JOURNAL OF INVERTEBRATE PATHOLOGY 9, 515–522 (1967)

# The Biology and Life History of *Nosema tracheophila* sp. n. (Protozoa:Cnidospora:Microsporidea) Found in *Coccinella septempunctata* Linnaeus (Coleoptera:Coccinellidae)

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*Accepted November 11, 1966*

The life cycle of a new microsporidian parasite, *Nosema tracheophila* sp. n. is described in adults of the coccinellid beetle, *Coccinella septempunctata* Linnaeus. The spore is ovoid in shape (4.0–5.3 × 2.2–3.1 μ); upon proper stimulation it extrudes a long polar filament (89–178 μ) from which a binucleate sporoplasm (2.8 × 1.5 μ) emerges. The sporoplasm divides, forming short chains of mononucleated schizonts (4.0 × 1.3 μ); the individual mononucleated schizonts then undergo nuclear division forming two, four, then eight nucleate meronts or daughter schizonts (3.1–4.4 × 1.8–2.2 μ). A diplokaryon stage was not observed; however, since the sporonts (4.2 × 3.1 μ) were observed and since they each give rise to one spore, this microsporidian is placed in the genus *Nosema*. The schizogonic stages are found primarily in hemocytes, while the sporogonic stages are in the connective tissue.

## INTRODUCTION

Microsporidia parasitizing species of Coleoptera were first described by Hesse (1905). Twelve species of Microsporidia have been reported to infect Coleoptera. Of these 12 species, 9 have been placed in the genus *Nosema;* only one *Nosema* has been reported in the host family Coccinellidae. Table 1 presents the species of *Nosema* reported infecting coleopteran hosts, their principal morphological characteristics, and sites of infection. The life stages of the Microsporidia investigated in this study were observed in adult specimens of *Coccinella septempunctata* Linnaeus, an aphidophagous species.

## METHODS AND MATERIALS

Adult *C. septempunctata* were reared using the pea aphid, *Macrosiphum pisi* (Kaltenbach) as a food source; the aphid was grown on living broad bean plants, *Vicia faba* Linnaeus.

Adult beetles were starved for 24 hr prior to oral inoculation, then fed *ad libum* a mixture of honey and spores. Infection was also accomplished by feeding the bodies of dead infected beetles to starved beetles.

Because infected hosts do not exhibit external signs of the disease, examination of tissue was necessary. Several methods were employed to detect the presence of developmental stages of the parasite in tissues. Wet mounts were prepared and examined using phase-contrast microscopy. Tissue smears were fixed with absolute methanol or 1% phosphate buffered osmium tetroxide and stained with 1 : 15 aqueous dilution of Giemsa.[1] Tissues to be

[1] Giemsa Stain Cat. No. So-G-28. Fischer Scientific Co., Chicago, Illinois.

TABLE 1

A COMPARISON OF *Nosema tracheophila* SP. N. WITH *Nosema* SPECIES REPORTED TO PARASITIZE COLEOPTERAN HOSTS

| Species | Spore shape | Spore size (length × width, μ) | Polar filament length (μ) | Principal tissue infected | Host species | Host family |
|---|---|---|---|---|---|---|
| *N. longifilum* Hesse 1905 | Oval | 4–5 × 3 | 85–90 | Fat body | *Otiorrhynchus fuscipes* | Curculionidae |
| *N. otiorrhynchi* Weiser 1951 | Cylindrical | 3.8–4 × 1.8–2<br>6–8 × 2 (rare) | Not observed | Malp. tubule epithelium | *Otiorrhynchus ligustici* | Curculionidae |
| *N. whitei* Weiser 1953 | Broadly oval | 3.5–5 × 2–2.5 | >160 | Fat body | *Tribolium castaneum* | Tenebrionidae |
| *N. melolonthae* Krieg 1955<br>Huger 1964 | Oval | 4–4.5 × 2.5–3 | 125–242 | Fat body | *Melolontha melolontha* | Scarabaeidae |
| *N. gibbsi* Gibbs 1956<br>Weiser 1961a | Oval | 3–4 × 2 | 100 | Fat body | *Gonocephalum arenarium* | Tenebrionidae |
| *N. typographi* Weiser 1959a | Oval | 2–3.5 × 3.6–5.3 | 63 | Fat body | *Ips typographus* | Scolytidae |
| *N. hippodamiae* Lipa and Steinhaus 1959 | Oval | 3.5–5.4 × 2.2–2.7 | Not observed | Midgut and fat body | *Hippodamia convergens* | Coccinellidae |
| *N. curvidentis* Weiser 1961b | Oval | 2.5–3.6 × 1.5–2 | Not observed | Fat body | *Pityokteines curvidens* | Scolytidae |
| *N. phyllotretae* Weiser 1961a | Oval | 4.2–6 × 2–3 | Not observed | Fat body | *Phyllotreta atra*<br>*P. undulata* | Chrysomelidae |
| *N. tracheophila* sp. n. | Oval | 4–5.3 × 2.2–3.1 | 89–178 | Tracheal epith. blood cells & conn. tissue | *Coccinella septempunctata* | Coccinellidae |

examined in section were first fixed with Bouin-Duboscq fixative and treated with chitinase according to Carlisle (1960) before embedding in Paraplast.[2] Sections were stained with Azan trichrome modified by Hubschman (1962) or Heidenhain's hematoxylin.

## Results

*Sites of Infection.* The microsporidia found in *C. septempunctata* infect three host tissues: hemocytes, tracheal epithelium, and connective tissue. The schizont forms were observed in circulating hemocytes. The spores were observed in tracheal epithelium and in connective tissue surrounding fat body, Malpighian tubules, gonads, and midgut musculature.

*Parasite Stages Observed from Smeared Material.* The polar filament is readily extruded from the spores in aqueous smears under pressure of a cover slip, particularly when the preparation begins to dry. The polar filament ranged in length from 89 to 178 $\mu$, averaging 121 $\mu$ (Fig. 2).

The binucleate planont (the emerged sporoplasm) was observed at the distal end of the extruded polar filament, and is shaped as a drop of fluid measuring approximately 2.8 $\times$ 1.5 $\mu$ (Fig. 7a).

*Stages Observed in Vitro and in Vivo.* The sporoplasm divides repeatedly, forming short chains of mononucleated rounded schizonts observed in vitro and in blood cells (Fig. 3), which upon separating yield individual schizonts (Fig. 7b,c). A chain of schizonts measured 1.3 $\times$ 4.0 $\mu$. The nuclei of these schizonts may undergo three synchronous divisions forming a large elongate cell measuring 3.1–4.4 $\times$ 1.8–2.2 $\mu$ with eight nuclei (Fig. 7c,d,e,f).

*Stages Observed in Sectioned Material.* Examination of paraffin sections revealed schizongonic and sporogonic stages (Figs. 4, 5, and 6) in connective tissue. The sporonts have the appearance of swollen spores, lacking the characteristic thick-walled outer spore coat and measuring approximately 4.2 $\times$ 3.1 $\mu$. Each sporont gives rise to one spore (Fig. 1).

The average spore size from a sample of 50 unfixed spores was 4.7 $\times$ 2.8 $\mu$, range 4.0–5.3 $\times$ 2.2–3.1 $\mu$. The spore size for 50 fixed spores averaged 3.7 $\times$ 2.3 $\mu$, range: 3.1–4.4 $\times$ 1.9–3.2 $\mu$.

Among the unfixed spores observed during the study, two were approximately twice as long as the other spores (8.8 $\times$ 2.6 $\mu$ and 9.6 $\times$ 3.0 $\mu$). Kramer (1960) and Weiser (1959b, 1961a) both observed similar atypical spores in microsporidian infections. Weiser refers to them as macrospores.

*Stages Not Observed.* Prevailing opinions of the cyclic development in the order Microsporidia indicate the octonucleate schizonts may divide, forming diplokaryae in which the nuclei eventually unite before the sporogonic phase of the cycle begins. These stages, considered to lead to the formation of the sporont in the order Microsporidia, were not observed in this study if they occur at all in the development of this species.

## Discussion

The taxonomic position of the microsporidian parasite found in the adult coccinellid beetles is considered in terms of recent classifications of the Protozoa (Honigberg *et al.*, 1964) and the Microsporidia (Weiser, 1961a).

Phylum Protozoa Goldfuss, 1818, emend. von Siebold, 1845
Subphylum Cnidospora Doflein, 1901
Class Microsporidea Corliss and Levine 1963
Order Microsporidia Balbiani, 1882
Suborder Monocnidea Leger and Hesse, 1922
Family Nosematidae Labbe, 1899
Genus *Nosema* Naegli, 1857

[2] Paraplast Media No. M 7370-S/P, Paraffin plus Plastic, from Scientific Products, Evanston, Illinois.

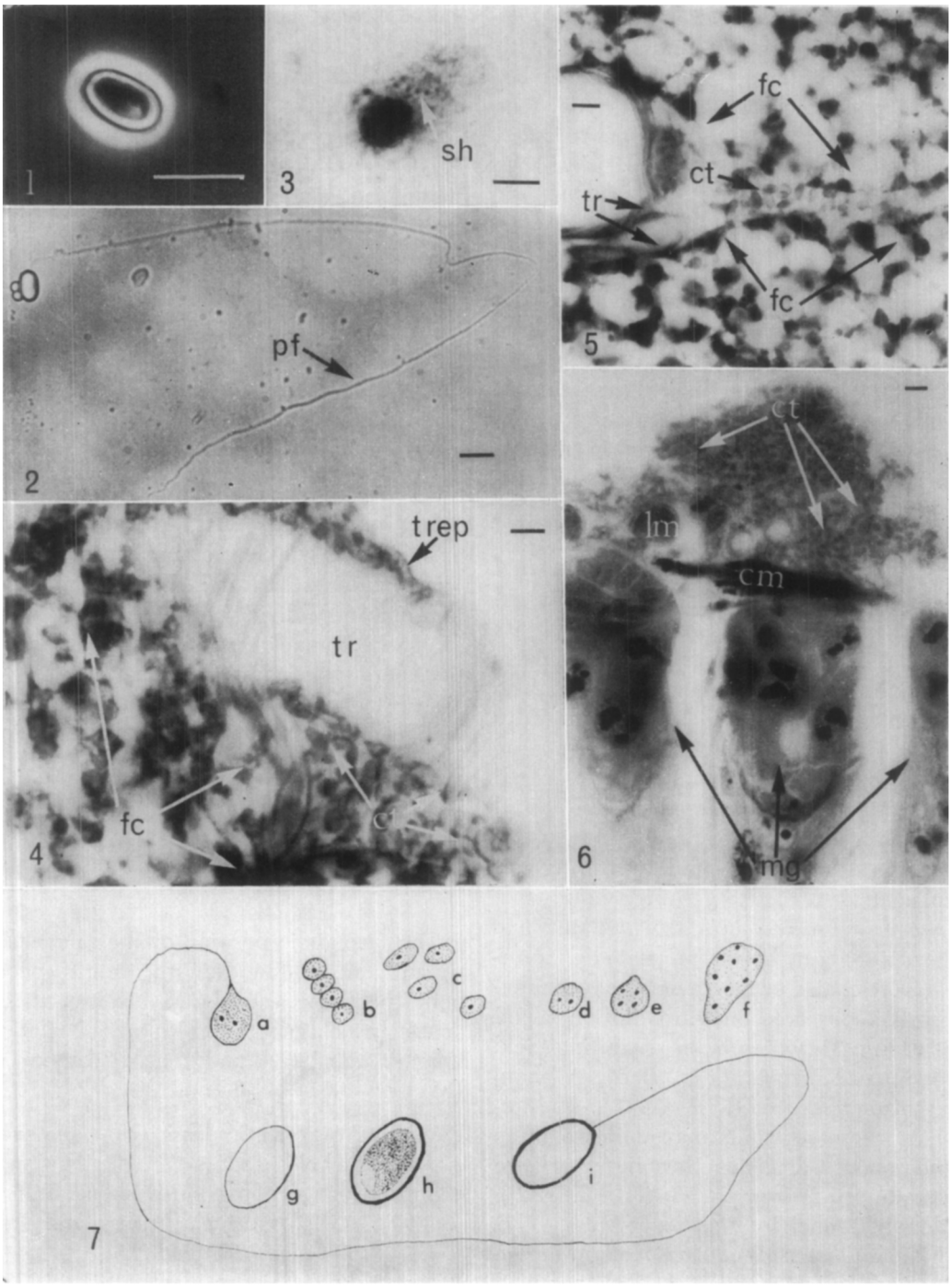


FIG. 1. Unfixed spore observed by phase-contrast microscopy.

FIG. 2. Unfixed spore with polar filament extruded.

FIG. 3. Hemocyte with chain of mononucleate schizonts in the cytoplasm; absolute methanol fixative, Giemsa stain.

The spore being shorter than three times its width places it in the family Nosematidae, and since the sporonts each form a single spore, the microsporidian is in the genus *Nosema*. A new species is proposed based upon the principle of ordinal specificity of the host, according to Weiser (1961a), and upon the comparison of *Nosema* spp. described from Coleoptera (Table 1).

According to Weiser (1961a), characteristics upon which microsporidian species may be based are as follows: host; the parasites in relation to the different tissues infected; the morphology of stages, especially the spores; the ease of polar filament extrusion; and the length of the polar filament. In light of these criteria, the microsporidian in this study appears similar only to *Nosema hippodamiae* Lipa and Steinhaus and then only by a common host family.

*Hippodamia convergens* Guerin, the host of *N. hippodamiae*, and *Coccinella septempunctata*, the host of the proposed new species, are both in the family Coccinellidae, and they have a similar dietary preference for aphids.

The two coccinellid species are often propagated simultaneously under the same insectary conditions; therefore, it is possible that only one species of microsporidian parasite is involved. However, there is good evidence that the microsporidia are different species. In heavy infections of the host, *C. septempunctata*, spores are found in connective tissue and tracheal epithelium (Figs. 4, 5, 6), never in the midgut cells or fat cells as has been reported for the nosema infection in *H. convergens* (Lipa and Steinhaus, 1959). The polar filaments of fresh spores found in *C. septempunctata* are easily extruded. This structure has not been observed in *N. hippodamiae*. Apparently it is relatively difficult to cause the extrusion of polar filaments in *N. hippodamiae*. Hence there are no comparative data for this structure in the *Nosema* spp. found in coccinellids. The morphology of the two microsporidia and infected host tissue sites are compared in Table 2. It should be noted that the presence of a polar filament is a requisite character for the order Microsporidia according to Kudo (1924) and Weiser (1961a), and confirmation of its presence should predicate species description.

The schizogonic and early sporogonic

FIG. 4. Section through edge of a fat lobe and longitudinal section of trachea. Note spores in the tracheal epithelium and in connective tissue surrounding fat cells and trachea. Bouin-Duboscq fixative, Azan trichrome stain.

FIG. 5. Section through a fat lobe revealing a junction of two fat cells and trachea held together by connective tissue. Note spores in the intercellular pocket of connective tissue between the fat cells and in the tracheal epithelium. Bouin-Duboscq fixative, Azan trichrome stain.

FIG. 6. Section through the midgut; midgut epithelial cells surrounded by circular and longitudinal muscle, tracheae, and connective tissue. Note the spores are located outside the midgut cells in the connective tissue. Bouin-Duboscq fixative, Heidenhain's hematoxylin.

FIG. 7. Diagram of stages in the life cycle of *Nosema tracheophila* sp. n.: (a) binucleate sporoplasm observed at end of polar filament; (b) chain of mononucleate, first division schizonts; (c) the chain yields mononucleate meronts; (d) each meront nucleus divides forming a binucleate meront; (e) a second division of each nucleus forms a tetranucleate meront; (f) a third division of each nucleus forms an octonucleate meront; (g) sporont with thin outer membrane showing characteristic swollen appearance when compared to spore; (h) thick-walled spore; (i) empty spore coat with extruded polar filament.

FIGS. 1–7.

*Symbols:* sp, spores; pf, polar filament; sh, schizonts; tr, trachea; trep, tracheal epithelium containing spores; ct, connective tissue containing spores; fc, fat cell; cm, circular muscle; lm, longitudinal muscle; mg, midgut cells. Scale, 5 $\mu$.

TABLE 2

COMPARISON OF THE MORPHOLOGY AND HOST TISSUES AFFECTED BY *Nosema hippodamiae* AND *Nosema tracheophila* sp. n.

| | Host | Spore morphology and size | Length of polar filament | Planont | Schizogony | Sporogony | Infected host tissue |
|---|---|---|---|---|---|---|---|
| *Nosema tracheophila* sp. n. | *C. septempunctata* | Ovoid 4.0–5.3 μ × 2.2–3.1 μ | 89–178 μ | Binucleated | Planont divides and forms a chain of mononucleate schizonts. Daughter schizonts form two, four, and eight nucleate stages | Single spore per sporont | Tracheal epithelium, blood cells, and connective tissue |
| *Nosema hippodamiae* | *H. convergens* | Ovoid 3.5–5.4 μ × 2.2–2.7 μ | Not observed | Mononucleated | No chain of mononucleate schizonts. Mononucleate planont becomes binucleate schizont. Only daughter schizonts containing two nuclei are formed | Single spore per sporont | Fat body and midgut cells |

stages but not the spores of the microsporidian parasite are found in hemocytes of *C. septempunctata*. If the asexual forms of the parasite observed in hemocytes are the result of phagocytosis or entry of planonts into hemocytes, the concentration of spores in connective tissue indicates that the residues of hemocytes are incorporated as connective tissue in the terminal histopathology of the infected hemocytes.

It is a possibility that formation of connective tissue is accelerated in insects infected with microsporidia due to the presence of a juvenile hormone like substance. Such a substance has been reported (Fisher and Sanborn, 1962) to be produced by a species of the genus *Nosema* infecting *Tribolium castaneum* (Herbst), also a coleopteran host. This would be in support of Wigglesworth (1959) who describes connective tissue formation by hemocytes as the result of hormonal stimulation. Thus a hormonal imbalance resulting from microsporidian infection in adult coccinellids could stimulate connective tissue formation and accelerate the incorporation of infected hemocytes into connective tissue.

The behavior of infected hemocytes in the *C. septempunctata* nosematosis may be contrasted with the result of Laigo and Paschke (1966) who demonstrated the complete cycle of a microsporidian parasite within circulating hemocytes of a lepidopteran host. A significant reduction in circulating hemocytes in the infected host was described by these authors. This may be the net result of the parasite stimulating removal of hemocytes to connective tissue formation.

## Conclusions

The data presented on affected tissue sites and parasite morphology are confirmatory evidence for a new species designation of the microsporidian parasite found in *C. septempunctata*. The abundance of sporogonic forms of the parasite detected in connective tissue and tracheal epithelium, both characteristically found in close association with the trachea, supports the specific designation of the species as *Nosema tracheophila* sp. n.

The lack of spores in the hemocytes, and the rare occurrence of asexual forms in the connective tissue, may be explained by the method of connective tissue formation in insects. Hemocytes may lose their integrity and become part of the enucleated, thin-stranded tissue mass surrounding organs in the hemocoel, termed connective tissue. Connective tissue formation may be stimulated by the presence of a foreign body in the hemocoel, for example, microsporidian schizonts, thus resulting in the transfer and concentration of sporogenous forms in connective tissue.

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