygenous females and intersex males; it was never observed in normal animals. It is possible to transmit the feminizing factor to normal males which acquire characters of intersexes (Ginsburger-Vogel and Carré-Lécuyer, 1976) or to normal females which become thelygenous (Table 1). Positive results are obtained with grafts of organs belonging to thelygenous females or intersex males. In positive cases, cells of *Marteilia* are present in the organs of the host. The same experiments were done filtering macerated organs with Millipore filters of various pore sizes. The feminizing effect is lost with holes <5 μm diameter. In this case, parasitic cells were not found in the animals receiving the filtered material.

These phenomena are temperature-sensitive. *Marteilia* sp. cells gradually disappear in thelygenous females reared at 22°C for 1 month or more.

Thus, this series of observations appears to support the hypothesis that *Marteilia* sp. is the feminizing factor.

Occurrence in Natural Populations

An indication of the presence of parasites in a population of *O. gammarellus* is the existence of intersex males and sex-ratio abnormalities.

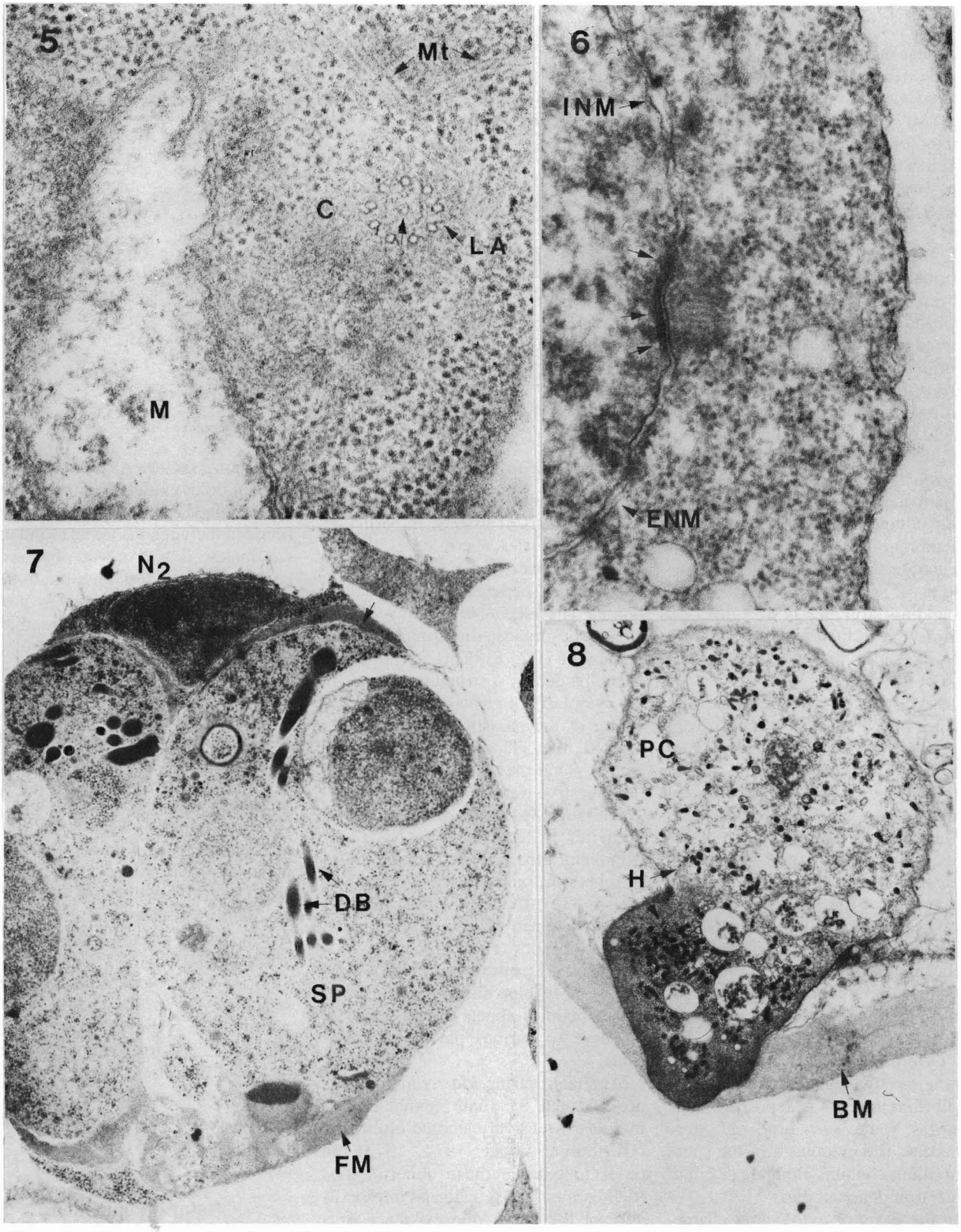

Figure 5.—Transverse section of a centriole (C) of *Marteilia* sp. Lateral arm (LA); cytoplasmic microtubules (Mt); mitochondria (M). Arrow indicates the central microtubule of the centriole. 80,000 ×. Figure 6.—Longitudinal section of the supposed kinetic center of *Marteilia refringens* showing the thickening (arrows) of the inner nuclear membrane (INM). External nuclear membrane (ENM). 80,000×. Figure 7.—Presporulation stage of *Marteilia* sp. Degenerative nucleus of secondary cell of sporangia (N_2); spore primordium (SP); dense bodies (DB); fibrillar material (FM). 21,000×. Figure 8.—Primary cell (PC) of *Marteilia* sp. digesting the basal membrane (BM) of an ovary Haplosporosomes (H). 11,000×.

Knowledge of both values (intersexuality rate and masculinity rate) can give an idea of parasite distribution among natural populations. *Orchestia gammarellus* is a species of the upper tide level which lives on pebble beaches or on the shores of estuaries, under stones or slimy gravels. Table 2 shows the rate of intersexes and the masculinity rate for different populations. Most populations of the seashore are characterized by a masculinity rate close to 50 percent and an intersexuality rate small or null. These populations are numerically important and live in abundant litters of decaying seaweeds. Seashore populations characterized by higher intersexuality rate (Ile verte, Dunes Ste Marguerite, Luc-sur-mer) are distinguished from other populations by scarcity of seaweed heaps and a smaller number of individuals. Estuarine populations are characterized by an even higher intersexuality rate, between 5 and 15 percent, and a masculinity rate generally lower than 50 percent. Also, it can be said that *Marteilia* sp. is particularly common in estuarine populations. It can be asked what are the external factors involved in this distribution? Salinity variations do not seem to be linked with it. Indeed, these animals are in contact with seawater only during the highest tides. On the contrary, the different populations live under varying thermal conditions. Decaying seaweeds represent, besides an abundant food supply, a shelter where temperature is higher and more stable than that of the surrounding air. Presence of intersexuality under experimental conditions is temperature-sensitive; therefore, differences of temperature could explain the absence of intersexes in some populations.

Table 2.—Masculinity and intersexuality rate in different populations.

Populations	Locality	Masculinity rate	Intersexuality rate
Seashore	Bloscon	53.45	0.3
	Ile verte	39.4	2.15
	Sainte Anne	54.31	0
	Ste Marguerite	48.23	0
	Baie de Cayola	49.28	0
	Luc-sur-mer	45.53	2.94
Estuaries	Penzé	29.98	15.91
	Aber-Wrach	46.66	12.58
	Aber-Benoît	42.12	4.87
	Aber-Ildut	31.73	5.66
	Sallenelles	11.8	15.62

Discussion

The species of *Marteilia* described here shows a feminizing effect on its host. This apparently nonpathological effect explains why this form was not seen before. In relation to structural likenesses and parallel distributions with *M. refringens,* the problem of their affinities must be considered at different levels: 1) Are they different species or could they be two different forms of the same species? 2) Are these species Haplosporida, as stated by Perkins (1976), or not? And if not, 3) to which group of protists do they belong? In the present state of knowledge, answers to these questions are certainly not definitive. Observed likenesses between *M. refringens* and the species found in *O. gammarellus,* particularly the mode of budding by internal cleavage, suggest that these forms are very similar and may belong to the same genus. The differing cytological characters (i.e., presence of striated inclusions in the cytoplasm of primary cells of *M. refringens,* absence in *Marteilia* sp., occurrence of dense bodies in the spore primordia of *Marteilia* sp., absence in *M. refringens*) seem insufficient to definitively conclude that they are different species. Different structures could be explained by the presence of the parasite in different hosts. Absence of centrioles in *M. refringens,* if confirmed, would present a strong argument favoring a separation into different species.

Acknowledgments

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