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Nosema calcarati n. sp. (Microsporidia), a new parasite of *Pityogenes calcaratus* Eichhoff (Col., Scolytidae)

By K. PURRINI and J. HALPERIN

Abstract

A new microsporidian *Nosema calcarati* is described from larvae, pupae and adult bark beetles *Pityogenes calcaratus*. The reproductive organs (male and female gonads) were the principal site of infection. The morphology of some stages in the life cycle of this microsporidian is described by light and electron microscopy. The fine structure was similar to that which has been reported for other microsporidian parasites of the genus *Nosema*, but there were singular features in the ultrastructure of the spore, especially of the posterior vacuole. This was bipartite and surrounded by a layer perforated by pores. Some data on prevalence of infection and host-parasite relationships are discussed.

1 Introduction

Various protozoan parasites were found during investigations of pathogenic agents in natural population of bark beetles, including *Pityogenes calcaratus* Eichhoff in Israel, 1980. The beetle *P. calcaratus*, is one of the most widely distributed and economically important pest infesting pine trees in this region (HALPERIN et al. 1982). In our material of *P. calcaratus*, three protozoan parasites: an amoeba (*Endamoeba* sp.), a neogregarine (*Ophryocystis* sp.), and a new microsporidian of the genus *Nosema* were found to infect the gut lumen *(Endamoeba)*, Malpighian tubules *(Ophryocystis)*, and reproductive organs *(Nosema)* of host animals. In the present paper only the *Nosema*, its morphology and development, examined at light and electron microscopic levels, is described.

2 Material and methods

In 1980 several samples of four species of bark beetles (350 specimens of *P. calcaratus*, 150 of *Orthotomicus erosus* Wall., 40 of *Tomicus destruens* Wall., and 20 of *Phloeosinus armatus* Reitter) infesting pine trees in Israel were collected and brought to the laboratories of Institute of Forest Zoology, University of Goettingen. They were examined for parasites by dissection of all organs using a light microscope. Distribution of parasites in the organs of host-animals were recorded. Dry smears were fixed with methanol and stained with Giemsa's stain. Infected reproductive organs of *P. calcaratus* were fixed in Bouin's fluid, sectioned (3–4 µm thick) and stained with Heidenhain's iron haematoxylin. The ultrathin sections (0.5–1.0 µm thick) of SPURRS blocks were stained with Toluidene blue. For electron microscopy, the dissected gonads were fixed in phosphate buffered glutaraldehyde (2 % glutaraldehyde in 0.1 M phosphate buffer pH 7.5) washed twice in 0.1 M cacodylate and 5 % sucrose; postfixed in 2 % osmium tetroxide in veronal acetate buffer and dehydrated in acetone series from 30 % to 100 %. After dehydration the material was placed in propylene oxide for 3 minutes. Finally the gonads were embedded in SPURR's low viscosity resin, sectioned and stained on, after contrasting with uranyl acetate and lead citrate. The sections were photographed using a PHILIPS EM 301 electron microscope.

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3 Results

3.1 Description of the parasite

3.1.1 Light microscopy (fig. 1–11)

Earliest forms observed were relatively large bi- and tetranucleate schizonts (figs. 1 and 2). Dividing diplokaryotic cells (diplokarya) with vesicular nuclei were also observed (figs. 3 and 4). The absence of other vegetative stages in our preparations were, we suppose, indicative of a short schizogony or this is perhaps due to the small dimensions of the host's reproductive organs (0.05 mm) which limits the space available for the parasite. In fresh (figs. 5 and 7) and stained preparations (figs. 8, 10, and 11) of infected tissues, the spores were oval. When fresh the spores measured 3.5–5.0 × 2.5–3.0 µm. In Giemsa's stained smears a large light aerea at the broad pole of the spore represent the posterior vacuole (fig. 8).

3.1.2 Electron microscopy (figs. 12–16)

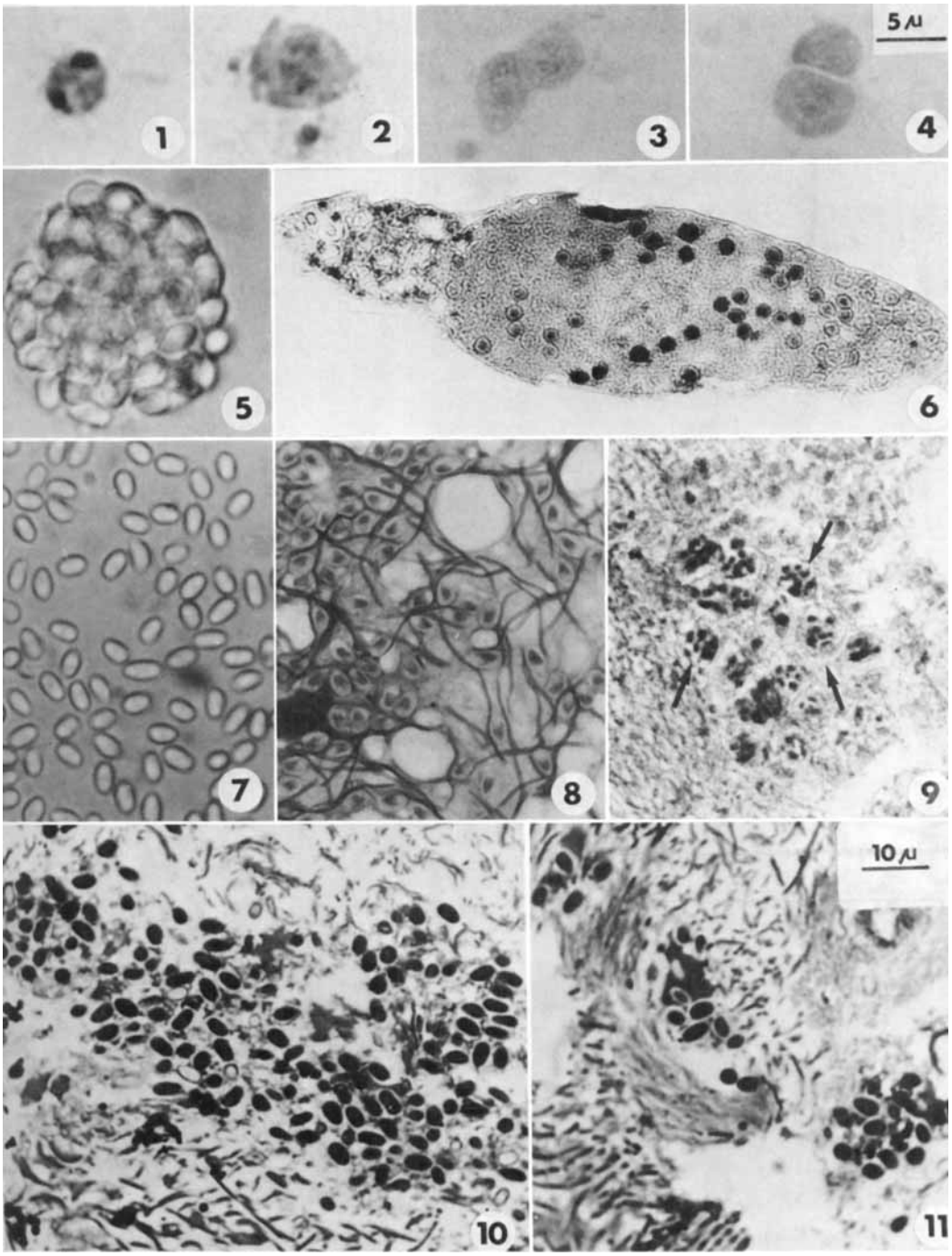
Figure 12 represent infected cell of the testes which appear as pseudocyst containing sporoblasts. They are characterized by an electron dense limiting layer, very electron dense coils of the developing polar filament, posterior vacuole of the same density and the presence of several parallel lamellae evidently belonging to the Golgi apparatus. Ultrastructure of the spore is represented in figs. 13–16. The spore has a coat of two layers of medium density, the outer one of which is being rugose. Beneath these two layers is a thick, transparent endospore. There are 12 coils of equal diameter of the polar filament in the spore. Two nuclei appearing as elongated bodies are centraly situated in the spore (fig. 13). The posterior vacuole shown in figs. 14–16, is one-fourth to one-fifth spore length, limited by a layer which is perforated by pores. The contents of the vacuole is a dark dense material (figs. 13 and 14) or irregularly granular (figs. 15 and 16) with some transparent aereas within it. Being unit zone at initial stages, the posterior vacuole becomes bipartite with spore maturation.

3.2 Host-parasite relationship and prevalence of infection

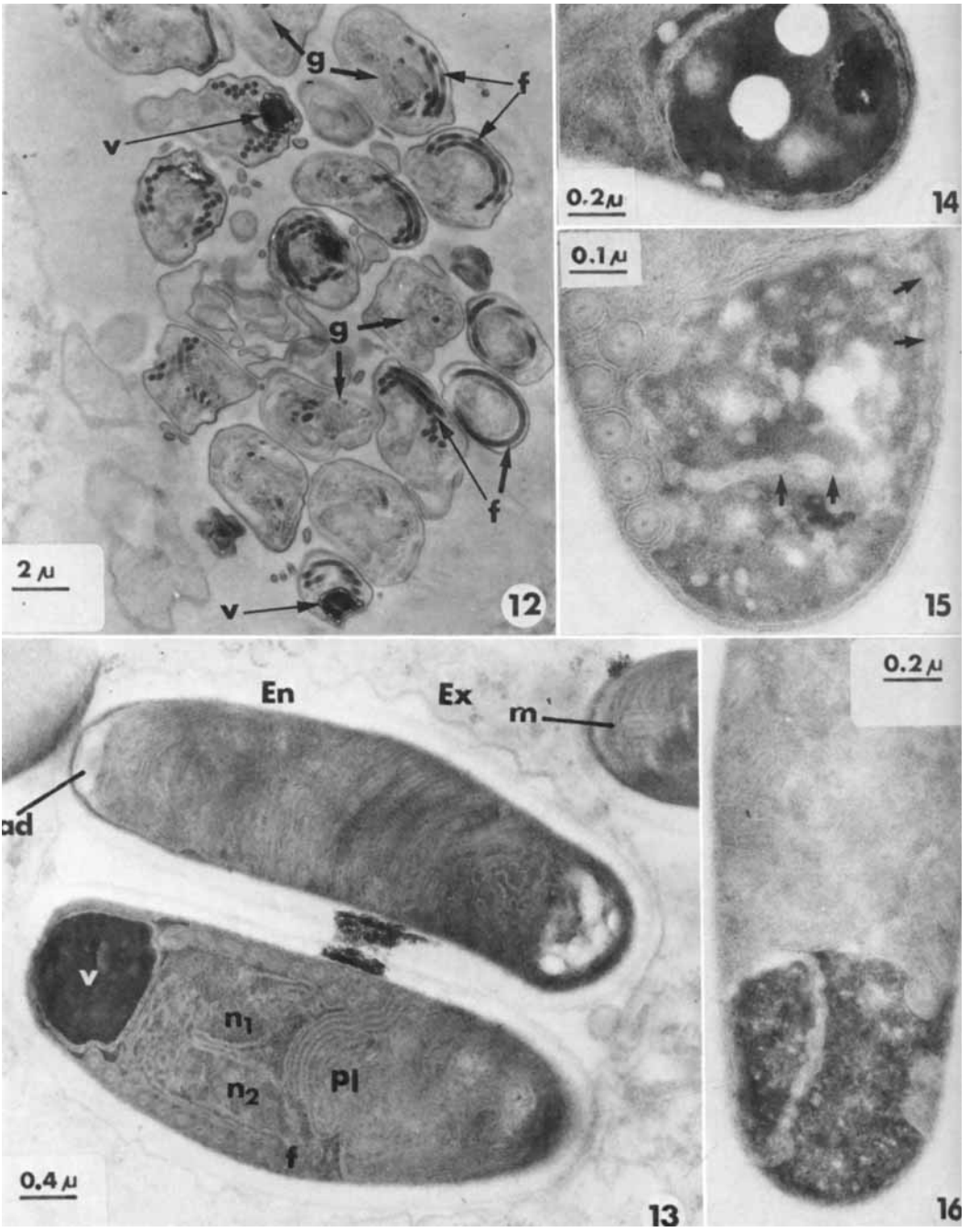
The microsporidian was found in male and female gonads of last instar of the larvae, pupae and adult *P. calcaratus*. The parasite did not induce any external symptoms in its host. The invaded ovaries appeared to by hypertrophied and transparent (fig. 6). Heavily infected cells of the testes appeared as pseudocysts containing the spores (fig. 5). Male gonads totally destroyed when heavily infected are represented in figs. 8, 10, and 11. Prevalence of infection caused by microsporidian parasite was considerably high, 50 % of 350 examined specimens of *P. calcaratus*.

4 Discussion

Our observations with the light microscope in the life cycle of microsporidian *N. calcarati* revealed binucleate and tetranucleate schizonts, and dividing



Figs. 1–11. Nosema calcarati n. sp. infecting gonads of *Pityogenes calcaratus*. Light microscopy. 1 Binucleate schizont; 2 Tetranucleate schizont; 3, 4 Dividing diplokarya with nuclei in paired arrangement; 5 Infected testes cell (pseudocyst) containing microsporidian spores; 6 Female gonads (ovarian) filled with microsporidian spores; 7, 8 Mature spores; 9 A part of healthy gonads spermatocytes visible (arrows); 10, 11 Destroyed male gonads filled with masses of spores. (Figs. 1–4: Giemsa's stain, 5 and 7: Fresh, 6 and 9: Heidenhain's iron haematoxylin, 10 and 11: Toluidene blue



Figs. 12–16. Nosema calcarati n. sp. infecting gonads of *Pityogenes calcaratus*. Electron microscopy. 12 Sporoblasts showing very electron dense coils of the developing polar filament (f), posterior vacuole (v), and some parallel lamellae possibly belonging to the Golgi apparatus (g); 13 Mature spore showing exospore (Ex), endospore (En), two nuclei (n_1, n_2), extrusion apparatus consisting of anchoring disc (ad), manubrium (m), and lamellar polaroplast (Pl), and the vacuole (v); 14 Posterior vacuole surrounded by a layer, a dark dense material of its content remarcable; 15 Bipartite posterior vacuole. The vacuole is surrounded by layer which appear to be perforated by pores (arrows). Its irregularly granular contents with some transparent aerea are evident; 16 Bipartite posterior vacuole

diplokarya with nuclei in constant paired arrangement. According to SPRAGUE and VERNICK (1971) this is essential characteristic of the genus *Nosema*.

At the electron microscope level, except for sporoblasts and spores, other developmental stages could not be observed. Generally, the fine structure of the sporoblasts and spores of *N. calcarati* resembled the microsporidians known from other arthropods, described by ISHIHARA (1970), SPRAGUE et al. (1968), SPRAGUE and VERNICK (1969), and VAVRA (1976). Our studies have underlined some new details of ultrastructure of the posterior vacuole which is one-fourth to one-fifth of the spore length. Similar to the microsporidian *Vavraia collembolae* n. sp. (PURRINI, unpubl.), the posterior vacuole of *N. calcarati*, being single (unit zone) at initial phases becames bipartite with spore maturation. The vacuole was surrounded by a layer, but its detailed structure was difficult to resolve. The layer appears as irregular sheth of electron transparent material perforated by pores and protruding from cytoplasm of the spore. The contents of posterior vacuole in our ultrathin sections was more electron dense than the rest of spore cytoplasm.

Species differentiation of the microsporidian infecting reproductive organs of bark beetles *P. calcaratus* has been based upon the development, site of infection and host-parasite relationships to the species listed in the table. Up to now, eight microsporidian parasites were reported from different bark beetles as a rather rare and focale infections. The *Nosema calcarati* n. sp. is proposed because of differences in the development, site of infection and host-parasite relationships from other microsporidians listed in the table.

Comparison of microsporidians reported from bark beetles (Scolytidae, Coleoptera)

Parasite	Host	Tissue	Spore (in microns)	Author	Locality
Nosema typographi	*Ips typographus*	fat body	3.6–5.3×2.0–3.5	WEISER 1955	Czechoslovakia
Nosema curvidentis	*Pityokteines curvidens*	fat body	2.5–3.6×1.5–2.0	WEISER 1961	Czechoslovakia
Pleistophora scolyti	*Scolytus scolytus*	midgut	3.0×2.0	WEISER 1968	Czechoslovakia
Stempellia scolyti	*S. scolytus*, *S. multistriatus*, *S. ensifer*, *S. pygmaeus*	midgut	2.6–3.6×1.0–2.0	LIPA 1968	Poland, USSR, Germany, Czechoslovakia
Nosema scolyti	*S. scolytus*, and other *Scolytus* spp.	Malpighian tubules, midgut	4.0–5.0×2.0–3.3	LIPA 1968	Poland, USSR, Germany
Nosema dendroctoni	*Dendroctonus pseudotsugae*	Malpighian tubules, fat body	2.6–3.0×2.0–1.0	WEISER 1970	Canada
Nosema dryocoetesi	*Dryocoetes autographus*	fat body, lymphocytes	2.5–4.0×2.0–2.3	PURRINI and ORMIERES 1981	Germany
Pleistophora xyloteri	*Xyloterus domesticus*	midgut, oenocytes	2.5–3.0×1.2–1.5	PURRINI and ORMIERES 1981	Germany
Nosema calcarati n. sp.	*Pityogenes calcaratus*	male and female gonads	3.5–5.0×2.5–3.0	this paper	Israel

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Zusammenfassung

Nosema calcarati n. sp. (Microsporidia), ein neuer Parasit von Pityogenes calcaratus Eichh. (Col., Scolytidae)

Eine neue Mikrosporidie, *Nosema calcarati* n. sp., aus den Larven, Puppen und Käfern von *Pityogenes calcaratus* Eichhoff wird beschrieben. Der Parasit befällt die Geschlechtsorgane der Wirte. Die Morphologie von einigen Entwicklungsstadien der Mikrosporidie wird auf Grund von licht- und elektronenmikroskopischen Untersuchungen dargelegt. Die Ultrastruktur der Sporoblasten und Sporen stimmt mit denen in anderen bekannten Mikrosporidien-Arten der Gattung *Nosema* überein. *N. calcarati* unterscheidet sich aber in einigen charakteristischen Merkmalen der Vakuole, wie die Ultradünnschnitte zeigen. Die Vakuole von *N. calcarati* war zweiteilig, umhüllt von einer durch Poren perforierten Schicht. Über die Infektionsrate der Mikrosporidie wird berichtet.

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