Nosema lepocreadii sp. n., a Parasite of Lepocreadium manteri (Digenea: Lepocreadiidae) from the Gut of the California Grunion, Leuresthes tenuis

Author(s): Elizabeth U. Canning, Andrew C. Olson and Jr.

Source: *The Journal of Parasitology*, Vol. 66, No. 1 (Feb., 1980), pp. 154-159
Published by: Allen Press on behalf of The American Society of Parasitologists
Stable URL: https://www.jstor.org/stable/3280609
Accessed: 04-05-2022 00:16 UTC

J. Parasitol., 66(1), 1980, pp. 154–159

NOSEMA LEPOCREADII SP. N., A PARASITE OF *LEPOCREADIUM MANTERI* (DIGENEA: LEPOCREADIIDAE) FROM THE GUT OF THE CALIFORNIA GRUNION, *LEURESTHES TENUIS*

Elizabeth U. Canning* and Andrew C. Olson, Jr.†

ABSTRACT: *Nosema lepocreadii* sp. n. is described from adult flukes *Lepocreadium manteri* Olson 1978 from California grunion. The microsporidia are parasitic in cells of the vitelline system. The arrangement of nuclei in diplokarya and the disporoblastic sporogony are characteristic of the genus *Nosema* Naegeli 1857. Schizonts with homogeneous cytoplasm and vesicular nuclei can be distinguished from sporonts that have banded cytoplasm and compact nuclei. Chain formation of schizonts or sporonts is common. Spores are 3.5 × 1.5 μm when fresh.

Lepocreadium manteri Olson 1978 (Digenea: Lepocreadiidae), is a parasite in the gut of the beach-spawning California grunion, *Leuresthes tenuis* (Ayres, 1860) (Osteichthyes: Atherinidae). While studying this parasite, we found that several grunion contained flukes which were hyperparasitized by microsporidia. The microsporidia were discovered during regular sampling of grunion as part of a study of the parasite fauna of these fish and in particular of *Lepocreadium manteri* Olson (1978). All the microsporidian material was collected and preserved in California by Olson.

In this report the hyperparasite, believed to belong to the genus *Nosema*, is described at the light microscope level. Electron microscopic observations will be presented in a later communication, where the ultrastructure will be compared with that of other *Nosema* species from trematodes.

MATERIALS AND METHODS

Grunion were collected by hand during their spawning runs at night on three beaches of San Diego Co., California, in June 1977 and March to May 1978. The fish were refrigerated and examined within 48 hr. *Lepocreadium* removed from the expanded anterior intestine ("stomach") were examined in physiological saline under slight coverslip pressure to detect larger worms without eggs and with some opacity, evidence for the presence of the microsporidia.

Infected flukes were teased apart on glass slides and smeared. Smears were dried, fixed in methanol, and stained in 2% Giemsa stain, buffered to pH 7.0, for 2.5 hr. Whole flukes were fixed in hot Bouin's, Carnoy's, or AFA fluids and were processed for embedding in 58° MP paraffin wax. Sections were cut at 5 μm and stained in Gram's stain or by the Giemsa Colophonium method. Fresh spores from disrupted flukes were suspended in seawater. Measurements against a 10-μm scale were made of spores photographed in a monolayer at a paraffin/seawater interface (Fig. 14).

RESULTS

Prevalence of infection

After detailed examination of the microsporidium began in 1978, complete records were kept of *Lepocreadium* infection and microsporidian infection for grunion collected from March to May 1978. The data are presented in Table I, which shows the prevalences of the two parasites in grunion collected on eight occasions, and in Table II, which gives the numbers of microsporidian-infected *Lepocreadium* in individual grunion.

The overall prevalence of *Nosema*-infected *Lepocreadium* was only 9.6% of the total *Lepocreadium* recovered from the 137 grunion, because many of the grunion contained no infected flukes. However, in individual fish, where hyperinfection occurred, it was common to find more than one fluke infected; usually several were infected and sometimes all of them, as in the fish collected on 11 March 1978.

Site of infection

Initially, infection was restricted to the vitelline system (Figs. 1, 2). Schizonts, sporonts, and spores were present together in the cytoplasm of individual cells (Fig. 3). Some of the follicles were so packed with spores that there was total replacement of normal tissue

Received for publication 16 February 1979.
* Department of Zoology, Imperial College, London, SW7 2AZ, England.
† Department of Zoology, San Diego State University, San Diego, California 92182.

TABLE I. *Prevalences of* Lepocreadium manteri *and* Nosema lepocreadii *in California grunion collected March–May 1978 from San Diego Co., California.*

Site	Date	Grunion examined	Grunion with *Lepocreadium*	Total *Lepocreadium*	*Lepocreadium* with *Nosema*	Grunion with infected *Lepocreadium*
San Diego Bay	March 11	19	9	94	69	6
	March 26	9	6	56	7	2
	April 9	15	14	185	5	3
	April 25	9	7	87	9	1
	May 8	27	24	320	3	2
	May 25	37	35	725	43	8
Mission Beach	May 9	15	10	64	9	5
Imperial Beach	May 26	6	5	76	10	2
Totals		137	110 (80.2%)	1,607	155 (9.6%)	29 (21.2%)

and spores also were present in the walls of the ducts and reservoir. Destruction of the vitelline follicles appeared to inhibit egg production, because no eggs were found in hyperparasitized flukes. Adult *Asymphylodora atherinopsidis* Annereaux 1947, which is a common fluke in the posterior intestine, and metacercariae of other flukes found in the brain, in cardiac muscle, and in the coelom, appeared to be free of microsporidian infection.

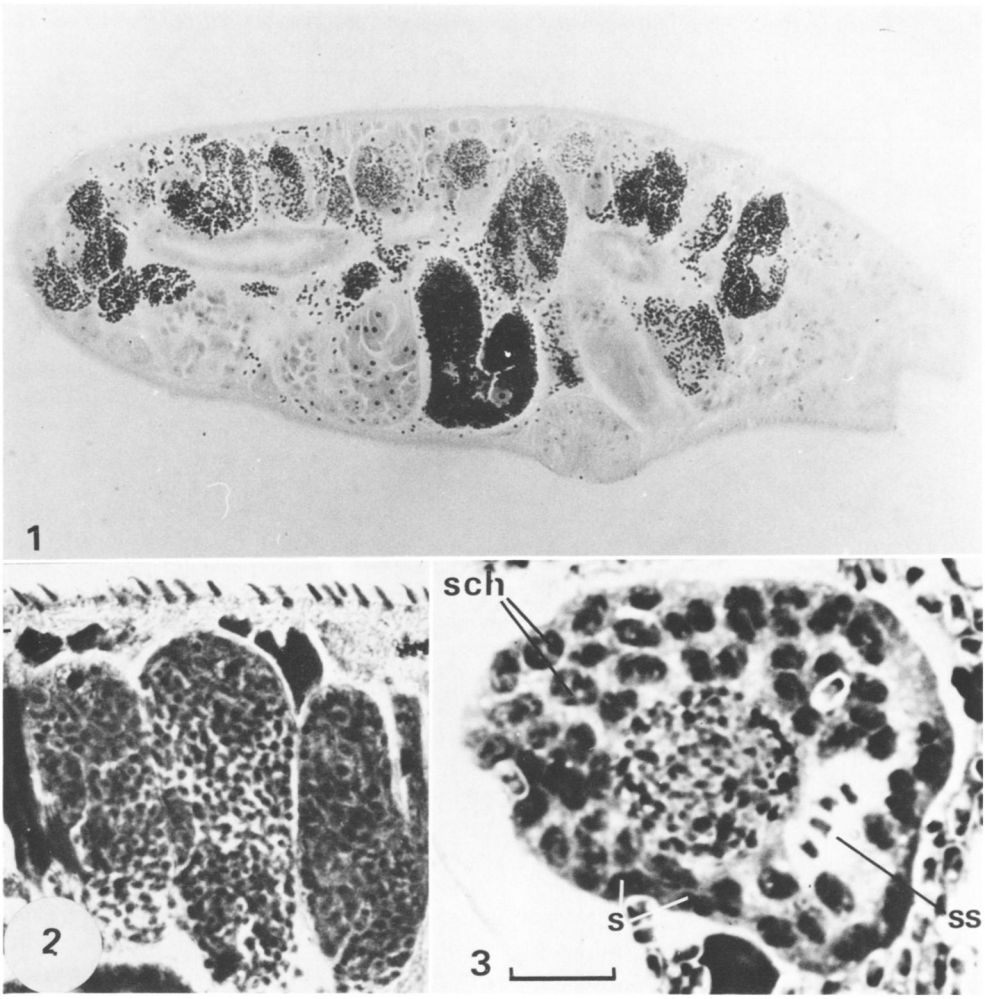
Development of the microsporidium

All stages of the microsporidium had nuclei in diplokaryon form. Several types of schizont were observed. The majority were elongate with the pairs of nuclei arranged along the length of the body so that cytoplasmic fission gave rise to merozoites in chain formation. The diplokarya could lie side by side or end to end with respect to the long axis of the chain. The cytoplasm was generally homogeneous except for a clear space around the nuclei. Elongate schizonts were of two types giving large merozoites 3.0 to 3.5 μm in diameter with vesicular nuclei (Figs. 6, 7) and small merozoites 2.5 μm in diameter with compact nuclei (Fig. 8). Along the course of the chains of large merozoites were expanded regions containing three to six diplokarya (Fig. 9). The third type of schizont, which had rounded or lobulate outlines (Figs. 4, 5) and several diplokarya, resembled the expanded regions of the large chains and may have separated from them during preparation of the smears.

Sporonts and sporoblasts were also produced in chains (Figs. 10–13), probably as a result of the sporonts developing from chains of schizonts. At first, each sporont was an elongate binucleate cell, 5.5 to 8.0 μm × 2.5 to 3.5 μm. Such sporonts could be distinguished from the schizonts by the arrangement of the nuclei that always lay end to end, along the longitudinal axis, and by the cytoplasm that stained unevenly as dark bands with pale areas between (Fig. 10). A final nuclear division gave two diplokarya which took up polar positions with the paired nuclei often lying transverse to the long axis of the sporont (Figs. 11, 12). Cytoplasmic fission resulted in two sporoblasts, in which the compact, rounded nuclei realigned themselves to lie end to end with respect to the long axis of the chain (Fig. 13). Sporoblasts were 4.0 × 2.0 μm. Sporont division could take place anywhere along the chain; thus, sporoblasts often were interpolated between undivided sporonts. Spores were binucleate, of an elongate elliptical shape, and 3.5 × 1.5 μm when fresh (Fig. 14).

TABLE II. *The numbers of* Nosema-*infected* Lepocreadium manteri *recovered from individual California grunion.*

Date	*Nosema*-infected/total *Lepocreadium*
March 11	1/1, 3/3, 4/4, 7/7, 7/29, 47/47 (6 fish)
March 26	3/40, 4/4 (2 fish)
April 9	1/1, 1/1, 3/27 (3 fish)
April 25	9/21 (1 fish)
May 8	1/5, 2/26 (2 fish)
May 25	1/6, 3/6, 1/10, 14/14, 4/15, 14/18, 2/22, 4/47 (8 fish)
May 9	1/1, 2/2, 1/3, 4/10, 1/6 (5 fish)
May 26	3/38, 7/30 (2 fish)

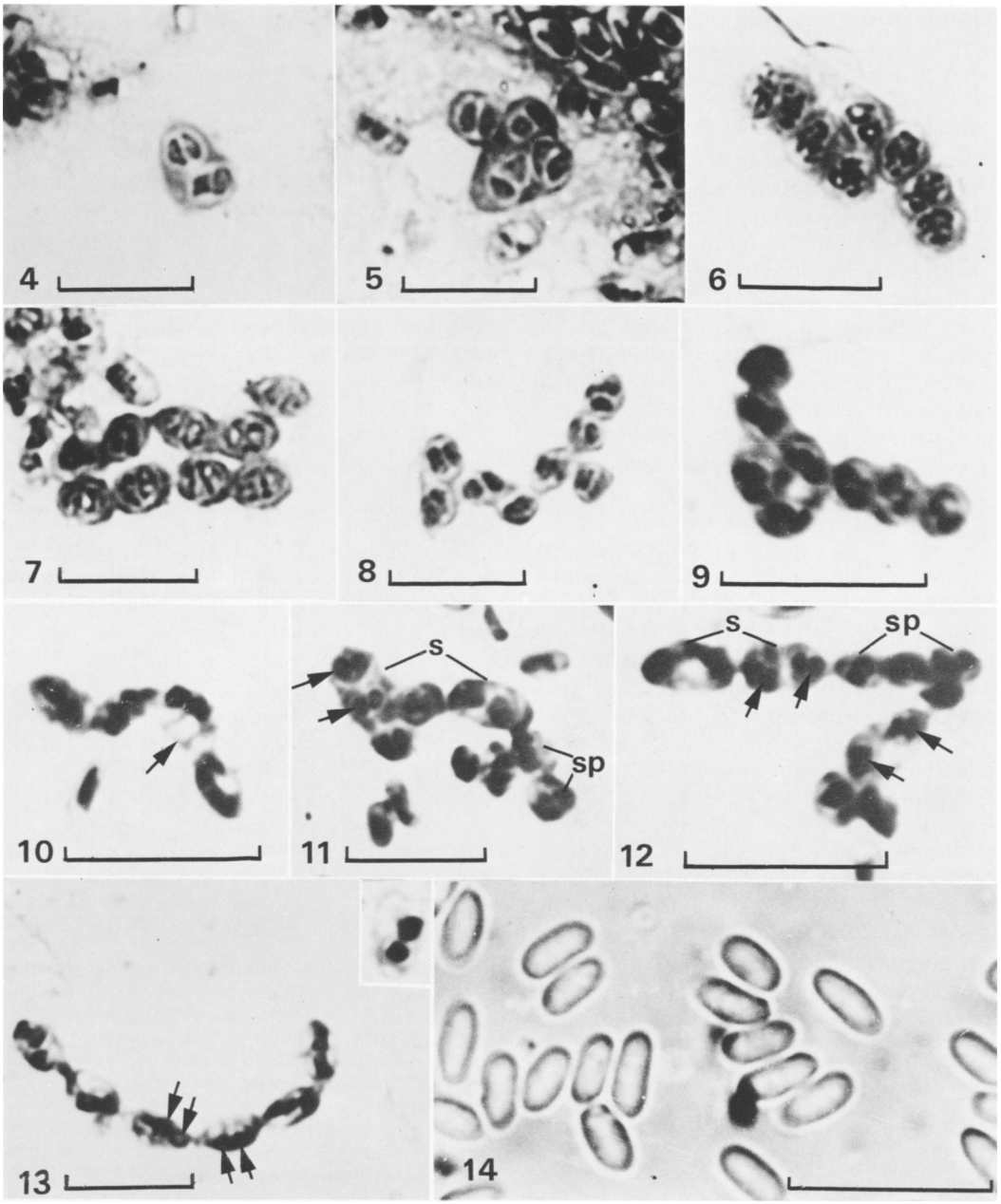


FIGURES 1–3. Sectional views of the fluke, *Lepocreadium manteri*. **1.** Longitudinal section showing spores of *Nosema lepocreadii* concentrated in the vitelline follicles. Isolated spores apparently in other organs have probably been displaced by sectioning. Grams stain, Light green. ×230. **2.** Peripheral layers showing the almost complete replacement of vitelline follicle tissue by spores of *N. lepocreadii* Giemsa/Colophonium. ×770. **3.** Cell of vitelline follicle intact after smearing the fluke, the cytoplasm is packed with schizonts (sch) sporonts (s), and spores (ss) of *N. lepocreadii*. Giemsa stain. Scale = 10 μm.

DISCUSSION

A characteristic feature of the microsporidium described herein is the production of long chains of individuals in the schizogonic and sporogonic sequences. The nuclei are in diplokaryon arrangement in all stages. Although sporoblasts are produced in chains, they are derived from sporonts by binary fission and are apparently devoid of a pansporoblast membrane. Chain formation of spores is characteristic of the genera *Perezia* Léger and Duboscq 1909, and *Ameson* Sprague 1977. Although schizogony is known incompletely in these genera, there is some evidence for the occurrence of diplokaryotic cells at least in the later stages of this sequence (Weidner, 1970; Ormières et al., 1977; Vivares and Sprague, 1979). In sporogony, the nuclei become isolated and elongate sporonts with up to eight nuclei are produced which give rise to uninucleate sporoblasts. Thus, the present species resembles these genera in schizogony but not sporogony.

The diplokaryotic organization and dispo-



FIGURES 4–14. Development of *Nosema lepocreadii* Giemsa-stained in smears except Figure 14, a fresh preparation. Scale = 10 μm. **4.** Schizont with two diplokarya. **5.** Lobed schizont with four diplokarya. **6, 7.** Chain formation of large schizonts: nuclei with pale centers, one diplokaryon per cell. **8.** Chain formation of small schizonts. **9.** Schizogony: most lobes of the chain with a single diplokaryon, one enlarged lobe with three diplokarya. **10.** A chain of four sporonts, each with a pair of compact rounded nuclei; the banded cytoplasm is clear in one sporont (arrow). **11, 12.** Division of sporonts (s) to give chains of sporoblasts (sp) each sporont, now with two pairs of nuclei (arrows), constricts centrally to give sporoblasts with a single pair. **13.** A chain of sporoblasts: the nuclei (arrows) are aligned to the long axis of the sporoblast. Insert: sporoblast showing clearly the position of the nuclei. **14.** Fresh spores.

roblastic sporogony are typical of the genus *Nosema* Naegeli 1857, to which the species from *L. manteri* is assigned. Chain formation of merozoites and sporoblasts generally is not featured by *Nosema* spp. but is not unknown. For example, in schizogony of *Nosema apis* Zander 1909, there is incomplete cytoplasmic division after karyogamy of diplokarya, which results in formation of multinucleate stages, from which diplokaryotic merozoites eventually separate (Grey et al., 1969).

Two types of merozoite in the present species were distinguished by size and nuclear organization. The compact nuclei of the small merozoites resembled the sporont nuclei, but the disparity in sizes of the two stages made it unlikely that these merozoites transformed directly into sporonts. In contrast, the size of the large merozoites was in accord with their being the precursors of sporonts but there was no clear indication of the point at which nuclear reorganization (from vesicular to compact) took place. Because these points could not be resolved by examination of the material available, no attempt has been made to place the schizogonic stages in sequence.

Nearly all microsporidia parasitic in trematodes have been described from the larval stages in molluscan hosts, and it is thought that some of these microsporidia, at least, are unable to continue their development when the larvae become adult, as for example in homeothermic vertebrates: *Nosema eurytremae* (Canning and Basch, 1968) Canning 1972 failed to develop in adult *Echinostoma malayanum* Leiper 1911 in rats (Lie and Nasemary, 1973), although it readily parasitized the rediae.

A few microsporidia have been reported as parasites of adult trematodes, but of these only *Nosema gigantica* Canning and Madhavi 1977 from adult *Allocreadium fasciatusi* Kakaji 1969 in a small freshwater fish can be attributed with any confidence to the genus *Nosema* (Canning and Madhavi, 1977). *Nosema gigantica*, with stained spores 7.9 × 4.9 μm, is clearly a different species from the microsporidium described here from an adult trematode in a marine fish.

Because it is unlikely that a single species would parasitize both marine and freshwater hosts, the present species is only compared further with those microsporidia, which may belong to the genus *Nosema*, described from marine hosts. *Microsporidium distomi* (Lutz and Splendore, 1908) Canning 1975 was reported from an adult trematode, probably *Glypthelmins linguatula* (Rudolphi 1819), in *Bufo marinus* (Linnaeus 1758). It was described inadequately, but its spores are distinctly smaller (2.0 × 0.8–1.0 μm) than those of the present species. *Nosema dollfusi* Sprague 1964, which was found in the sporocysts of *Bucephalus cuculus* McCrady 1874 in *Crassostrea virginica* Gmelin 1791, and *Microsporidium spelotremae* (Guyénot, Naville and Ponse, 1925) Canning 1975 from the metacercariae of *Spelotrema carcini* Lebour 1908 in the crab *Carcinus maenas* (Linnaeus 1758) have spores of the same general shape and size as the present species. The measurements given by Guyénot et al. (1925) for *M. spelotremae* are 3.5 × 1.5 μm for fresh spores, and by Sprague (1964) for *N. dollfusi* are 3.0 × 1.7 μm for stained spores. Unfortunately details are lacking for both species on vegetative development, sporogony, and nuclear complement. However, it should be noted that these parasites appeared to complete their development in the larval trematodes, because it was mainly spores that were found. In contrast, the presence of a full range of schizogonic and sporogonic stages of *Nosema* in *L. manteri* suggests that the infection was acquired by the adult fluke when present in the gut of the grunion.

For the present, it seems best to consider the parasite of *L. manteri* as a new species for which the name *Nosema lepocreadii* sp. n. is proposed. The projected electron microscope studies will enable a further comparison to be made with *M. spelotremae*, of which some ultrastructural details are available (Stanier et al., 1968).

The common occurrence of more than one infected fluke in a single fish indicated that infection was unlikely to have occurred by casual ingestion of the microsporidian spores dispersed in seawater. The prevalence of infection is similar to that reported for *Unikaryon allocreadii* Canning and Madhavi 1977 in *A. fasciatusi*. Because the larvae of *A. fasciatusi* had been found uninfected in the snail *Amnicola travancorica* Benson 1960 and in copepods, which serve as the two intermdiate hosts, it was proposed that the spores, which infected the adult flukes, had been derived from an invertebrate host serving as food for

the fish. Although it was not stated, by inference, there would be an alternation of hosts. Because the larval stages of *L. manteri* are unknown, it is not known whether the larvae are infected. However the presence of large numbers of schizogonic stages in the adult flukes is indicative of the early stages of infection and it is probable that, like *Allocreadium*, infection of *Lepocreadium* takes place in the gut of the fish, via an intermediate or paratenic host of the microsporidium. The grunion is a relatively small planktivore growing to a length of 184 mm. The most conspicuous food items during this study have been copepods. It is interesting to note, not only that copepods are common hosts to microsporidia (Sprague, 1977), but also that copepods were a major food item for *Aplocheilus melastigma* McClelland 1839 which harbored the aforementioned microsporidian-infected *A. fasciatusi* (Canning and Madhavi, 1977). It would be useful to examine some of the marine copepods collected from California for microsporidia to see if a link could be established between them and the presently described species.

ACKNOWLEDGMENT

We should like to thank J. Paul Nicholas for invaluable technical help and preparation of the plates.

LITERATURE CITED

CANNING, E. U. 1972. *Nosema eurytremae* a replacement name for the secondary homonym *Perezia helminthorum* Canning and Basch, a parasite of digenean larvae. J Invert Pathol **20:** 371.

———. 1975. The microsporidian parasites of Platyhelminthes: Their morphology, development, transmission and pathogenicity. Commonw Inst Helminthol Misc Publ **2:** 1–32.

———, AND P. F. BASCH. 1968. *Perezia helminthorum* sp. nov., a microsporidian hyperparasite of trematode larvae from Malaysian snails. Parasitology **58:** 341–347.

———, AND R. MADHAVI. 1977. Studies on two new species of Microsporida hyperparasitic in adult *Allocreadium fasciatusi* (Trematoda, Allocreadiidae). Parasitology **75:** 293–300.

GREY, F. H., A. CALI, AND J. D. BRIGGS. 1969. Intracellular stages in the life cycle of the microsporidian *Nosema apis*. J Invert Pathol **14:** 391–394.

GUYÉNOT, E., A. NAVILLE, AND K. PONSE. 1925. Deux microsporidies parasites de trematodes. Rev Suisse Biol **31:** 399–421.

LIE, K. J., AND M. NASEMARY. 1973. Transmission of *Nosema eurytremae* (Microsporida, Nosematidae) to various trematode larvae. Z Parasitenkd. **41:** 109–117.

LUTZ, A., AND A. SPLENDORE. 1908. Ueber pébrine und verwandte Mikrosporidien. Zentralbl Bakteriol Parasitenkd-Infectionskr Hyg Abt 1: Orig Reihe A **46:** 311–315.

OLSON, A. C., JR. 1978. *Lepocreadium manteri* sp. n. (Trematoda: Lepocreadiidae) from the California grunion, *Leuresthes tenuis*, and its hyperparasitic microsporidan. Proc Helminthol Soc Wash **45:** 155–157.

ORMIÈRES, R., C. LOUBES, AND J. MAURAND. 1977. Etude ultrastructurale de la microsporidie *Perezia lankesteriae* Léger et Duboscq, 1909 espèce-type: Validation du genre *Perezia*. Protistologica **13:** 101–112.

SPRAGUE, V. 1964. *Nosema dollfusi* n. sp. (Microsporidia, Nosematidae), a hyperparasite of *Bucephalus cuculus* in *Crassostrea virginica*. J Protozool **11:** 381–385.

———. 1977. The zoological distribution of the Microsporidia. In L. A. Bulla and T. C. Cheng (eds.), Comparative pathobiology. Vol. 2. Systematics of the Microsporidia. Plenum Press, New York, New York, 510 p.

STANIER, J. E., M. A. WOODHOUSE, AND R. L. GRIFFIN. 1968. The fine structure of the spore of *Nosema spelotremae*, a microsporidian parasite of a *Spelotrema* metacercaria encysted in the crab *Carcinus maenas*. J Invertebr Pathol **12:** 73–82.

VIVARES, C. P., AND V. SPRAGUE. 1979. The fine structure of *Ameson pulvis* (Microspora, Microsporida) and its implications regarding classification and chromosome cycle. J Invertebr Pathol **33:** 40–52.

WEIDNER, E. 1970. Ultrastructural study of microsporidian development. I. *Nosema* sp. Sprague, 1965 in *Callinectes sapidus* Rathbun. Z Zellforsch Mikrosk Anat **105:** 33–54.