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Orthosomella lambdinae n. sp. (Microsporida: Unikaryonidae) from the Spring Hemlock Looper, *Lambdina athasaria* (Lepidoptera: Geometridae)

THEODORE G. ANDREADIS, CHRIS T. MAIER, AND CAROL R. LEMMON

The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven, Connecticut 06504

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A new species of microsporidia, *Orthosomella lambdinae* n. sp. (Microsporida: Unikaryonidae), is described from larvae of the spring hemlock looper, *Lambdina athasaria* (Lepidoptera: Geometridae). The principal site of infection is the midgut epithelium. The microsporidium is free within the hyaloplasm of the host cell and has unpaired nuclei in all stages of development. Schizonts are rounded to irregular in shape with a variable number of nuclei (2–29, median = 6). They are limited by a simple plasmalemma and divide by plasmotomy. Sporogonial plasmodia are elongate and sausage-shaped with a variable number (2–18, median = 6) of linearly arranged nuclei. They are surrounded by an electron-dense surface coat and give rise to moniliform chains of uninucleated sporoblasts with a corresponding number of nuclei. Sporoblasts concurrently undergo sporogenesis prior to cytoplasmic cleavage into free spores. Mature spores are uninucleate and oblong to slightly reniform. They measure 2.8 × 1.5 μm (live) and have 7–8 turns of the polar filament. The natural prevalence of *O. lambdinae* in larval populations of *L. athasaria* was found to range from 2.0 to 18.4% (6.8% overall).

KEY WORDS: *Orthosomella lambdinae;* microsporidia; *Lambdina athasaria;* spring hemlock looper; morphology; taxomony; ultrastructure; field prevalence.

INTRODUCTION

The genus *Orthosomella* Canning, Wigley & Barker, 1991 is a replacement name for *Orthosoma* Canning, Wigley and Barker, 1983, preoccupied. The type species by monotype is *Orthosomella operophterae* (Canning, 1960) Canning, Wigley & Barker, 1991 and its host is the winter moth, *Operophtera brumata* (L.) (Lepidoptera, Geometridae) (Canning, 1960; Canning *et al.,* 1983). The description of the genus is based on a single species and includes the following: all stages of merogony (schizogony) and sporogony with unpaired (unikaryotic) nuclei surrounded by an electron-dense surface coat in direct contact with the host cell cytoplasm; and sporogony with bi-, tetra-, or octosporoblastic sporogonial plasmodia, giving rise to two, four, or eight sporoblasts held together in chains (Canning *et al.,* 1991).

Between 1989 and 1992, a major infestation of spring hemlock looper, *Lambdina athasaria* (Walker) (Lepidoptera, Geometridae), occurred in hemlock forests throughout many areas of Connecticut (Maier *et al.,* 1993). This was the first outbreak of *L. athasaria* in the state in more than 50 years (Friend, 1941, 1942) and it was coincident with scattered outbreaks in other areas of the northeastern US as well (Grehan *et al.,* 1992). In response to this outbreak, a survey for natural enemies of *L. athasaria* was initiated. In the course of this investigation we discovered a new microsporidium with characteristics of the genus *Orthosomella.* Accordingly, we present a description of the life cycle stages of this microsporidium by light and electron microscopy and, based on morphological and developmental differences with *O. operophterae,* propose the establishment of a new species, *Orthosomella lambdinae* n. sp. Data on the natural prevalence of *O. lambdina* infection in larval field populations is also detailed. To our knowledge, this is the first complete description of a microsporidium from a member of the genus *Lambdina.*

MATERIALS AND METHODS

Life Cycle Studies

All life cycle investigations were conducted with naturally infected, field-collected larvae obtained from Devil's Hopyard State Park (East Haddam, CT) during July–August, 1993. General characterization of parasite development was determined from microscopic examination (×1000) of Giemsa-stained (10% solution) smears of infected tissues that were dissected from living second through fifth instar larvae. Measurements of mature spores were made on 50 stained and live spores, each with an ocular micrometer (×1000). Live

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spores were viewed in whole wet-mount preparations of infected tissues with phase-contrast microscopy.

For the ultrastructural studies, infected tissues were dissected from living larvae and fixed overnight at 4°C in a 2.5% (v/v) glutaraldehyde/2% (v/v) paraformaldehyde solution containing 0.1% (w/v) $CaCl_2$ and 1% (w/v) sucrose in 100 mM Na cacodylate buffer (pH 7.4). Specimens were postfixed in 1% (w/v) OsO_4, stained *en bloc* in 2% (w/v) uranyl acetate, dehydrated through an ethanol and acetone series, and embedded in a LX-112/Araldite mixture. Thin sections were poststained with 5% (w/v) uranyl acetate in 50% (v/v) methanol followed by Reynold's lead citrate and examined in a Zeiss EM 10C electron microscope at an accelerating voltage of 80 kV.

Field Studies

The natural epizootiology of *O. lambdinae* was assessed in a wild larval population of *L. athasaria.* This study was conducted during 1993 in a hemlock-dominated forest (80–90% of the canopy) at the same location as described above. The sample area was approximately 15 ha in size and had experienced 2 successive years of defoliation by *L. athasaria.* Observations were initiated at the onset of larval hatch (July 8) and continued every other week until pupation (September 16). Biweekly prevalence rates of infection were obtained from microscopic examination of Giemsa-stained smears of approximately 100 larvae for each sample date. Larvae were randomly collected from both the upper and the lower portions of the canopy. To eliminate possible horizontal transmission of the microsporidium following collection, all larvae were isolated in 30-ml plastic cups and promptly examined for infection the next day.

The relative abundance of *L. athasaria* was also determined for the same period. This was achieved by pruning two 0.5-m terminal branches from 10 trees in the sample area and counting the number of larvae present. In July and August, when early instars were present, foliage samples were placed in a clear plastic bag and brought to the laboratory, where they were beaten over a white sheet to dislodge larvae that may have been overlooked. In September, when larger instars were present, larvae were counted immediately after pruning by beating branches over a white sheet placed directly on the ground. Instar determinations were made from measurements of head capsule widths. These were obtained with an ocular micrometer fitted to a stereomicroscope.

RESULTS

Light Microscopy Studies

Orthosomella lambdinae was exclusively found within the midgut epithelial cells of *L. athasaria* larvae. All stages of development observed in Giemsa-stained smears had unpaired nuclei. Schizonts (Figs. 2–4) were recognizable by their uniform deep blue cytoplasm. They were rounded and irregular in shape and contained a highly variable number of nuclei ranging from 2 to 29 (mean = 7, median = 6) (Fig. 1). The odd number of nuclei observed in many schizonts seemed to indicate that schizonts underwent asynchronous nuclear division to produce large multinucleated plasmodia. Binary division of schizonts was not observed and schizogony appeared to occur by repeated plasmotomy of some of the smaller multinucleated plasmodia (Figs. 2 and 3).

Irregularly shaped plasmodia with one or more radiating finger-like projections were also observed in the Giemsa-stained smears (Figs. 4–6). In most cases, these projections appeared to result from simple elongation of the multinucleated schizont plasmodia, rather than from additional nuclear division. These were therefore interpreted as transitional stages leading to sporogenesis.

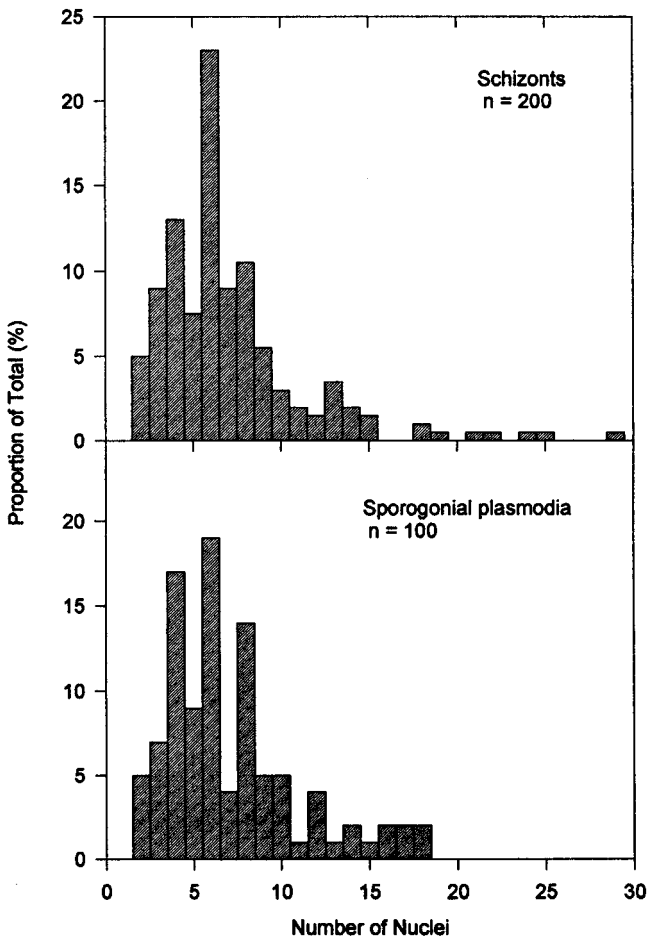
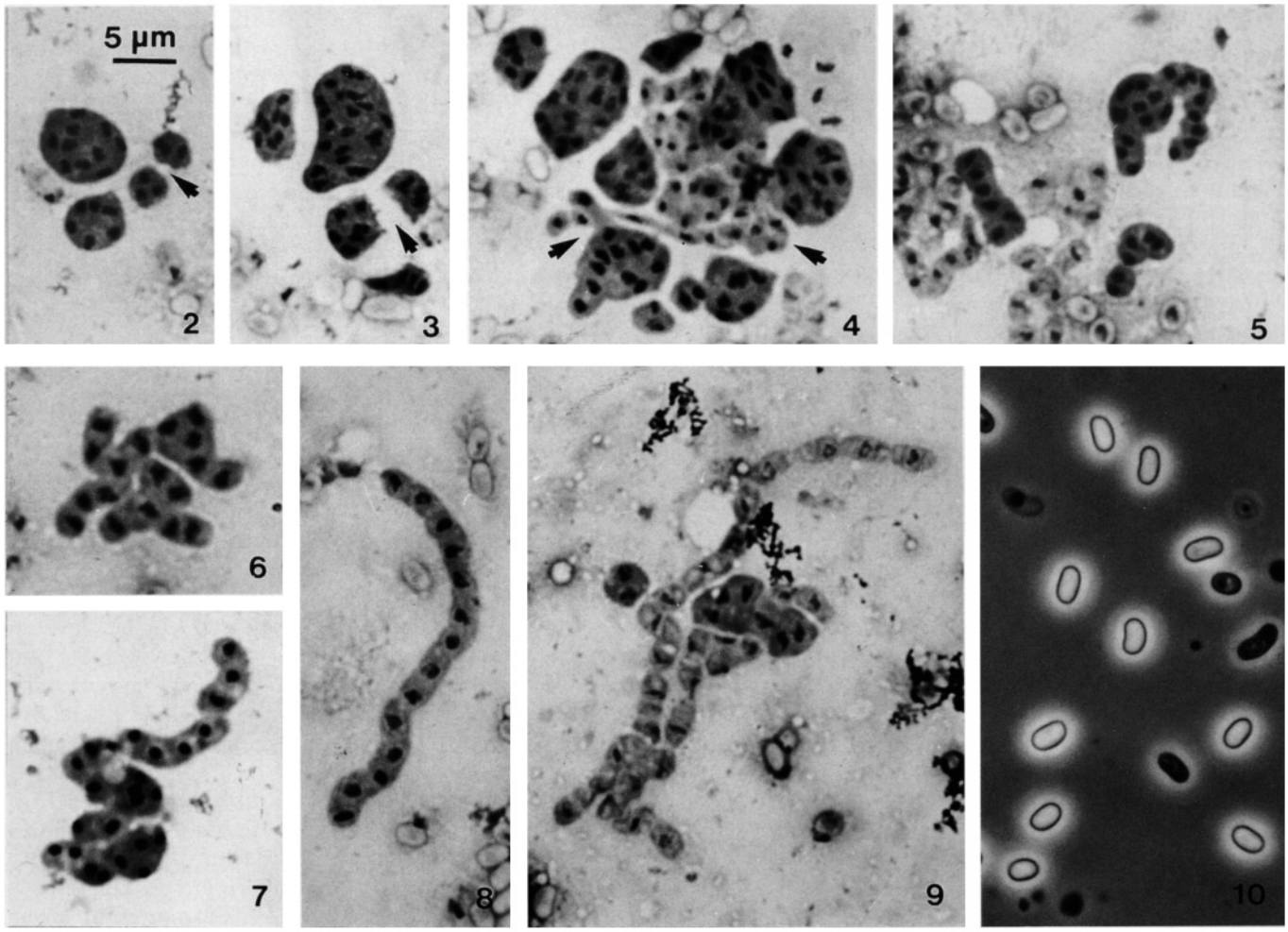


FIG. 1. Comparative distribution of multinucleated schizonts and sporogonial plasmodia of *Orthosomella lambdinae* as enumerated in Giemsa-stained smears of infected midguts from *Lambdina athasaria.*



FIGS. 2–10. Developmental stages of *Orthosomella lambdinae* as seen in Giemsa-stained smears and phase-contrast microscopy (Fig. 10), ×1,800.

FIGS. 2 and 3. Schizonts. Arrows indicate possible dividing forms.

FIG. 4. Aggregation of several large multinucleated schizonts (dark-stained cytoplasm) intermixed with the less intensely stained, irregular plasmodia (arrows).

FIGS. 5 and 6. Transitional sporogonial plasmodia.

FIGS. 7 and 8. Sausage-shaped sporogonial plasmodia.

FIG. 9. Moniliform chain of uninucleate sporoblasts.

FIG. 10. Mature spores (live).

Stages believed to be sporogonial plasmodia were seen as elongate and sausage-shaped with a less intensely stained cytoplasm (Figs. 7 and 8). These characteristically contained a variable number of linearly arranged nuclei ranging from 2 to 18 (mean = 7, median = 6) (Fig. 1). Sporogonial plasmodia gave rise to moniliform chains of uninucleate sporoblasts with a corresponding number of nuclei (Fig. 9). This was followed by sporogenesis and finally by cytoplasmic cleavage into free spores.

Mature spores (Fig. 10) were uninucleate and oblong to slightly reniform. They measured 2.6 × 1.5 μm in stained smears (n = 50) and 2.8 × 1.5 μm when live (n = 50).

Ultrastructural Studies

All stages of *O. lambdinae* observed at the ultrastructural level had unpaired nuclei. Schizonts were elongate oval to irregular in shape with one or more nuclei depending on the profile of the section (Fig. 11). They were limited by a simple unit membrane (plasmalemma) (Fig. 12) and were free within the hyaloplasm of the host cell. The cytoplasm of the schizont was homogeneous and contained numerous free ribosomes, a zone of Golgi, and irregularly arranged cisternae of rough and smooth endoplasmic reticulum. Signs of nuclear division (i.e., spindle plaques, microtubules, and polar vesicles) were apparent (Fig. 13) but schi-

zonts undergoing cytoplasmic division (Fig. 14) were only occasionally observed in thin section.

Transitional stages leading to sporogenesis were frequently seen in close association with the schizonts. These stages were generally elongate and were distinguished by the presence of patchy thickenings of the plasmalemma, which was clearly of parasite origin (Fig. 15). The nuclei of these stages were linearly arranged and the cytoplasm contained prominent parallel cisternae of rough endoplasmic reticulum.

Stages identified as fully formed sporogonial plasmodia were similarly elongate and were surrounded by a distinct electron-dense surface coat that appeared to represent early formation of the exospore (Fig. 16). The nuclei of these stages were evenly spaced and in many cases separated by cytoplasmic constrictions. Signs of nuclear division were also apparent (Fig. 17), thus indicating that some karyokinesis may accompany cell elongation and the deposition of the electron-dense surface coat.

Sporogenesis was observed to occur well before the cytokinetic portion of sporogony was complete. This was marked by the formation of numerous vacuoles within the cytoplasm of the cell and further thickening of the surface coat into an exospore (Fig. 18). These events were subsequently followed by the appearance of an elaborate Golgi network and the development of the polar filament (Fig. 19). Moniliform chains of uninucleate sporoblasts concurrently undergoing the final stages of sporogenesis were commonly observed (Fig. 20).

Mature spores (Figs. 21–24) were uninucleate and possessed a lamellate polaroplast with narrow tightly compressed lamellae anteriorly and wider less regularly arranged lamellae posteriorly. It occupied the anterior third of the spore. The polar filament was isofilar and singly coiled with 7–8 turns. The exospore was very thin (10 nm) and the endospore moderately thick (50 nm). The posterior vacuole occupied one-fourth of the spore. It was oval and filled with a membranous posterosome (Fig. 24).

Field Studies

The relative abundance of *L. athasaria* and natural prevalence of *O. lambdinae* are shown in Table 1. Early instar larvae were detected in the first week in July but no infection with *O. lambdinae* was found until July 22. A maximum infection rate of nearly 20% was observed in fourth and fifth instar larvae collected in mid-August. However, infection rates declined steadily throughout September to a low of only 2% just prior to pupation. We did not examine pupae. Larval density declined steadily during the same period of time that infection with *O. lambdinae* was increasing; however, there was no overt pathology that could be attributed to infection. The overall prevalence rate was 6.8% (n = 577).

DISCUSSION

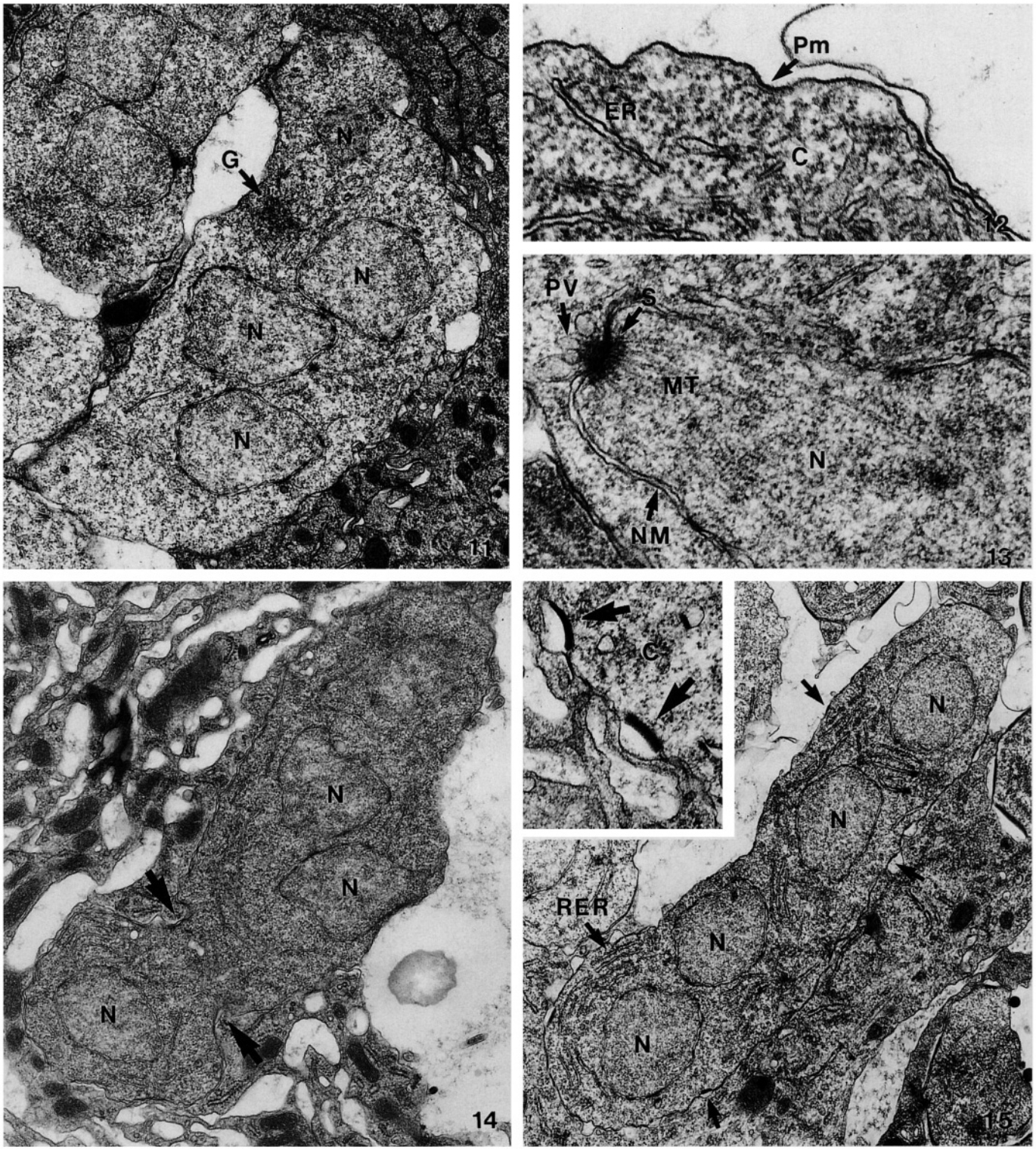
The microsporidium from *L. athasaria* described herein exhibits several morphological and developmental features with the type species *O. operophterae,* which would logically place it within the genus *Orthosomella* (Canning *et al.,* 1983, 1991; Sprague *et al.,* 1992). These similarities include all stages of schizogony and sporogony with unpaired nuclei; development in direct contact with the host cell cytoplasm; and multinucleate, ribbon (sausage)-shaped sporonts (sporogonial plasmodia) with linearly arranged nuclei giving rise to moniliform chains of unikaryotic sporoblasts and free spores.

Attributes that distinguish *O. lambdinae* from *O. operophterae* are summarized in Table 2 and reviewed immediately below. According to Canning *et al.* (1983), schizonts of *O. operophterae* are rounded, uni-, bi-, or tetranucleate and divide by binary fission. Our observations indicate that schizonts of *O. lambdinae* are more irregular in shape, have numerous nuclei (up to 29, with a median of 6), and divide by repeated plasmotomy. Furthermore, schizonts of *O. lambdinae* are limited by a simple plasmalemma and are not surrounded by an electron-dense surface coat that has been reported for *O. operophterae* (Canning *et al.,* 1985). The latter is an important finding because the universal presence of a thick surface coat in all stages of development is included in the current description of the genus. We accordingly propose that this feature be deleted as a generic characteristic to accommodate the present species.

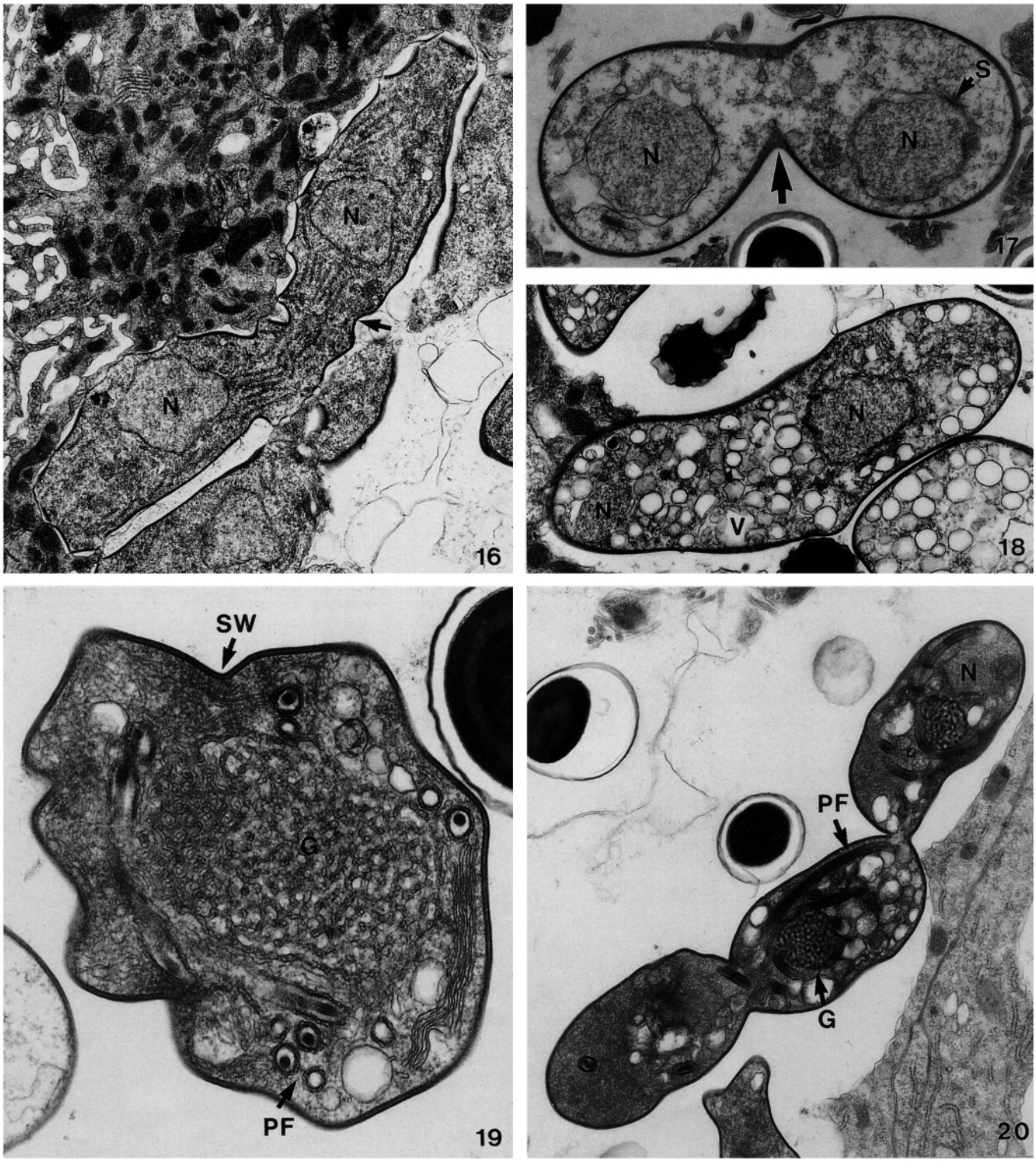
TABLE 1

Prevalence of *Orthosomella lambdinae* in a Larval Population of *Lambdina athasaria* at Devil's Hopyard State Park in East Haddam, Connecticut, 1993

Date collected	Dominant larval instars	Mean ± SE no. larvae/branch	No. collected and examined	Percentage infected with *O. lambdinae*
July 8	L_1, L_2	3.3 ± 0.5	119	0
July 22	L_2, L_3	1.3 ± 0.4	107	7.5
August 5	L_3, L_4	0.6 ± 0.2	106	4.7
August 19	L_4, L_5	0.2 ± 0.2	98	18.4
September 2	L_5, L_6	0.2 ± 0.1	48	12.5
September 16	L_6	0.1 ± 0.1	99	2.0



FIGS. 11–15. Electron micrographs of *Orthosomella lambdinae.*
FIG. 11. Multinucleate schizont (×16,500).
FIG. 12. High magnification of schizont cytoplasm (C) and plasmalemma (Pm) (×73,700).
FIG. 13. Dividing schizont nucleus (N) (×53,600).
FIG. 14. Schizont undergoing cytoplasmic division (arrows) (×15,800).
FIG. 15. Transitional stage leading to sporogonial plasmodia (×14,500). Note electron-dense thickenings on the plasmalemma (arrows). Inset ×35,500. ER, endoplasmic reticulum; G, Golgi apparatus; MT, microtubules; NM, nuclear membrane; PV, polar vesicles; RER, rough endoplasmic reticulum; S, spindle plaque.



FIGS. 16–20. Electron micrographs of *Orthosomella lambdinae.*

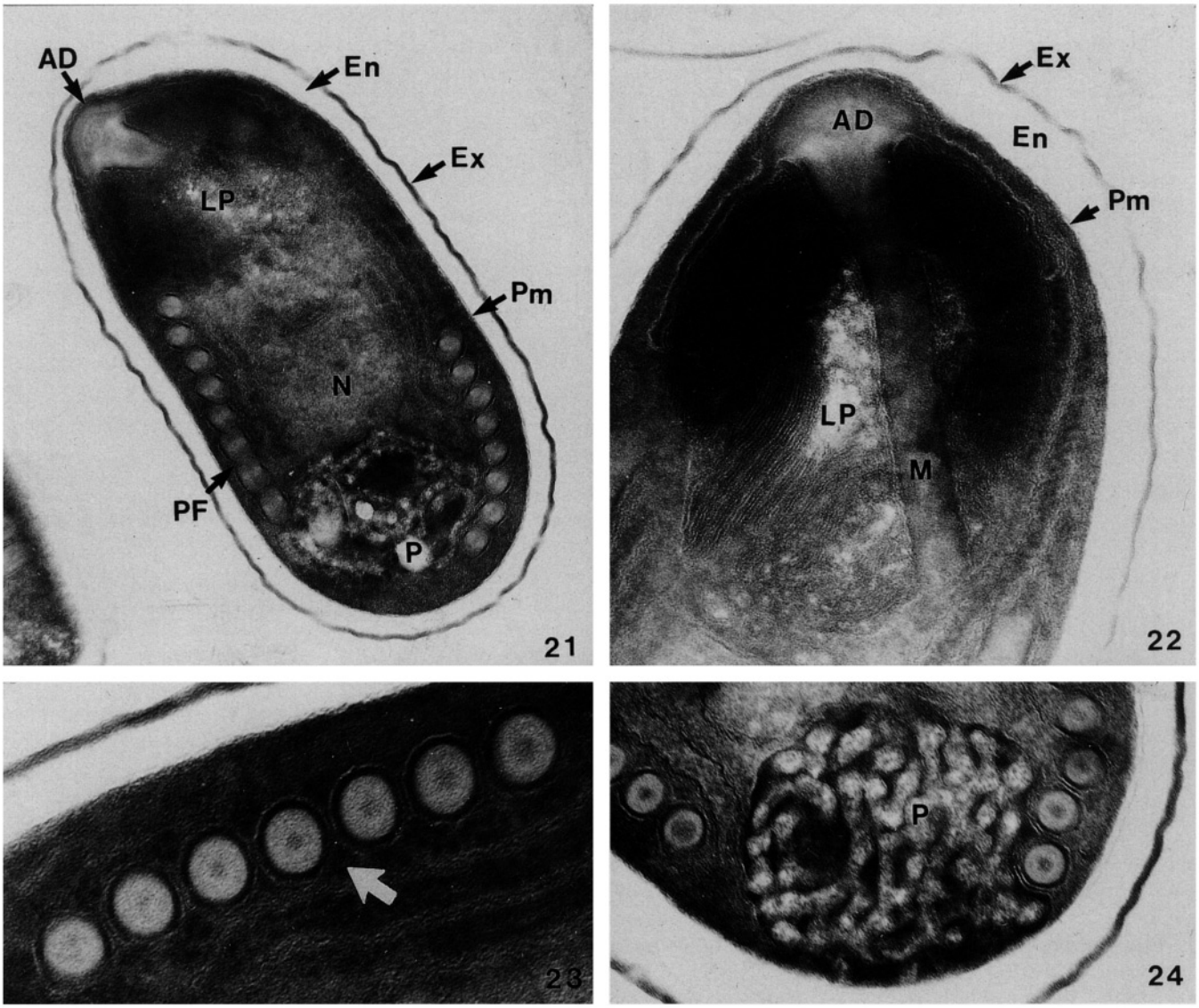
FIG. 16. Portion of a sporogonial plasmodium (×11,800). Note cytoplasmic constriction and electron-dense surface coat (early exospore) (arrow).

FIG. 17. Sporogonial stage showing signs of nuclear and cytoplasmic division (large arrow) (×17,500).

FIG. 18. Late stage sporogonial plasmodium showing numerous vacuoles (V) within the cytoplasm (×16,400).

FIG. 19. Sporoblast in advanced stage of sporogenesis showing reticulate Golgi apparatus (G) and differentiation of polar filament (PF) and exospore of spore wall (SW) (×42,000).

FIG. 20. Moniliform chain of sporoblasts in the process of sporogenesis (×16,300). N, nucleus; PF, polar filament; S, spindle plaque.



FIGS. 21–24. Electron micrographs of mature spores of *Orthosomella lambdinae.*
FIG. 21. Sagittal section of entire spore (×52,200).
FIG. 22. Higher magnification of polaroplast region (×93,700).
FIG. 23. Polar filament (arrow) (×144,700).
FIG. 24. Posterosome (P) (×88,200). AD, anchoring disc; En, endospore; Ex, exospore; LP, lamellar polaroplast; M, manubroid portion of the polar filament; N, nucleus; PF, polar filament; Pm, plasmalemma.

Differences in the nucleation of sporogonial plasmodia were apparent as well. In *O. operophterae* the sausage-shaped stages have 2, 4, 8, or rarely 12 linearly arranged nuclei, and plasmodia with odd numbers of nuclei are rare (Canning *et al.,* 1983). In *O. lambdinae,* sporogonial plasmodia generally possess a greater number of nuclei (up to 18, with 6 as the most common), and stages with odd numbers are typical. The odd number of nuclei suggests asynchronous nuclear division.

Sporogony and sporogenesis also appear to be significantly different. In *O. operophterae,* the cytokinetic portion of sporogony is complete before sporogenesis is initiated; sporogonial plasmodia divide into binucleate forms, which then divide into uninucleate sporoblasts (Canning *et al.,* 1983). In *O. lambdinae,* the sporogonial plasmodia do not undergo cytoplasmic division before they initiate sporogenesis but rather transform directly into a chain (or a few shorter chains) of sporoblasts with a corresponding number of nuclei. These never become isolated sporoblasts but undergo sporogenesis, after which the young spores separate; thus sporogony and sporogenesis are concurrent. We also note that the "whorl-like" membranous structures ob-

TABLE 2

Summary of Morphological and Developmental Characters Used to Distinguish *Orthosomella operophtera* and *Orthosomella lambdinae*

	Orthosomella operophterae	*Orthosomella lambdinae*
Host	*Operophtera brumata*	*Lambdina athasaria*
Site of infection	Silk gland, gut and other tissues	Midgut
Schizonts	Rounded, usually uni-, bi-, or tetranucleate	Irregular and multinucleate with up to 29 nuclei (most with 6)
Schizont surface	Plasmalemma covered by an electron-dense surface coat	Plasmalemma not covered
Schizogony	Binary fission	Plasmotomy of paucinucleate plasmodia
Sporogonial plasmodia	Moniliform, with 2, 4, 8, or rarely 12 linearly arranged nuclei	Moniliform, ribbon-shaped, with up to 18 linearly arranged nuclei (most with 6)
Sporogony	Plasmodia divide into binucleate forms and these divide into uninucleate sporoblasts; complete before sporogenesis	Plasmodia divide into uninucleate forms (young spores) directly; accompanied by sporogenesis
Spore	3.5 × 1.3 μm polar tube with 6–7 turns	2.8 × 1.5 μm polar tube with 7–8 turns

served by Canning *et al.* (1985) in the cytoplasm of almost all vegetative stages of *O. operophterae* were never seen in any stages of *O. lambdinae.*

A comprehensive comparison of the ultrastructural morphologies of the spores of the two species is not possible because of the poor preservation of *O. operophterae* (Canning *et al.,* 1985, Fig. 27). However, differences in spore size (*O. lambdinae* is 0.7 μm shorter in length on average) and in the number of turns in the polar filament (7–8 vs 6–7) were evident. The structure of the polaroplast and spore wall appeared to be superficially similar.

The natural prevalence of *O. lambdinae* infection in larval populations of *L. athasaria* (6.8% overall) was considerably less than the 30–40 and 26% recorded for *O. operophterae* in the winter moth, *O. brumata* (Canning *et al.,* 1985). Unfortunately, we were unable to obtain any experimental data on infectivity, virulence, or transmission. However, based on the site of host infection (midgut epithelium) and lack of obvious mortality in the field, we strongly suspect that *O. lambdinae* is orally transmitted between larvae of the same generation via ingestion of spores and is only mildly to moderately pathogenic. There is no published data on the pathogenicity of *O. operophterae* to *O. brumata;* however, vertical transmission via ingestion of spore-contaminated eggs has been reported (Canning and Barker, 1982).

TAXONOMIC SUMMARY

Orthosomella (Canning, 1960) Canning, Wigley & Barker, *1991, Amended Diagnosis*

All stages of schizogony and sporogony with unpaired nuclei.

Development free within the hyaloplasm of the host cell.

Sporogonial plasmodia multinucleate and sausage-shaped with linearly arranged nuclei giving rise to moniliform chains of unikaryotic sporoblasts and free spores.

Orthosomella lambdinae, n.sp.

Type host. *Lambdina athasaria* (Walker) (Lepidoptera, Geometridae).

Transmission. No data but presumed to be *per os.*

Site of infection. Midgut epithelium of larva.

Interface. No interfacial envelope before appearance of exospore at the onset of sporogenesis.

Schizogony. All stages with unpaired nuclei. Schizont plasmodia rounded to irregular in shape with a variable number of nuclei (2–29, mean = 7, median =

6). Limited by a simple plasmalemma and division by plasmotomy of paucinucleate plasmodia.

Schizogony–sporogony transition. Transitional stages irregularly shaped, multinucleated plasmodia with finger-like projections. Plasmalemma with patchy thickenings.

Sporogony. Sporogonial plasmodia elongate and sausage-shaped with a variable number (2–18, mean = 7, median = 6) of linearly arranged nuclei. They become constricted between nuclei to produce moniliform chains of uninucleated sporoblasts with a corresponding number of nuclei. Sporoblasts become covered by an electron-dense coat that forms the exospore. Sporoblasts concurrently undergo sporogenesis prior to cytoplasmic cleavage into free spores.

Spore. Uninucleate. Oblong to slightly reniform and measuring 2.6 × 1.5 μm (stained) and 2.8 × 1.5 μm (live). Exospore is thin (10 nm), and exospore is moderately thick (50 nm). Polaroplast lamellate with two regions and occupying anterior third of spore. Polar filament isofilar and singly coiled with 7–8 turns. Posterior vacuole oval, filled with a membranous complex (posterosome) and occupying one-fourth of the spore.

Type locality and prevalence. Devil's Hopyard State Park (East Haddam, CT). Natural prevalence of 2.0–18.4% (6.8% overall) in larval populations.

Deposition of type materials. A syntype slide has been deposited in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, DC (USNM No. 47824). Additional syntype slides and type specimens embedded in plastic resin are also in the collection of the senior author.

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