

lysosomal pathway of protein degradation in isolated rat hepatocytes by ammonia, methylamine, chloroquine and leupeptin. *Europ. J. Biochem.*, **95**:215-225.

43. Von Brand, T. 1979. *Biochemistry and Physiology of Endoparasites*. Elsevier/North Holland Biomedical Press, Amsterdam. P. 13.

44. Weir, D. M. 1978. *Handbook of Experimental Immunology*. Blackwell Scientific Publications, Oxford. Pp. 35.1-35.20.

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## Description of a New Species of Microsporidia from *Muscidifurax raptor* (Hymenoptera: Pteromalidae), a Pupal Parasitoid of Muscoid Flies

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**ABSTRACT.** A microsporidian parasite, *Nosema muscidifuraci*s n. sp., has been found in *Muscidifurax raptor*, a parasitoid of muscoid flies. Stages of the parasite developed in direct contact with the host cell cytoplasm and were detected in midgut epithelium, Malpighian tubules, ovaries (including oocytes) and fat body of larvae and adults. Spores were also detected within eggs deposited on the host. Light and electron microscopy revealed a developmental cycle with diplokaryotic stages dividing by binary fission and disporous sporulation sequences producing diplokaryotic spores of three morphological classes, differing significantly only in length of the polar filament. Two of the classes were found in larvae, pupae and adults. One of these, with about five turns in the coiled polar filament, is presumed to be responsible for transmission from cell to cell within the host (autoinfection) and the other, with about 10 turns, responsible for transmission from host to host. A third class, with about 15 turns in the polar filament, was found in eggs of *M. raptor*. It is, presumably, either involved in initiation and spread of the infection at eclosion or is responsible for horizontal transmission to a new host individual when eggs are cannibalized.

**Supplementary key words.** Biological control, Microspora, taxonomy, ultrastructure.

HERE are many reports on host-parasitoid-pathogen relationships where hymenopterous parasitoids are susceptible to microsporidia of the host [2]. But we know of only one report of microsporidia that are specific for parasitic hymenoptera [3]: *Nosema campoletidis* Brooks & Cranford, 1972 in *Campoletis sonorensis* (Cameron) and *N. cardiochilis* Brooks & Cranford, 1972 in *Cardiochiles nigriceps* Viereck were described with light microscopy and details of the host-pathogen relationships presented. These organisms exhibited chronic effects on the parasitoid host, were transovarially transmitted and did not infect the lepidopterous hosts. Recently, an undescribed microsporidium was reported from *Muscidifurax raptor* Girault & Sanders, a parasitoid of muscoid flies [5, 18]. This new microsporidium adversely affected the fitness of *M. raptor*, was transovarially transmitted and the muscoid host was not infected. This study describes the developmental sequences and morphological features of this new and unusual microsporidium and presents additional information on the host-pathogen relationship. A new species is proposed and its affinities to other microsporidia specific to hymenopterous parasitoids are discussed.

### MATERIALS AND METHODS

**Cultures.** Uninfected colonies of *Muscidifurax raptor* and colonies infected with the undescribed microsporidium were established from dairy farms in central New York (strain "MR87" of ref. 5). Infected female parasitoids were allowed to oviposit in the puparia of *Musca domestica* L. and dissected at various times post-exposure. Infected eggs, larvae and adults of *M. raptor* were obtained in this manner.

**Tissue preparation.** Development of the pathogen was followed and characterized at the light level with Giemsa-stained smears according to Hazard et al. [8]. For electron microscopy, eggs and tissue from larvae and adults were fixed for 2.5 h at

room temperature in 2.5% (v/v) glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 0.1% CaCl<sub>2</sub> and postfixed in 1% aqueous OsO<sub>4</sub> (w/v). These tissues were dehydrated through an ascending ethanol and acetone series and embedded in Epon-Araldite. Thin sections were post-stained with methanolic uranyl acetate followed by lead citrate.

### RESULTS

**Development in larvae and adults.** This part of the life cycle involved the concurrent production of two kinds of diplokaryotic spores. One spore was distinguished by a short polar filament and was presumably involved in transmission to new host cells (autoinfection). The other was distinguished by a longer polar filament and presumably involved in transmission to a new host individual. Presporulation stages for the two developmental sequences could not be distinguished at either the light or EM level. Therefore, a general description of the presporulation stages is given without an attempt to assign them to a particular sequence.

**Presporulation development.** Uninucleate, binucleate and diplokaryotic cells were present throughout development of the pathogen in larvae and adults. Uninucleate schizonts stained intensely and were generally of two sizes. They were spherical in shape with a large centrally located nucleus (Fig. 1, 2). Stages that appeared to be binucleate (not diplokaryotic) were uncommon (Fig. 3, 4). Diplokaryotic meronts were the predominant forms throughout development with considerable variation in size and shape. The earliest recognizable meronts were spherical to elongate with densely stained cytoplasm and compact nuclei (Fig. 5, 19). Multiplication was primarily by binary division (Fig. 6, 7) but octonucleate plasmodia that divided by multiple fission were observed (Fig. 8).

**Sporogony.** Transitional forms were larger than meronts, slightly elliptical to fusiform in shape with a lightly stained cytoplasm (Fig. 9, 10, 20). The plasmalemma of these forms became distinctly thicker during the transition and was accom-

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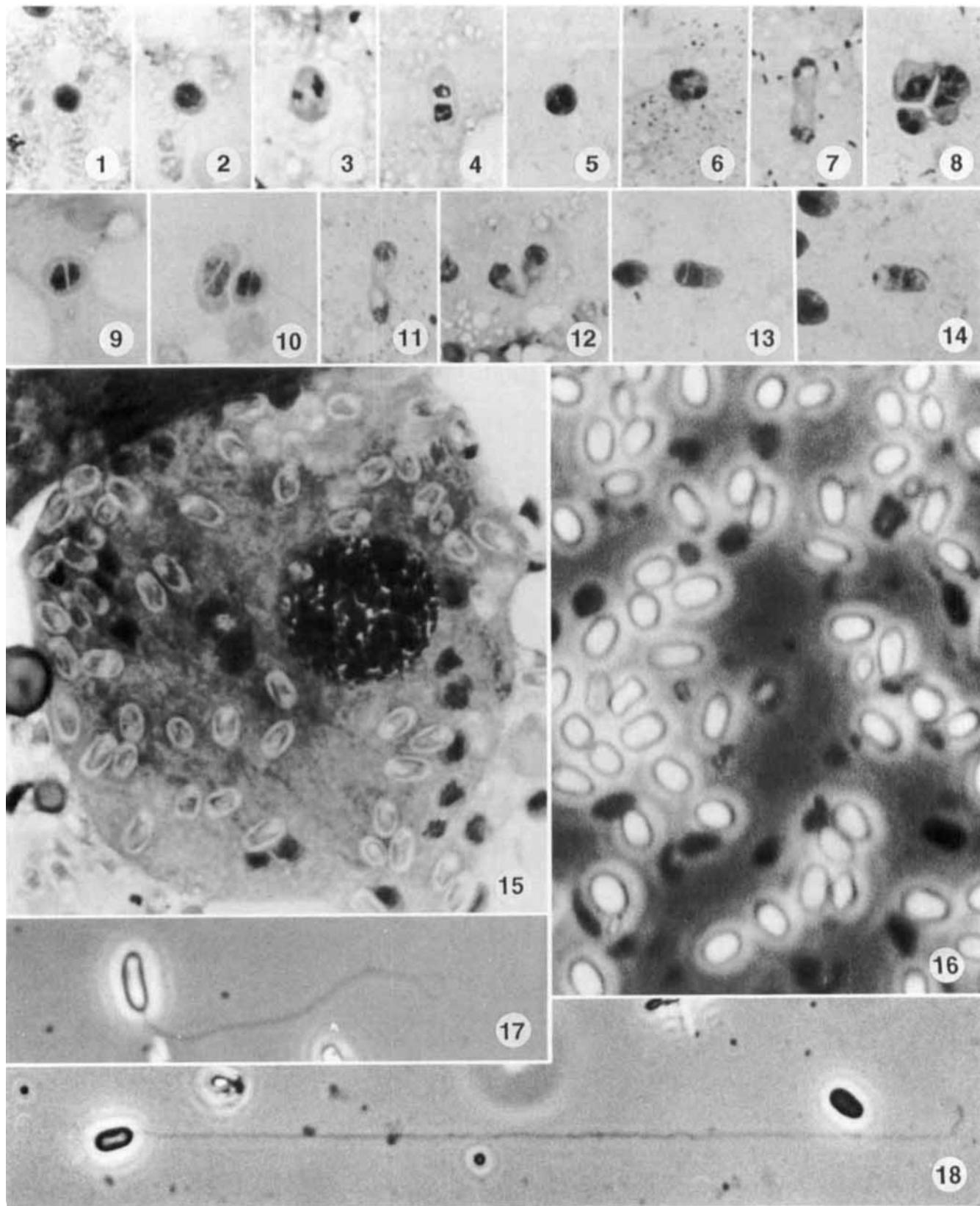


Fig. 1-18. Photomicrographs of stages of *N. muscidifuracis* in larvae and adults of *M. raptor*. Giemsa-stained and  $\times 2,000$  excepting Fig. 16-18, which are fresh with magnification as indicated. 1. Small uninucleate schizont. 2. Large uninucleate schizont. 3, 4. Binucleate stages. 5. Quadrinucleate meront. 6. Diplokaryotic meront dividing. 7. Cytokinesis of meront. 8. Dividing octonucleate sporogonial plasmodium. 9. Diplokaryotic sporont. 10. Fusiform sporont. 11. Dividing sporont. 12. Cytokinesis of sporont. 13. Early diplokaryotic sporoblast. 14. Immature diplokaryotic spore. 15. Diplokaryotic spores in cytoplasm of host cell. 16. Diplokaryotic spores released from adult *M. domestica*.  $\times 2,000$ . 17. Germinated diplokaryotic spore with short polar tube.  $\times 1,600$ . 18. Germinated diplokaryotic spore with long polar tube.  $\times 1,600$ .

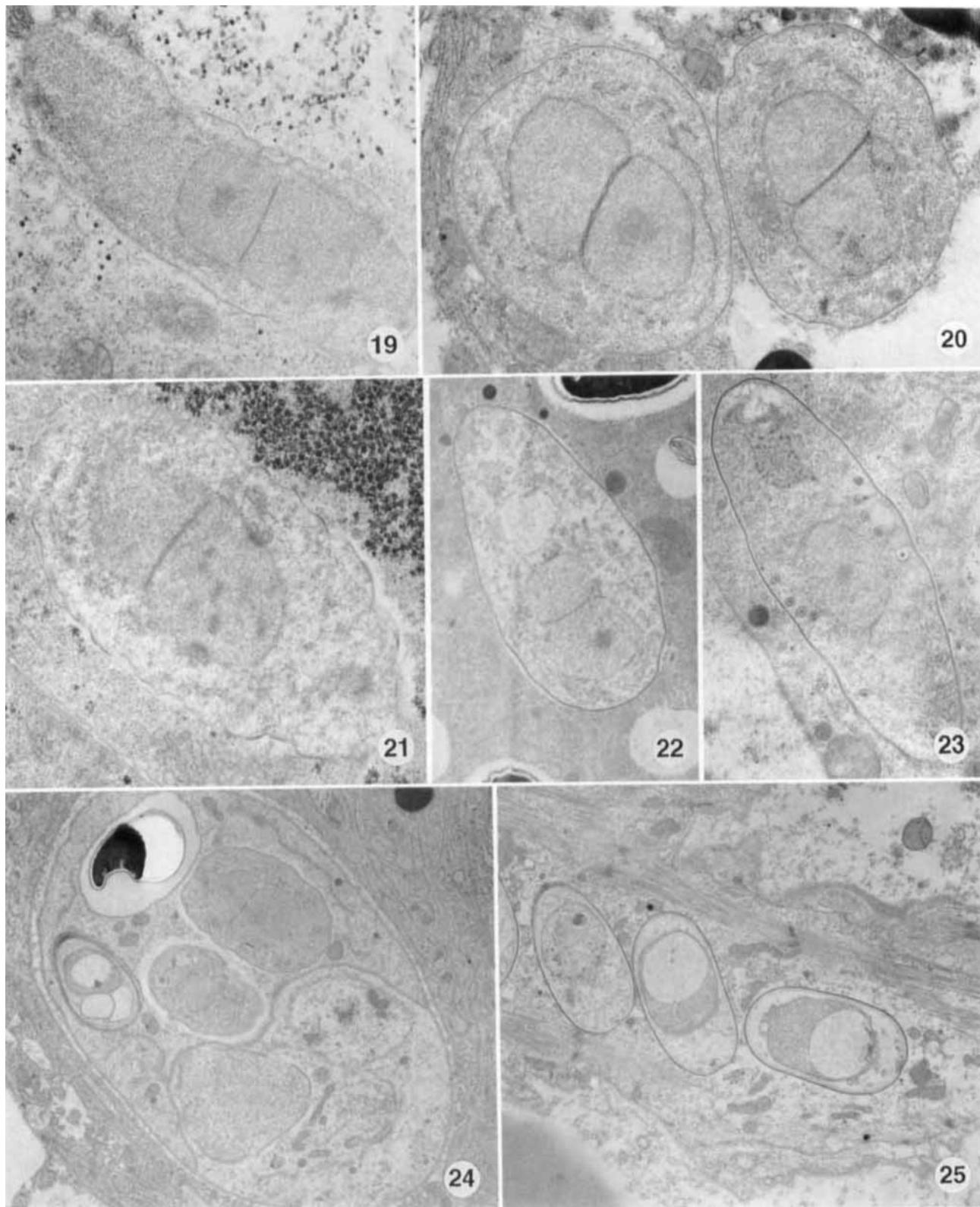


Fig. 19-25. Electron micrographs of selected stages of *N. muscidifuracis* in larvae and adults of *M. raptor*. 19. Elongate diplokaryotic meront.  $\times 13,000$ . 20. Early diplokaryotic sporont.  $\times 15,000$ . 21. Dividing diplokaryotic sporont.  $\times 12,000$ . 22. Early diplokaryotic sporoblast.  $\times 12,000$ . 23. Late sporoblast.  $\times 15,000$ . 24. Infected host cell with sporont, spore and germinated spore.  $\times 8,000$ . 25. Intracellular germinated spores.  $\times 10,000$ .

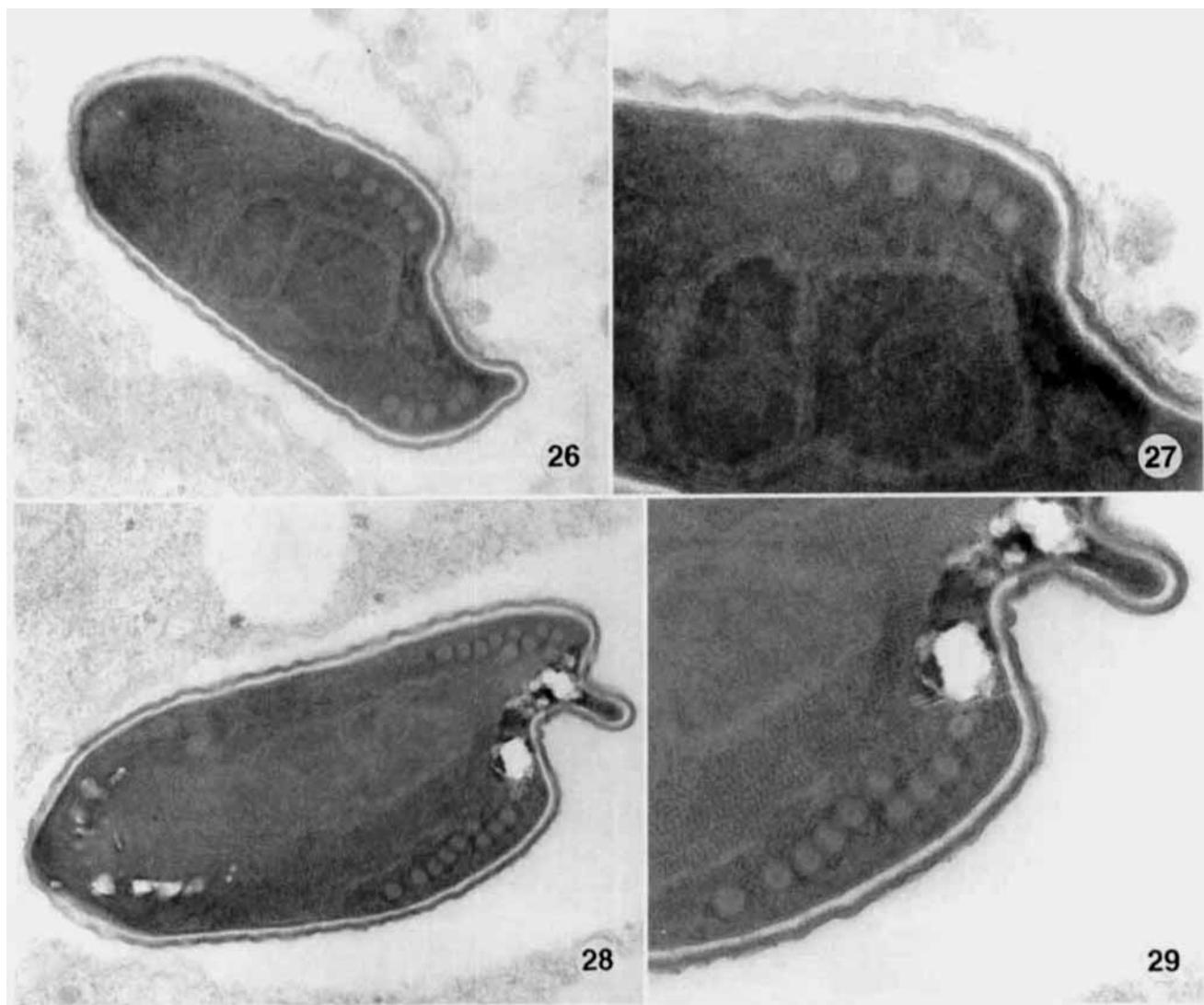


Fig. 26-29. Electron micrographs of diplokaryotic spores of *N. muscidifuracis* in larvae and adults of *M. raptor*. 26. Spore with short polar filament.  $\times 30,000$ . 27. Details of spore wall and coils of the polar filament of spore in Fig. 26.  $\times 60,000$ . 28. Spore with long polar filament.  $\times 30,000$ . 29. Details of spore wall and coils of the polar filament of spore in Fig. 28.  $\times 60,000$ .

panied by a reduction in the number of free ribosomes in the cytoplasm (Fig. 20). Sporogony was disporoblastic (Fig. 11, 12, 21). Early sporoblasts were elongate with the diplokaryon positioned at one end of the cell (Fig. 13, 22). Later sporoblasts had a small chromophilic granule at one pole (Fig. 14) which perhaps represented the Golgi body involved in the production of the polar filament (Fig. 23). Certain host cells were found to contain sporonts, spores and germinated spores (Fig. 24, 25).

**Spores.** Spores were generally ovoidal (Fig. 15, 16) and measured  $5.4 \pm .5 \times 3.0 \pm .2 \mu\text{m}$  ( $n = 32$ ). Two kinds of diplokaryotic spores were distinguished at the ultrastructural level. One spore had a short polar filament making approximately five turns (Fig. 17, 26, 27). The other spore had a longer polar filament with approximately 9–10 turns (Fig. 18, 28, 29). Both spores had an isofilar polar filament and laminate polaroplasts. Germinated spores were commonly found in all areas of the material examined (Fig. 17, 18, 24, 25).

**Development in eggs.** Transovarially infected eggs from *M. raptor* contained vegetative stages and spores. The chorion of the egg proved to be a barrier to fixation and embedding media.

This resulted in poor ultrastructural details of the developmental stages but some useful information was obtained.

**Presporulation development.** Development within the egg was restricted to a region of vacuolated cytoplasm between the blastoderm and the yolk sac (Fig. 30). The predominant forms were diplokaryotic meronts that divided by binary fission (Fig. 35) but some stages appeared to be uninucleate (Fig. 31, 34).

**Sporogony.** Fusiform diplokaryotic stages were presumably the final products of merogony (Fig. 36) and the initial stages of sporogony or sporonts. Sporogony presumed disporoblastic.

**Spores.** One kind of diplokaryotic spore was observed in the eggs (Fig. 32, 33, 37, 38). Fresh spores were elongate ovoidal and measured  $6.0 \pm .8 \times 3.2 \pm .3 \mu\text{m}$  ( $n = 32$ ). The polar filament was isofilar and made approximately 15–16 turns about the posterior vacuole (Fig. 33). The spore wall was distinctive in lacking an obvious exospore (Fig. 32). This could be because the exospore merges imperceptibly into the surrounding cytoplasm or attributable to poor preservation that also precluded a more detailed description of the spore structure. Germinated spores were not observed in the egg.

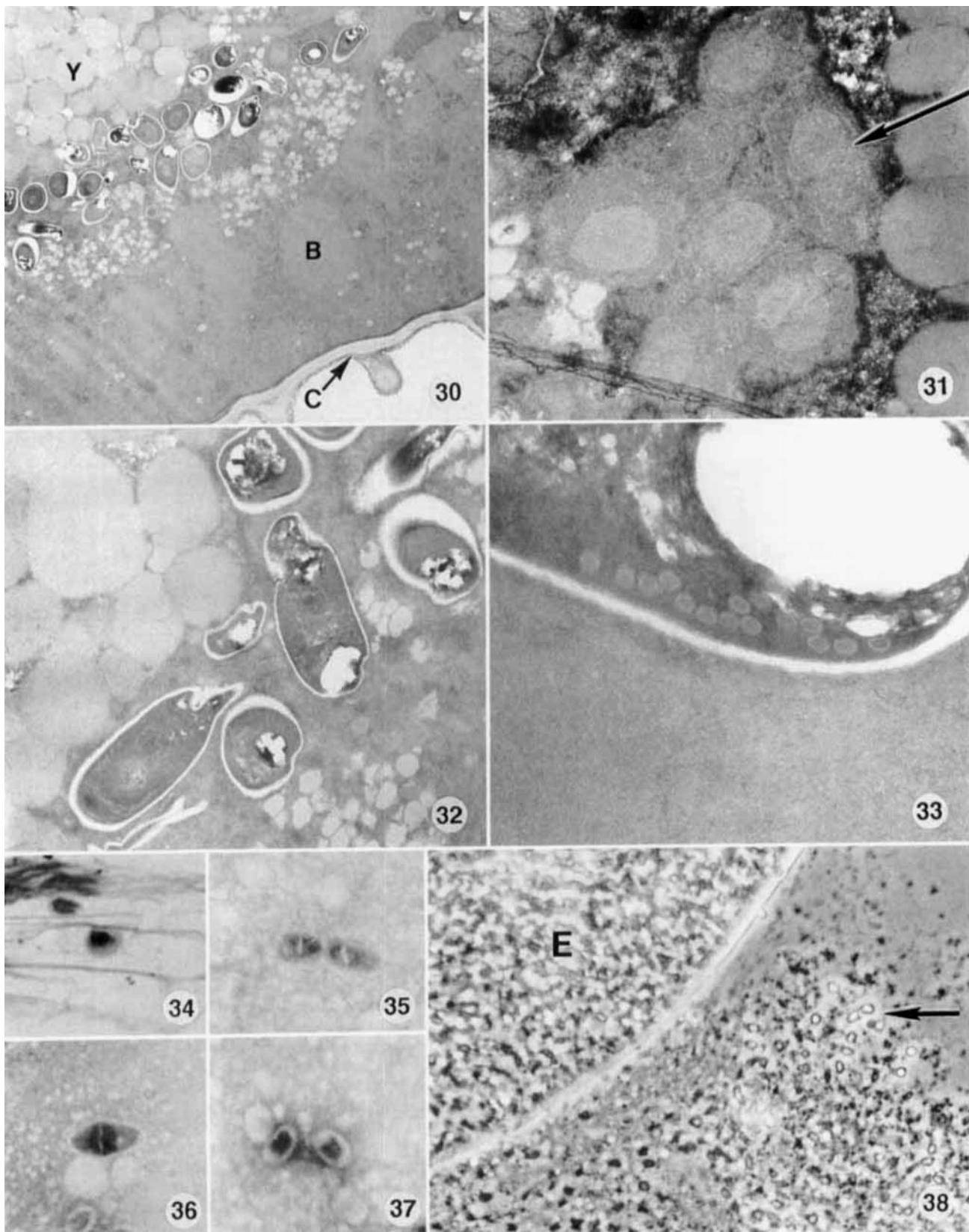


Fig. 30-38. Electron and photomicrographs of developmental stages and spores of *N. muscidifuracis* in eggs of *M. raptor*. Fig. 34-37 are Giemsa-stained and  $\times 2,000$ , Fig. 38 is fresh and  $\times 850$ . 30. Spores and developmental stages in the vacuolated cytoplasm of the egg between the blastoderm (B) and yolk (Y). Chorion (C).  $\times 3,500$ . 31. Group of vegetative stages, one appearing to be diplokaryotic (arrow).  $\times 15,000$ . 32. Group of diplokaryotic spores.  $\times 12,000$ . 33. Details of the spore wall and coils of the polar filament of diplokaryotic spores.  $\times 50,000$ . 34. Uninucleate schizont. 35. Dividing diplokaryotic meront. 36. Fusiform diplokaryotic stage. 37. Diplokaryotic spores. 38. Diplokaryotic spores (arrow) released from the egg. E, egg.

## DISCUSSION

The taxonomic features of this new microsporidium are in general agreement with those for the genus *Nosema* Naegeli, 1857. Specifically, these are: presence of uninucleate cells, diplokaryotic meronts that divide by binary fission or multiple division of paucinucleate plasmodia, disporoblastic sporogony, different kinds of diplokaryotic spores distinguished by length of polar filament, and all stages developing in direct contact with the host cell cytoplasm [14]. We therefore assign this new microsporidium the species name *Nosema muscidifuracis* n. sp., the specific name being after its host *Muscidifurax raptor*.

This and other studies [5, 6, 18] have indicated an intimate relationship between *N. muscidifuracis* and *M. raptor*. Systemic infections in larval, pupal and adult parasitoids produce large numbers of spores but adults survive to reproduce. There are profound effects on the fecundity and longevity of adult *M. raptor* and these adversely affect the reproductive success of infected parasitoids [5, 18]. The high prevalence levels of *N. muscidifuracis* found in natural populations of *M. raptor* [18] can only be explained by efficient mechanisms of transmissions. Transovarial transmission is 100% efficient and is the main mechanism for maintenance of this pathogen within *M. raptor* populations [18]. But because of the detrimental effects of infection, transovarial transmission alone cannot maintain *N. muscidifuracis* in natural populations of *M. raptor* [4]. Periodic horizontal transmission events must occur for *N. muscidifuracis* to enter healthy individuals of *M. raptor*. Transmission from the muscoid host to the parasitoid is not possible because the former is refractory to infection by *N. muscidifuracis* [6, 18]. Cannibalism of infected *M. raptor* appears to be the main pathway for horizontal transmission of *N. muscidifuracis* to both larvae and adults [6]. Cannibalism as a result of superparasitism provides the primary mechanism for the horizontal infection of larval parasitoids. Laboratory studies have found that when host-parasitoid ratios are high, *M. raptor* does not superparasitize hosts. When hosts are scarce, superparasitism occurs [12, 16]. Superparasitism also occurs in the field and increases as the overall rate of parasitism increases; moreover, it is present at substantial levels when the rate of parasitism is low (< 25%). At high rates of parasitism (> 75%) most parasitoids that emerge do so from parasitized hosts [11, 13]. When superparasitism occurs, only one or no adult parasitoids emerge. This is regulated by the aggressive behavior of some young parasitoid larvae. The newly hatched larva searches the host and consumes eggs and less aggressive larvae [12, 17]. Larval infection occurs during superparasitism when healthy and infected eggs are deposited in the same host. Cannibalism of infected eggs and/or larvae results in the parasitoid receiving an infective dose of spores to initiate a new line of infected *M. raptor*.

Adult parasitoids feed by wounding the pupal host with the ovipositor and then ingesting the released fluids. Adult wounding of hosts parasitized by *M. raptor* results in cannibalism of the infected larva and cause the adult parasitoid to consume an infectious dose of spores. Presumably, these infected adults can transmit the pathogen to progeny to initiate a new generation of infected individuals. Alternative mechanisms for the infection of adult parasitoids in nature are unknown; however, as adult parasitoids readily become infected by feeding on honey contaminated with spores [6], other pathways seem likely.

Hazard and Weiser [7] made the important discovery of "two distinctly different types of spores . . ." in the life cycle of species they assigned to the genus *Thelohania*. Since then, the word "types" has acquired a rather specific meaning; it signifies kinds of spores differing as distinctly as the typical ovo-cylindrical, diplokaryotic spores of *Nosema* and the typically pyriform, uni-

nucleate spores of *Thelohania*. Iwano and Ishihara [10] used "types" in a more restricted sense by applying it to sub-groups of spores within a "*Nosema-like*" "type." It is clear that there is a problem with distinguishing different kinds of spores and finding satisfactory names. Sprague et al. [14] warned against the confusion associated with the way "types" is used. There is an urgent need for appropriate and unambiguous terminology for the many and various categories of spores that occur in microsporidia. To avoid further confusion, we have used the unambiguous term "classes" to distinguish the different diplokaryotic spores found in *N. muscidifuracis*.

The first clear evidence for different "types" of diplokaryotic spores in a *Nosema* sp. was presented by Iwano & Ishihara [9] in cell cultures and later verified for the type host *N. bombycis* Naegeli, 1857 [10]. The first diplokaryotic spore (termed "FC type") germinated immediately after formation and was morphologically distinctive from a second diplokaryotic spore formed later in development. This second spore (termed "MC type") did not germinate within the cells and had a longer polar filament and a thicker endospore [10]. Although there was a clear separation in time between their formation, both spore classes could be found occurring together. The authors speculated that the first spore was responsible for spread of the parasite within the host (autoinfection) while the other spore was for transmission to a new host individual. The two classes of diplokaryotic spores observed for *N. muscidifuracis* in larvae and adults are similar to those described for *N. bombycis* in both morphology and function. Germinated spores within tissues suggest that one spore was responsible for autoinfection while the other likely plays a role in transmission from host to host. Both spores exhibited morphological differences expressed in the length of the polar filament. While the two spores of *N. bombycis* were, for the most part, formed sequentially, those in *N. muscidifuracis* were produced concurrently. This difference could be attributable to differences in the biology of the lepidopterous and parasitoid hosts with the availability of both classes of spores on a continuous basis necessary for transmission within and between hosts.

The presence of spores within the eggs of hymenopterous parasitoids has been reported for microsporidia that are specific for the parasitoid [3] and for species found in both the parasitoid and the lepidopterous host [1]. These spores were believed to be involved in transovarial transmission to progeny of infected females and there was no evidence to suggest that these spores were of a different class. For *N. muscidifuracis*, ultrastructural details of spores in the egg showed that they were of a different class than either of the ones found in larvae and adults. Because vegetative stages were also found in eggs and could initiate the infection at eclosion, it is conceivable that spores in the egg do not play a role in transovarial transmission. The sole or alternative function of these spores may be for horizontal transmission to a new generation of hosts when eggs are cannibalized during superparasitism. Regardless, it is unclear whether the presence of a third class of diplokaryotic spore in *N. muscidifuracis* is unique to this species or has gone undetected in other members of *Nosema*.

With information on the development and host-pathogen relationships of three species of microsporidia specific for their parasitoid hosts, some common themes have emerged and others are implied. *Nosema muscidifuracis*, *N. campoletidis* and *N. cardiochilis* exhibited chronic effects on the parasitoid and were transovarially transmitted to progeny [3]. While horizontal transmission appears to be an important aspect of pathogen maintenance for *N. muscidifuracis*, it has not been demonstrated for *N. campoletidis* or *N. cardiochilis*. Infections caused by *N. campoletidis* in larvae, pupae and adults were systemic and heavy but no obvious pathologic effect on the parasitoid host

was detected [3]. Because of this, it was suggested that transovarial transmission was the only mechanism necessary for maintenance of *N. campoletidis* in natural populations of the parasitoid. In light of our findings, the existence of horizontal transmission pathways for *N. campoletidis* and *N. cardiochilis* seem highly probable. It is unlikely that maintenance of these species in nature could occur by transovarial transmission alone.

For each species, diplokaryotic spores were produced in all life cycle stages of the parasitoid hosts (larvae, pupae, adults and eggs) by a typical *Nosema*-type development. Ultrastructural information is not available for either *Nosema campoletidis* or *N. cardiochilis* to determine whether different classes of diplokaryotic spores are present as found for *N. muscidifuracis*. Documentation of the different classes of diplokaryotic spores for these species, as well as horizontal transmission testing, would verify the closely related nature of this group of pathogens in parasitic Hymenoptera. The susceptibility and specificity of these species for their respective parasitoid host would further demonstrate that each of these pathogens is a distinct species.

Ribosomal DNA sequence analysis data has proven to be a useful tool for species identification as well as understanding phylogenetic relationships for microsporidia and should be a part of species descriptions [15]. Spores of *N. muscidifuracis* will be submitted for rDNA analysis and it is suggested that all authors of new species submit material for analysis to researchers active in this area.

#### TAXONOMIC SUMMARY

##### *Nosema muscidifuracis* n. sp.

*Microsporidium* sp. Geden, Smith, Long & Rutz, 1992, *Entomol. Soc. Am.*, **85**:179-187. Zchori-Fein, Geden & Rutz, 1992, *J. Invertebr. Pathol.*, **60**:292-298.

**Type host.** *Muscidifurax raptor* Girault & Saunders (Hymenoptera, Pteromalidae), a parasitoid of *Musca domestica* L. (Diptera, Muscidae).

**Transmission.** Per os, transovarial and autoinfection. Transovarial within the egg and per os when infected egg ingested by healthy parasitoid larvae or when healthy adults ingest spores from larvae. Spread in larvae and adults by germination of spores within host cells (autoinfection).

**Site of infection.** Midgut, Malpighian tubules, ovaries (including oocytes) and fat body of adults; vegetative stages and spores within eggs [18]. Systemic in larvae.

**Interface.** Parasites of all stages in direct contact with host cell cytoplasm.

**Other parasite-host cell relations.** Parasites usually in cytoplasm and randomly dispersed. Development within the egg was restricted to a region of vacuolated cytoplasm between the blastoderm and the yolk sac. Heavily infected cells may become distended.

**The haplophase.** Uninucleate and binucleate cells were present in eggs, larvae and adults.

**Merogony.** Diplokaryotic stages were the predominant form throughout development with considerable variation in size and shape. Multiplication was primarily by binary division but octonucleate plasmodia that divided by multiple fission were observed.

**Merogony-sporogony transition.** Merogony ends with fusiform diplokaryotic cells.

**Sporogony.** Transitional forms were larger than meronts, slightly elliptical to fusiform in shape. Sporogony was disporoblastic.

**Spore.** Spores from larvae and pupae measured  $5.4 \pm .5 \times 3.0 \pm .2 \mu\text{m}$  ( $n = 32$ ); spores from eggs measured  $6.0 \pm .8 \times 3.2 \pm .3 \mu\text{m}$  ( $n = 32$ ). Three morphological classes, all ovoidal

in shape and diplokaryotic. Spores in larvae and adults were of two classes distinguishable only at the ultrastructural level. One spore had a short polar filament making approximately five turns. The other spore had a longer polar filament with approximately 9–10 turns. Both spore types had isofilar polar filaments and laminate polaroplasts. Diplokaryotic spores of only one class were observed in the eggs. The polar filament was isofilar and made approximately 15–16 turns about the posterior vacuole. The spore wall was distinctive in lacking an obvious exospore.

**Type locality.** "Dairy farms in central New York."

**Deposition of type specimens.** Type slides have been deposited at the International Protozoan Type Slide Collection, Smithsonian Institution (USNM Nos. 43224, 43225, and 47725). Spores of *N. muscidifuracis* will be submitted for rDNA analysis to Dr. Charles R. Vossbrinck, Office of Agricultural Entomology, 147 PABL, 1201 West Gregory, Urbana, Illinois 61801.

**Etymology.** Specific name after *Muscidifurax raptor* because of the intimate host-pathogen relationship.

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#### LITERATURE CITED

1. Blunck, H., Krieg, A. & Scholyteck, E. 1959. Weitere Untersuchungen über die Mikrosporidien von Pieriden und Parasiten und Hyperparasiten. *Z. Pflanzenkrankheiten*, **66**:129–142.
2. Brooks, W. M. 1993. Host-parasitoid-pathogen interactions. In: Beckage, N. E., Thompson, S. N. & Federici, B. A. (ed.), *Parasites and Pathogens of Insects*, Vol. 2: Pathogens. Academic Press, New York. Pp. 231–272.
3. Brooks, W. M. & Cranford, J. D. 1972. Microsporidioses of the hymenopterous parasites, *Campoletis sonorensis* and *Cardiochiles nigriceps*, larval parasites of *Heliothis* species. *J. Invertebr. Pathol.*, **20**: 77–94.
4. Fine, P. E. M. 1975. Vectors and vertical transmission: an epidemiologic perspective. *Ann. N.Y. Acad. Sci.*, **266**:173–194.
5. Geden, C. J., Smith, L., Long, S. J. & Rutz, D. A. 1992. Rapid deterioration of searching behavior, host destruction, and fecundity of *Muscidifurax raptor* (Hymenoptera: Pteromalidae) in culture. *Annals, Entomol. Soc. Am.*, **85**:179–187.
6. Geden, C. J., Long, S. J., Rutz, D. A. & Bechel, J. J. 1994. *Nosema* disease of the muscid fly parasitoid of *Muscidifurax raptor* (Hymenoptera: Pteromalidae): prevalence, patterns of transmission, and treatment. *Biocontrol Science and Technology*. (in press)
7. Hazard, E. I. & Weiser, J. 1968. Spores of *Thelohania* in adult female *Anopheles*: development and transovarial transmission, and re-descriptions *T. legeri* Hesse and *T. obesa* Kudo. *J. Protozool.*, **15**:817–823.
8. Hazard, E. I., Ellis, E. A. & Joslyn, D. J. 1981. Identification of microsporidia. In: Burges, H. D. (ed.), *Microbial Control of Pests and Plant Diseases 1970–1980*. Academic Press, New York. Pp. 163–182.
9. Iwano, H. & Ishihara, R. 1989. Intracellular germination of spores of *Nosema* sp. immediately after their formation. *J. Invertebr. Pathol.*, **54**:125–127.
10. Iwano, H. & Ishihara, R. 1991. Dimorphism of spores of *Nosema* spp. in cultured cell. *J. Invertebr. Pathol.*, **57**:211–219.
11. Petersen, J. J. 1986. Evaluating the impact of pteromalid parasites on filth fly populations associated with confined livestock installations. *Misc. Publ. Entomol. Soc. Amer.*, **61**:52–56.
12. Podoler, H. & Mendel, Z. 1977. Analysis of solitariness in a parasite-host system (*Muscidifurax raptor*, Hymenoptera: Pteromalidae—*Ceratitis capitata*, Diptera: Tephritidae). *Ecol. Entomol.*, **2**:153–160.
13. Propp, G. D. & Morgan, P. B. 1985. Effect of host distribution on parasitoidism of house-fly (Diptera: Muscidae) pupae by *Spalangia*

- spp. and *Muscidifurax raptor* (Hymenoptera: Pteromalidae). *Can. Entomol.*, **117**:515–524.
14. Sprague, V., Becnel, J. J. & Hazard, E. I. 1992. Taxonomy of phylum Microspora. *Crit. Rev. Microbiol.*, **18**:285–395.
  15. Vossbrinck, C. R., Baker, M. D., Didier, E. S., Debrunner-Vossbrinck, B. A. & Shadduck, J. A. 1993. Ribosomal DNA sequences of *Encephalitozoon hellem* and *Encephalitozoon cuniculi*: species identification and phylogenetic construction. *J. Euk. Microbiol.*, **40**:354–362.
  16. Wylie, H. G. 1971. Ovipositional restraint of *Muscidifurax raptor* (Hymenoptera: Pteromalidae) on parasitized housefly pupae. *Can. Entomol.*, **103**:1537–1544.
  17. Wylie, H. G. 1972. Larval competition among three hymenopterous parasite species on multiparasitized housefly (Diptera) pupae. *Can. Entomol.*, **104**:1181–1190.
  18. Zchori-Fein, E., Geden, C. J. & Rutz, D. A. 1992. Microsporidioses of *Muscidifurax raptor* (Hymenoptera: Pteromalidae) and other pteromalid parasitoids of muscoid flies. *J. Invertebr. Pathol.*, **60**:292–298.

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## Divisional Morphogenesis in *Amphisiliellides illuvialis* N. Sp., *Paramamphisiella caudata* (Hemberger) and *Hemiamphisiella terricola* Foissner, and Redefinition of the Amphisiliellidae (Ciliophora, Hypotrichida)

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**ABSTRACT.** Classification of hypotrich ciliates is bewildering, possibly due to many unrecognized convergencies and the lack of detailed ontogenetic data in most species. A puzzling case are hypotrichs which have an obliquely extending “median cirral row” on the ventral surface between the right and left marginal cirral row. Such species are often assigned to the poorly defined family Amphisiliellidae. However, we show by a comparative analysis of the divisional morphogenesis of three amphisiliellid morphotypes and by a reevaluation of literature data that a median cirral row can be formed by at least four non-homologous processes. These data are used to define the “amphisiliellid median cirral row” (a row containing all or most cirri from at least two rightmost anlagen, arranged one behind the other during cytokinesis), to redefine the Amphisiliellidae (Euhypotrichina with an amphisiliellid median cirral row of which the anterior segment is formed by cirri of the rightmost ventral anlage and the posterior segment by cirri of the second ventral anlage from right; a middle segment may be formed by neighboring anlagen), and to improve the diagnoses of the seven genera assigned by us to this family. Attempts to reconstruct the evolution within the Amphisiliellidae failed, in spite of the detailed morphological and ontogenetic data available. We thus conclude that such data, although highly valuable, are insufficient to light up the supergeneric phylogeny of hypotrich ciliates. For this, molecular markers and investigations of the morphogenetic processes at electron microscopic level appear indispensable.

**Supplementary key words.** *Amphisiliella*, *Gastrostyla*, *Lamnostyla*, *Paragastrostyla*, phylogeny, *Pseudouroleptus*, *Tachysoma*, taxonomy.

MANY hypotrichs have an obliquely extending cirral row on the ventral surface between the right and left marginal row. Using ontogenetic data, Borror & Wicklow recognized that this “median cirral row” is of different origin in several species, despite the superficial interphase similarity of such hypotrichs (Borror, A. C. & Wicklow, B. J. 1982. Non-homology of median rows in hypotrichs with only three longitudinal rows of cirri. *J. Protozool.*, **29**:285A). However, data were too scarce and bewildering for a conclusive interpretation, i.e. for establishing higher taxa. In spite of this, Small & Lynn [23], having hardly more data, installed the new family Amphisiliellidae. Their definition “at least 1 or more frontoventral cirral files extends well past mid-ventrum” applies to almost any hypotrich taxon. Furthermore, their new family is a junior synonym of Jankowski’s [21] Amphisiliellidae and includes genera, e.g. *Kahliella* and *Onychodromus*, which clearly belong to other families.

A wealth of data on hypotrichs with a median cirral row has become available since Borror and Wicklow’s (1982 abstract) classic formulation of the problem [3, 9, 27, 32]. These findings and those presented in our paper provide a firm basis for a comprehensive redefinition of the Amphisiliellidae.

## MATERIALS AND METHODS

*Amphisiliellides illuvialis* n. sp. was discovered 7 December 1991 in a litter sample from a disused pigpen in village of Schrötten, Deutsch Goritz, Austria. A second population (Fig. 21–24) was found near Eching (Bavaria) in a tiny track puddle, which was heavily polluted by sewage from an activated sludge plant. This population was very similar to the type material and was not studied in detail.

*Paramamphisiella caudata* (Hemberger, 1985) was found on the Shimba Hills near Mombasa (Kenya, Africa) in July 1985. It was isolated from a sandy soil collected near a small river in the vicinity of the Sheldrick waterfalls.

*Hemiamphisiella terricola* Foissner, 1988 was found in February 1987 in mosses covering the soil of an autochthonous pine forest (*Callitris* sp.) near Adelaide (Tailem Bend; Australia).

All species were isolated from raw cultures set up with the non-flooded Petri dish method [13]. Pure cultures were started with several individuals in lettuce-medium enriched with some squeezed wheat grains. Body shapes of living specimens were drawn from slides without coverslip. Details were studied on slightly to heavily squeezed individuals using an oil immersion objective and interference contrast optics. The infraciliature was revealed by Foissner’s [16] protargol protocol. Drawings were made with the help of a camera lucida.

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