Microsporidian Infections of Amphipods with Special Reference to Host-Parasite Relationships: A Review

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ABSTRACT—A survey of the 10 species of microsporidians associated with amphipod crustaceans is presented. Specific features for their recognition are given on taxonomy, morphology, spore size, sites of infection, and host range. Some aspects of the host-parasite relationships are considered. In several species, transovarian transfer appears to be a major route of transmission. Previous results on microsporidians indicate that they do not cause fatal diseases in their amphipod hosts. A sex-determining influence on the progeny of Gammarus duebeni after infection by Octosporea effeminans or Thelohania herediteria is indicated. Both of these microsporidians are transmitted through the eggs to the host's offspring which, in general, all develop into females. Some results and problems of this effect on sex ratio are discussed.

INTRODUCTION

Microsporida are cytozoic parasites with a wide host range, comprising all phyla of the animal kingdom from protozoans to mammals. They are important pathogens of arthropods, especially insects, since they cause severe diseases in several members of this group. Compared with the numerous detailed investigations of microsporidioses in insects, very few studies are devoted to microsporidian infections in crustaceans, and to the effects they have on their hosts. Since Kudo's review (1924), no comprehensive and critical report on the taxonomy and biology of the microsporidians detected in crustaceans has been published, except for a survey recently presented by Sprague (1970a) on protozoan parasites in decapod Crustacea. Sprague recorded less than two dozen microsporidian species, mainly from marine hosts, with some known to cause diseases in commercially important decapods.

Due to several inadequate descriptions and some taxonomic confusion, it is difficult to estimate the exact number of microsporidian species occurring in crustaceans. Moreover, the widely scattered literature supplies little or no information on host-parasite relationships. Therefore, only scanty data are available for an assessment of their pathological significance.

This paper is confined to the microsporidian species parasitic in amphipod crustaceans, and presents a survey of their taxonomy, specific morphological characters, sites of infection, and host range. In addition, various aspects of the host-parasite relationships are considered.

TAXONOMIC SURVEY

Microsporidia are protozoan parasites which are characterized by the formation of spores equipped with an extrusible polar filament. Their life cycle commences with the liberation of a uninucleate or bi-nucleate sporoplasm from the spore with subsequent entry into a host cell. An intracellular multiplicative phase (schizogony) is followed by the development of spores (sporogony). The resistant spores infect new hosts.

Among the criteria employed for the separation and identification of microsporidian species, the most significant characters are shape and size of the spores. Besides the morphometrics of the spores, the various stages of the life cycle, the mode of sporogony, the sites of infection, and the host specificity are of diagnostic importance. However, since some original descriptions are vague and fail to provide adequate detail, a satisfactory review of all species recorded in amphipods cannot be presented. Nevertheless, an attempt has been made to list all species described and to summarize specific features for their recognition (Table 1). The list is based on a survey presented by Lipa (1967), but includes several additions and corrections. In several species the dimensions of fresh spores are given, but in others it is doubtful whether size refers to fixed or fresh spores.

The microsporidians recorded from amphipods belong to 10 species from 5 genera. Most host species belong to the genus *Gammarus* which occurs in freshwater as well as brackish and marine habitats. Some additional data for characterization of the microsporidian genera (cf. Weiser, 1961) and species are briefly presented below.

Genus *Nosema* Nageli, 1857

The essential characteristic of this genus is that only one spore is produced by each sporont.

Nosema gammari van Ryckeghem has been described from only one specimen of *Gammarus pulex*, found in Belgium (van Ryckeghem, 1930). A diffuse infection of the host muscles was observed, but no further details on the life history have been reported for this parasite. In England, Pixell-Goodrich (1929, 1956) noted *Nosema* sp. in *G. pulex*, but no detailed characteristics of this species have been published.

Nosema kozhovi Lipa was recorded (Lipa, 1967) in *Brandtia lata lata* collected on the shores of Lake Baikal (Soviet Union). All tissues were infected with especially heavy concentrations in the gut epithelium, adipose tissue, and muscles. Parasitized animals had a milky-white appearance.

Genus *Thelohania* Henneguy, 1892

This genus develops eight spores from a single pansporoblast and the spores may remain together within a common membrane.

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Table 1.—List of microsporidian species parasitic in amphipod crustaceans.

Microsporidian species	Host species	Sites of infection	Size of spores (μm)	References
Nosema gammari van Ryckeghem	*Gammarus pulex* L.	peri- and intermuscular tissue	1.5 by 0.75	van Ryckeghem (1930)
Nosema kozhovi Lipa	*Brandtia lata lata* (Dybowski)	gut epithelium, adipose tissue, muscles	3.3-3.9 by 2.1-2.2 (fresh)	Lipa (1967)
Thelohania mülleri Stempell	*Gammarus pulex* L., *Gammarus chevreuxi* Sexton	muscles	4-5 by 2 (fresh)	Pfeiffer (1895), Stempell (1902), Léger and Hesse (1917), Jírovec (1936), Pixell Goodrich (1929, 1956), Bulnheim (1971a)
Thelohania mülleri var. *minuta* van Ryckeghem	*Gammarus pulex* L.	muscles	3 by 1.5	van Rycheghem (1930)
Thelohania vandeli Poisson	*Niphargus stygius* Schiödte	muscles (?)	6-6.5 by max. 3	Poisson (1924)
Thelohania herediteria Bulnheim	*Gammarus duebeni* Lilljeborg	muscles, ovaries	4.5-6 by 1.9-2.7 (fresh)	Bulnheim (1969, 1971a)
Stempellia mülleri (Pfeiffer) [syn.: *Glugea mülleri* Pfeiffer (partim), *Plistophora mülleri* (Pfeiffer), *Thelohania giraudi* Léger and Hesse, *Plistophora blochmanni* Zwölfer]	*Gammarus pulex* L., *Gammarus locusta* (L.), *Gammarus oceanicus* Segerstråle, *Gammarus salinus* Spooner, *Gammarus zaddachi* Sexton, *Gammarus duebeni* Lilljeborg, *Niphargus ilidzensis* Schäfer	abdominal muscles	4-5.5 by 2.5-3 (fresh)	Pfeiffer (1895), Labbé (1899), Stempell (1902), Léger and Hesse (1917), Debaisieux (1919, 1928), Zwölfer (1926a, 1926b), Géorgévitch (1929), van Ryckeghem (1930), Bulnheim (1971b)
Octosporea gammari van Ryckeghem	*Gammarus pulex* L.	excretory system, heart epithelium	4-6 by 1.2-2	van Ryckeghem (1930), Jírovec (1943)
Octosporea effeminans Bulnheim and Vávra	*Gammarus duebeni* Lilljeborg	ovarial tissue, adipose tissue	4-10 by 1.5-2.5 (fresh)	Bulnheim (1967, 1969, 1970), Bulnheim and Vávra (1968)
Bacillidium niphargi (syn.: *Mrazekia niphargi* Poisson)	*Niphargus stygius* Schiödte	?	8-9 by 2	Poisson (1924), Jírovec (1936)

Thelohania mulleri Stempell, a parasite of *G. pulex*, was described in detail by Stempell (1902). Evidently the same microsporidian was found in *Gammarus chevreuxi* by Pixell-Goodrich (1929). The papers of Léger and Hesse (1917), van Ryckeghem (1930), Jírovec (1936) and Bulnheim (1971a) also deal with this species. Initially, *T. mulleri* was confused with *Stempellia mulleri*, as outlined in a subsequent paragraph. The infected host displays a spotted, whitish, opaque coloring. Both the body muscles and the muscles of the appendages become heavily infected. The spores are typically pyriform in shape, but anomalies of spore formation occasionally may occur with the development of four or even two spores that are significantly larger than the others within a pansporoblast. Host records derive from Germany, England, and Belgium.

T. mulleri var. *minuta* van Ryckeghem is a smaller microsporidian than *S. mulleri*, with the same characteristics of spores and sites of infection (van Ryckeghem, 1930). Without re-examining both forms, it is difficult to decide whether they represent distinct species.

Thelohania herediteria Bulnheim is clearly distinguishable from *T. mulleri* by its larger size and more cylindrical spores (Fig. 1). It invades the muscles and ovaries of female *Gammarus duebeni*, but does not occur in males

(Bulnheim, 1971a). Although the posterior part of the body is more heavily parasitized than the anterior, infection of the host cannot be recognized macroscopically. The only record of this species is from the River Elbe estuary in Germany.

Genus *Stempellia* Leger & Hesse, 1922

In this genus, 4, 8, 16, or 32 spores develop within one pansporoblast.

Stempellia mulleri (Pfeiffer) has a rather complicated taxonomic history. Pfeiffer (1895) described a species from *G. pulex* named *Glugea mulleri*, which is similar to *S. mulleri*. Later on it was named *Plistophora mulleri* (Pfeiffer) by Labbé (1899) and *T. mulleri* (Pfeiffer) by Stempell (1902). Debaisieux (1919) and Zwölfer (1926a) stated that the form discern. One species is to be regarded as *T. mulleri*, the other as *S. mulleri*. The latter appears to be identical with the descriptions of *Thelohania giraudi* (Léger and Hesse, 1917), *G. mulleri* descriptions of *Thelohania giraudi* (Léger and Hesse, 1971), *G. mulleri* (Debaisieux, 1919, 1928) and *Plistophora blochmanni* (Zwölfer, 1926a, 1926b). Géorgévitch (1929) recognized this synonymy and named the microsporidian in question *P. mulleri* (Pfeiffer). According to the numbers of spores produced within one pansporoblast,

it must be transferred to the genus *Stempellia*. The spores have an ovoid shape (Fig. 2). *S. mulleri* has a wide host range as demonstrated by several infection experiments (Bulnheim, 1971b). Therefore, it can be expected that this parasite may invade amphipods other than those listed in Table 1. Host records derive from Germany (freshwater and euryhaline species), Belgium, and Yugoslavia. *S. mulleri* can be easily recognized by the presence of one or more white tubular masses of parasites which are arranged in the abdominal muscles of its hosts and separated from one another by 2-3 mm.

Genus *Octosporea* Flu, 1911

Species of the genus *Octosporea* have cylindrical spores which may be slightly arched. The spore width is one-third or less of the spore length, and all stages of the life cycle are binucleate.

Octosporea gammari van Ryckeghem was detected in one specimen of *G. pulex* from the region of Louvain in Belgium. Van Ryckeghem (1930) noticed in this specimen a dual infection of *O. gammari* and *T. mulleri*. Jírovec (1943) later observed the same microsporidian near Prague, Czechoslovakia. It invades the excretory system and the tissue around the heart.

Octosporea effeminans Bulnheim and Vávra (Fig. 3) has been found in the
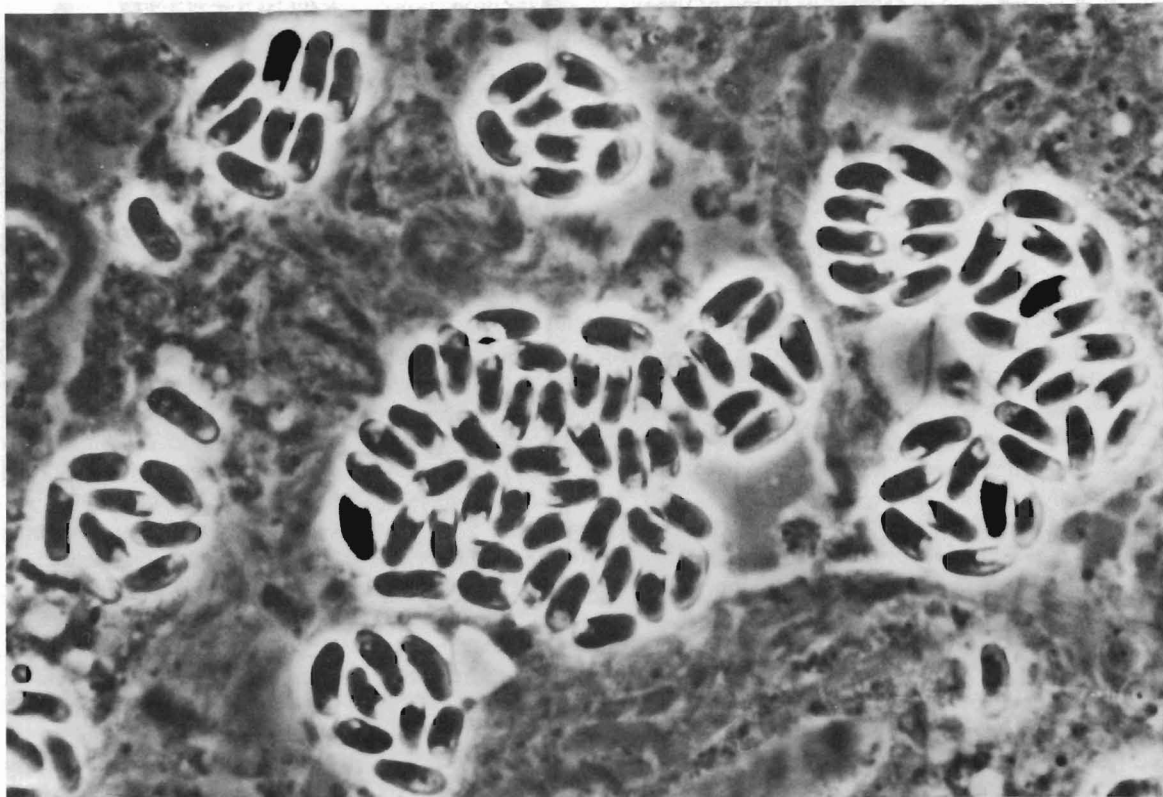
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Figure 1.—*Thelohania herediteria*. Fresh spores from infected *Gammarus duebeni*. (Phase contrast, 2,000×).

ovaries and adipose tissue of the brackish-water species *G. duebeni* (Bulnheim and Vávra, 1968). This parasite occurs only in females and, sometimes, in intersexes. This parasite was found in populations of *G. duebeni* from the River Elbe estuary in Germany and the Baltic Sea. Dual infections by this species and by *T. herediteria* have been observed in a few cases (Bulnheim, 1971b).

Genus *Bacillidium* Janda, 1928

The genus *Bacillidium* is characterized by very long, rod-shaped spores. In contrast to members of the genus *Mrazekia*, they have no tails (Weiser, 1961). However, Sprague (1970b) pointed out that the genus *Bacillidium* was falsely distinguished from *Mrazekia* by comparison with a species incorrectly regarded as the type of the latter genus and is a junior subjective synonym of *Mrazekia*.

Bacillidium niphargi (Poisson) has been reported to occur in the subterranean amphipod *Niphargus stygius*. It was first named *Mrazekia niphargi* by Poisson (1924), but the spores did not possess tails. Jírovec (1936), therefore,

assigned this microsporidian to the genus *Bacillidium* and named it *B. niphargi*. Unfortunately, no further biological data have been recorded on this parasite.

Unnamed Species

In the muscles and heart of *Talitrus* sp., Mercier (1906) observed a microsporidian. Anders (1957) detected a microsporidian, whose taxonomic position could not be clearly identified, associated with *G. pulex subterraneus*. He found this parasite in intersexual specimens. Recent studies by the author have demonstrated that *Gammarus locusta* and *Gammarus salinus* may also be attacked by *Thelohania* sp. which have smaller spores than *T. herediteria* but similar morphology and sites of infection. Whether or not they represent a new species requires careful investigation and comparison with the known species of the genus *Thelohania*.

From these observations it is obvious that a considerable diversity of microsporidian species exists in amphipods, and that many new microsporidian

species will be discovered in this crustacean group.

TRANSMISSION

In general, the infection of a susceptible host is initiated by the ingestion of spores. It is assumed that the digestive fluids then cause the extrusion of the polar filament and the discharge of the sporoplasm which penetrates a host cell. Transovarian transfer represents a route of infection which is probably the major method of transmission of microsporidians in amphipods studied thus far.

An experimental infection of laboratory-reared gammarids by uptake of spores with food was achieved in *S. mulleri* (Bulnheim, 1971b). Although Zwölfer (1926a) failed to infect *G. pulex* in this way, Bulnheim (1971b) transmitted this species by feeding starved gammarids with infected musculature from other specimens to demonstrate the wide range of possible hosts for this microsporidian.

In contrast to *S. mulleri*, which does not invade the ovaries, several other species have been shown to be transferred through the eggs of their hosts.

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This mode of transmission was recorded in *O. effeminans* and *T. herediteria* (Bulnheim and Vávra, 1968; Bulnheim, 1971a). Further investigations and re-examination provided evidence that transovarian transfer also occurred in *Thelohania* sp. associated with other representatives of the genus *Gammarus*: *G. locusta*, *G. salinus*, and *G. pulex*. However, the taxonomic position of *Thelohania* sp. from the latter amphipods could not be clarified, but the microsporidian found in *G. pulex* was assigned to *T. mulleri* var. *minuta*, which was briefly described by van Ryckeghem (1930).

In the microsporidians transferred transovarially, infection of the oocytes and multiplication by schizogony is intimately associated with the reproductive cycle of the female hosts. Their ovaries are long paired tubes which contain oogonia and three layers of different-sized oocytes. Generally, the oocytes are not infected before they are arranged in the third layer in order to undergo intensive cytoplasmic growth. As a rule, only vegetative stages, mainly binucleate schizonts, can be found in the oocytes. The propagation of the parasites within the ooplasm proceeds until the formation of yolk granules takes place. When the eggs are released into the brood pouch and fertilized, the parasites undergo a second period of schizogonic multiplication which leads to a strong infiltration of the developing embryo by vegetative stages.

Attempts to achieve peroral transfer of the microsporidians *O. effeminans* and *T. herediteria* to their host *G. duebeni* did not succeed. Although fresh and dried tissues contaminated by spores were offered to the test animals, an experimental infection could not be accomplished. The reasons for this failure are not clear, but may be due to the fact that extrusion of the filament from the spore cannot be instigated by chemical or mechanical stimuli in these species. Nevertheless, a natural infection by uptake of spores scattered in the aquatic environment cannot be excluded. However, transovarian transfer of vegetative stages appears to be the most important and effective way of regularly transmitting an infection.

PATHOGENICITY

Epizootic diseases such as microsporidian infections may cause various histopathological alterations, often resulting in an enormous hypertrophy. However, no evidence of such pronounced pathological changes from microsporidian infections in amphipods has been provided. As a result of progressive multiplication of the parasites, the muscles become filled up with ripe spores, ultimately destroying the host muscle fibers. In some gammerids infected with members of the genera *Nosema* and *Thelohania*, Pixell-Goodrich (1929) observed yellow or brownish chitinoid nodules which constituted necrotic muscle fibers that were detached from the surrounding tissues. These muscles were then attacked by phagocytes which also ingested spores. Owing to the secretion of chitinoid substance by these hemocytes, the areas where the attack was most advanced exhibited the darkest brown color.

A similar defense mechanism of the host appears to occur in *G. pulex* infected by *S. mulleri*. The large masses
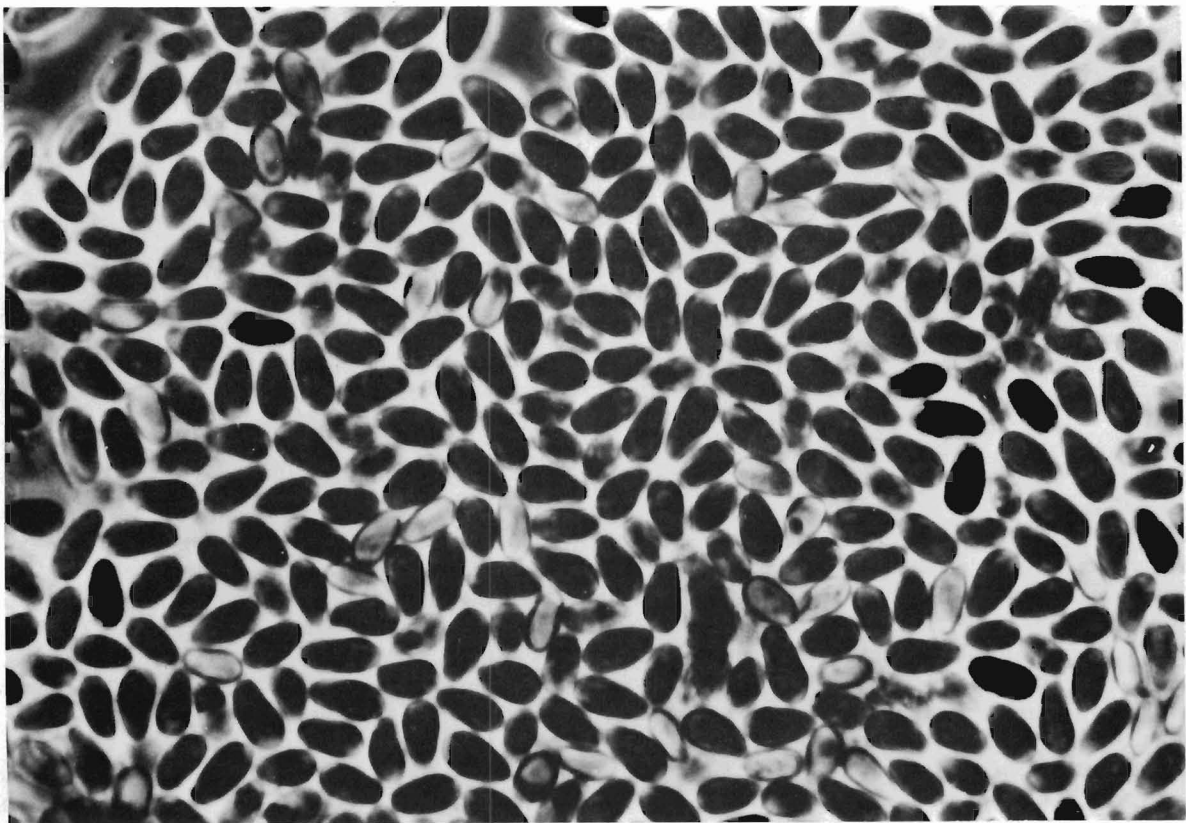

Figure 2.—*Stempeilia mulleri*. Fresh spores from infected *Gammarus salinus*, some with extruded sporoplasm. (Phase contrast, 2,000×).
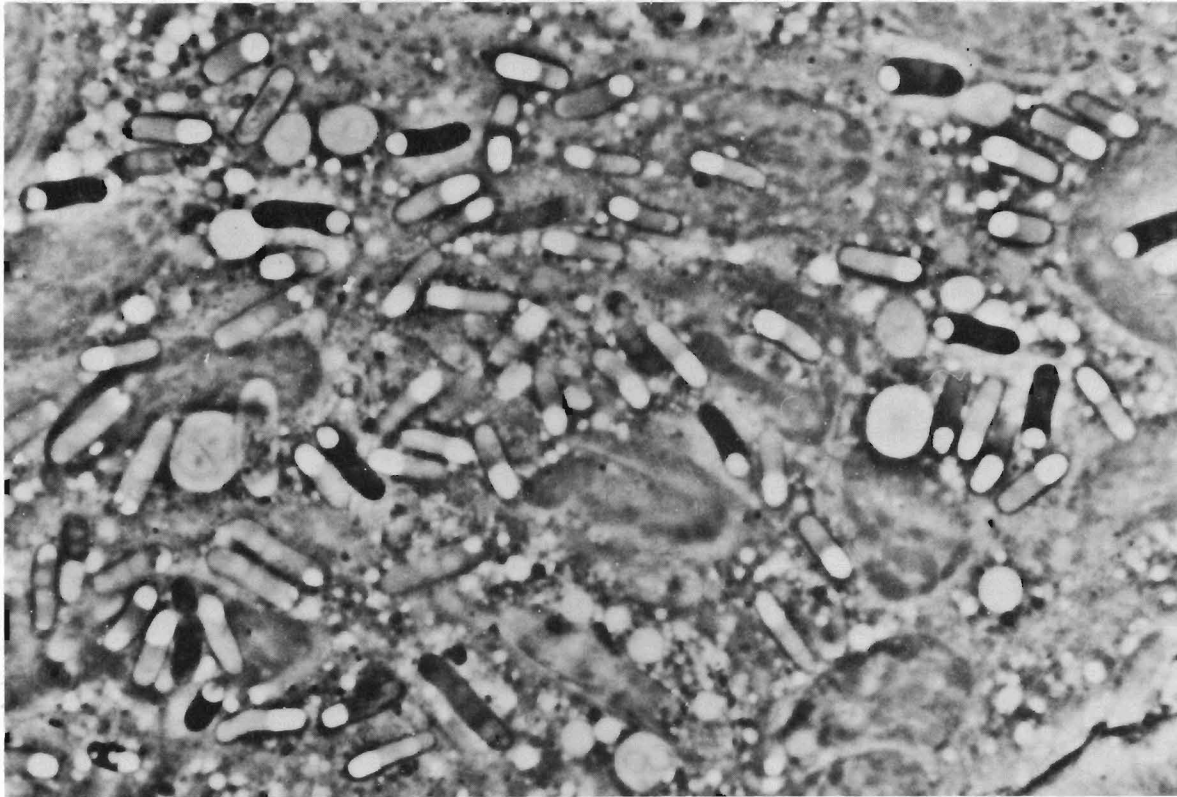
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Figure 3.—*Octosporea effeminans*. Fresh spores and sporonts together with a binucleate schizont (left) from infected *Gammarus duebeni*. (Phase contrast, 2,000×).

of parasites distributed within the abdominal muscles are sometimes surrounded by a brownish envelope, as observed by Zwölfer (1926a). He also noticed partially digested spores and sporoblasts at the periphery of such zones and suggested that the connective tissue of the host might be responsible for this encapsulation. Only severe infections, which lead to a complete breakdown of the muscles, can cause this reaction by the host (Zwölfer, 1926a). Similar responses in tissues invaded other than muscles have not been reported.

With respect to the effects of the parasites on their hosts, some findings have been recorded which indicate that none of the microsporidian species which infect amphipods cause fatal diseases, and thus do not seriously affect the life functions of their hosts. Lipa (1967) stated that specimens of *B. lata lata* infected by *N. kozhovi* display reduced locomotory activity compared with healthy individuals. Reduced capacity for locomotion and increased mortality was observed by Zwölfer (1926a) in *G. pulex* infected by *T. mulleri*. Stempell (1902), however, did

not find any apparent pathogenic effects due to these microsporidians. *S. mulleri* has no noticeable pathogenic effect on its hosts (Zwölfer, 1926a), as confirmed by my own observations. Two microsporidian species, *O. effeminans* and *T. herediteria*, associated with *G. duebeni* have no pathological effects. Infected and noninfected specimens of *G. duebeni* have been reared for many generations in the laboratory. Several life functions have been studied and compared, such as brood size, mortality of embryos, juveniles and adult individuals, growth intensity, onset of maturity, life span, molting frequency, and metabolic rates, with no significant differences observed between infected and healthy amphipods (Bulnheim and Vávra, 1968; Bulnheim, 1971a). Results of investigations on standard metabolism as a function of size in noninfected *G. duebeni* juveniles and females compared with those infected by *T. herediteria* are presented in Figure 4 (cf. Bulnheim, 1972b for further experimental details). Despite the rather severe infection of the host, only a slight reduction in the respiratory rate was established with increasing size.

Except for a reduced resistance to temperature stress noticed in specimens infected by *T. herediteria*, no further differences in the life functions tested have been revealed. Thus, the results obtained from studies on *O. effeminans* and *T. herediteria* document the relationships between parasite and host to be rather well balanced.

INFLUENCE ON SEX DETERMINATION

Another aspect of the host-parasite relationship to be considered is the influence on the determination and inheritance of sex. This effect, elucidated by several studies on microsporidian infections in the brackish-water crustacean *G. duebeni* (Bulnheim, 1967, 1969, 1970, 1971a, 1972a; Bulnheim and Vávra, 1968), represents a peculiarity without parallel among Microsporidia.

G. duebeni exhibits considerable deviations from the normal 1:1 sex ratio. Rearing experiments demonstrated females produced young of both sexes and other females which give birth only to female offspring. Females of such strains mated with males from strains of

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different origin again produced broods consisting exclusively or predominantly of females, but accompanied by a few males or intersexes. Since this sex-ratio condition was transferred through the maternal line, cytoplasmatic inheritance was first assumed (Traut, 1962).

During an investigation conducted to find the suggested extra-chromosomal elements affecting sex determination, the occurrence of transovarially transferred microsporidians was detected. Since these were found almost exclusively in females, but sometimes in intersexes, it was supposed that the parasites might be involved in the mechanism of sex determination. In order to obtain definite proof of this assumption, the sex ratio of female progeny from a noninfected strain was compared with that from experimentally infected females. Females derived from one brood of two noninfected strains were divided into two groups. The females of one group were infected by inoculation of contaminated ovarial tissue into the body cavity, while the other group was not infected and served as a control. After pairing with males about 2 months later, offspring from the two groups were reared and sexed. The results of this experiment, listed in Table 2, clearly demonstrate that the experimental infection, compared with the control, resulted in shifting the sex ratio significantly toward females. The female descendants of the experimentally infected females proved to be contaminated and produced only unisexual offspring.

The microsporidian responsible for this sex-ratio condition was shown to be *O. effeminans* (Bulnheim, 1967; Bulnheim and Vávra, 1968). Later, another microsporidian, *T. herediteria*, was detected in *G. duebeni* females, which affects sex determination of its host in the same way when transmitted via the eggs to the offspring through successive gen-erations (Bulnheim, 1969, 1971a). For this reason, males do not harbor these parasites.

It appeared of interest to obtain information on whether or not sex determination could be established in other amphipods infected with microsporidians. Therefore, the following amphipods were studied: *G. locusta*, *G. salinus*, and *G. pulex*. They were all infected with *Thelohania* sp. whose taxonomic position at the species level, as previously mentioned, is still uncertain. *Thelohania* sp. are transferred transovarially and, thus, the young are infected at birth before sexual differentiation is initiated. In *G. locusta* and *G. salinus*, breeding experiments with specimens attacked by the microsporidians were conducted in the laboratory, whereas parasitized individuals of *G. pulex* were collected in the field. In all cases studied, it became evident that females as well as males are associated with microsporidians. Therefore, a sex-determining influence by *Thelohania* sp. on their hosts can be excluded.

With regard to the particular host-parasite relationship detected in the two microsporidian species associated with *G. duebeni* females, the question arises: what is the underlying mechanism of the host's response to the infection? The suggestion that the microsporidians might be involved in influencing genetic processes controlling determination and inheritance of sex in *G. duebeni*, which was initially taken into consideration, could not be confirmed. Detailed studies on the mode of sex determination in this amphipod revealed a lack of heteromorphic sex chromosomes. From the results obtained by breeding experiments with non-infected individuals, it can be concluded that sex determination is governed by a balanced polygenic system of sex genes, whereby environmental factors such as different photo-periods may have a pronounced influence on sex ratio (Bulnheim, 1969, 1972a).

Considering the mode of the sex determining influence by *O. effeminans* and *T. herediteria*, the assumption is proposed that the microsporidians might affect the differentiation of the androgenic gland during postembryonic development. This gland, situated at the vas deferens, is the source of the male sex hormone in Malacostraca and controls the differentiation of primary and secondary male sex characters, and if it does not develop its hormonal function the gonads develop into ovaries (Charniaux-Cotton, 1956, 1965).

Observations on infected intersexes with rudimentary androgenic glands and. later on, from experimentally infected males, demonstrate that this organ or its anlage are not attacked by the parasites. Therefore, it is probable that any substances or byproducts the microsporidians excrete during their multiplication might inhibit the differentiation of this gland. The idea that the parasites might cause such inhibitory effects was supported by another finding. As mentioned above, males of *G. duebeni* have not been shown to be parasitized by either *O. effeminans* or *T. herediteria*. Therefore, experimental infection of adult males was undertaken in order to test whether it exerts an influence on their sexual organization. Since attempts at peroral transfer failed, an inoculation was accomplished by transplantation of contaminated tissues of females into the male body cavity. After several molts, the male test animals infected by *O. effeminans* exhibited some alterations in their external sex characters. It was found that the size of the gnathopods, uropods, and antennae was gradually reduced and development of oostegites, which represent secondary sex characters of females, was observed. Thus, the males attained an intersexual appearance with respect to external characters. However, the male gonads, although invaded by microsporidians, displayed no structural changes.

This finding indicates that interference of microsporidians with the male sex hormone produced by the androgenic gland might be responsible for the sexual alterations observed. On the other hand, a species-specific effect of the microsporidians upon their host

Table 2.—Results of breeding experiments (15°C, 10⁰/₀₀ salinity) in *Gammarus duebeni* infected with *Octosporea effeminans*.[1]

	Strain	No. of couples	No. of broods	No. of juveniles	Sex ratio ♀ : ♂	Mortality (%)
Uninfected controls	da	3	9	555	224:231	18
	db	7	25	1,066	215:665 94 intersexes	16
Infected amphipods	da	3	9	458	393:10	12
	db	7	25	650	568:0 3 intersexes	12

[1]Females divided from one brood of two noninfected strains (da, db) were divided into two groups. The females of the one group were experimentally infected by transplantation of contaminated ovarial tissue, whereas the other group served as a control to compare sex ratio of descendants in both series. (Data from Bulnheim, 1967).
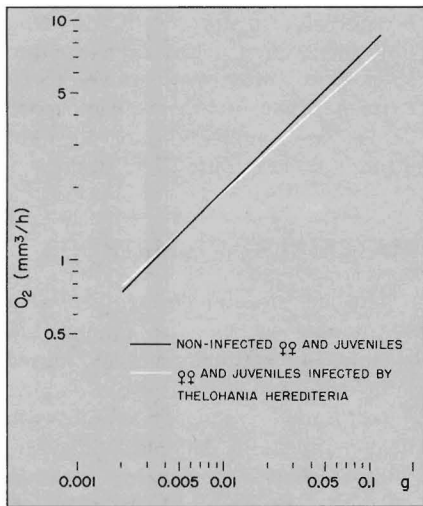
Figure 4.—Relationship between oxygen-uptake rates (standard metabolism) and size (wet weight) of *Gammarus duebeni* ♀♀ and juveniles infected by *Thelohania heredieria* in comparison with ♀♀ and juveniles free of microsporidians (15°C, 10⁰/₀₀ S). Consumption of O_2 is expressed by the regression equations log y = log 24.35 + 0.57 log x (infected specimens) and log y = log 30.63 + 0.607 log x (noninfected specimens). There are no significant differences (p>0.1) between the slopes of the two regression lines.

cannot be excluded. This became obvious when *T. heredieria*, experimentally transferred to *G. duebeni* males, did not induce any changes in either external or internal sex characters. The assumption of a species-specific action is confirmed by observations with *S. mulleri* in association with *G. duebeni*, where neither a sex-determining influence on the host nor a modification of sex characters occurs.

It should be mentioned that a hormonal involvement of a microsporidian has been detected in insects. In a study on endocrinological implications of *Tribolium* larvae infected by *Nosema* sp., Fisher and Sanborn (1964) demonstrated the ability of the parasites to produce a substance with juvenile hormone activity in their hosts.

The various aspects which pertain to the relationships between microsporidian infection and sex determination in *G. duebeni* cannot be discussed in full detail in this presentation. Additional results obtained from these investigations will be submitted in a forthcoming publication. In addition, the interrelations found between environmental factors, multiplication of the microsporidians, and sex differentiation of the host have not been outlined in this report, since recent studies provide detailed information on this subject (Bulnheim, 1969, In press).

Reference should be made to host-parasite interactions between some *Thelohania* sp. and mosquitoes. In *Culex tarsalis*, and probably also in other representatives of *Culicidae*, infection by *Thelohania* sp. is fatal to male larvae. Sporogony only occurs in male hosts which usually succumb with progressive infiltration by the parasites, whereas females acquire benign infections and harbor only schizogonic stages which are transmitted transovarially (Kellen and Wills, 1962; Kellen et al., 1965). The same responses have been reported in *Anopheles* sp. infected with *Thelohania legeri*, which is carried via the eggs to their progeny. In each generation the infected female larvae develop into adults, while infected male larvae die (Hazard and Weiser, 1968). In contrast to the situation found in the progeny of *G. duebeni* females associated with *O. effeminans* or *T. heredieria*, the shift of sex ratio in mosquitoes is caused by selective mortality of the offspring during the larval stages.

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