die Temperaturen unter 17 °C sanken, hörte ihre Fortpflanzung beinahe auf. Charakteristisch für *A. graminis*-Populationen sind ein Höhepunkt im Sommer in der semi-ariden Zone und 2 Höhepunkte, im Sommer und Winter, in der ariden Zone.

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On three new sporozoan parasites of bark beetles (Scolytidae, Coleoptera)

By K. PURRINI and R. ORMIÈRES

Abstract

Three new sporozoan parasites: one neogregarine *Ophryocystis hylesini* n. sp. (Ophryocystidae, Neogregarinida), and two microsporidians, *Pleistophora xyloteri* n. sp. (Pleistophoridae, Microsporidia), and *Nosema dryocoetesi* n. sp. (Nosematidae, Microsporidia) were described from natural populations of three species of bark beetles, *Hylesinus fraxini* Panz., *Xyloterus domesticus* L., and *Dryocoetes autographus* Ratz. (Scolytidae, Coleoptera).

1 Introduction

Various parasites were discovered during investigations of pathogenic organisms present in certain forest insects including the bark beetles in Lower Saxony (Federal Republic of Germany), 1978–1979. In a survey of the patho-

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gens present in natural populations of three species of bark beetles, *Hylesinus fraxini* Panz., *Xyloterus domesticus* L., and *Dryocoetes autographus* Ratz. reported the presence of three new sporozoan parasites: one neogregarine and two microsporidians.

In this paper studies on the morphology, development and distribution of the parasites, host range, and pathogenicity are described.

2 Material and methods

Specimens of *H. fraxini*, *X. domesticus*, and *D. autographus* (larvae, pupae and adults) were collected by the first author in June and July of 1978, October and November of 1979 at different localities in Lower Saxony, Federal Republic of Germany. Samples were brought to the laboratory at the Institute of Forest Zoology, University of Goettingen and investigated by dissecting and separate examination of all organs. When spores of microsporidians and sporocysts of neogregarine were found, the material was spread in dry smears for Giemsa or wet smears for Heidenhains iron haematoxylin staining. In more common infections, some animals were fixed in Bouin and processed in paraffin sections 6–7 μm thick, stained with Heidenhains iron haematoxylin. The size of developmental stages of parasites were evaluated in fresh and stained preparations with a light microscope using a normal micrometer ocular. Data on hosts, localities, frequency of infections and infected tissues were recorded. In rare infections it was neither possible to prepare material for section preparations nor to provide experimentally vegetative stages when they were not present in origine isolates. With microsporidian parasites, parts of the Giemsa stained smears were treated with boiling 10 % HCl and restained for 2–3 minutes with Giemsa to differentiate the nuclei. In this way spores of *Pleistophora* in the bark beetle, *X. domesticus* were distinguished when only the spores were present.

3 Results

Ophryocystis hylesini n. sp. (Plate I, figs. 1–22)
Host: *Hylesinus fraxini* Panz.
Tissue: Malpighian tubules
Locality: Bleckede, Lower Saxony (Federal Republic of Germany), 1978

This parasite, recorded from live and dead adults of the bark beetle, *H. fraxini*, (Ophryocystidae, Neogregarinidae).

The infection was localized in the cavity of Malpighian tubules; they were disintegrated and filled with vegetative stages and cysts of the parasite. The presence of a considerable number of developmental stages and cysts (although some elements of the life cycle of the parasite were absent) permits us to describe it in detail. Some young stages, typical of the genus *Ophryocystis*, the radicelles which constricted during fixation and which could be seen only in live material, could not be observed in our Giemsa and Heidenhains iron haematoxylin stained preparations. The vegetative stages, directly developed from sporozoits, are spherical with cytoplasm formed a honey-comb like, measuring 7–8 μm in diameter (figs. 1, 2, 22). Growth of these stages continued during syzygy. The fusion of two of these stages gives rise to a connected copula (syzygy) measuring 10 × 8 μm (figs. 3, 4, and 16–18). The separation line in the syzygy is flattened. At the beginning both stages have only a single nucleus which multiplies and stages with two nuclei in each of them are produced (figs. 5, 6). Only one of filial nuclei gives rise to a second division,

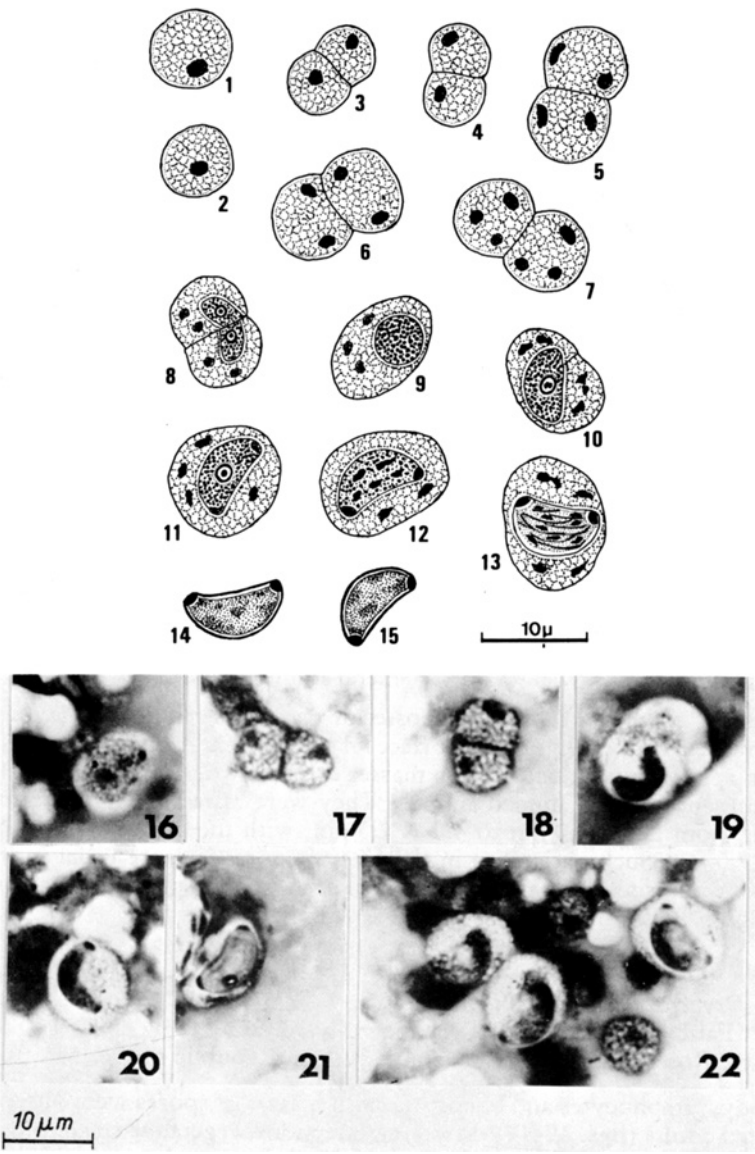

Plate I. Figs. 1–22: Ophryocystis hylesini n. sp. (figs. 1–15: drawings; figs. 16–22: microphotographs, Heidenhains iron haematoxylin, ca 3000×). – 1, 2 = mature vegetative stages (individuals); 3, 4 = two uninucleate stages forming copula; 5, 6 = two binucleate individuals in copula; 7 = copula with 6 nuclei, one nucleus in each individuals divides; 8 = formation of two gametes; 9 = spherical sporoblast in gametocyst; 10, 11 = elongated sporoblasts in gametocysts where residues disappear; 12, 13 = two gametocysts, each of them containing a single sporocyst. Sporozoits are produced inside the sporocysts; 14, 15 = free thick-walled sporocysts with a thick part at both poles; 16–18 = copula of two uninucleate stages (individuals); 19–21 = three gametocysts, each containing a single sporocyst of "half-moon like" form; 22 = two rounded vegetative stages and three gametocysts, each containing a single sporocyst of "half-moon like" form

and three nuclei in each of copulated vegetative stages (altogether six nuclei in copula) are produced again. Four of the six newly produced nuclei in the syzygy (fig. 7) degenerate, and the other two nuclei are isolated near the place of their copulation with a portion of cytoplasm, each of them producing a single gamete (fig. 8). Copulation of two gametes creates one copula which develops in a spherical sporoblast with granular cytoplasm, measuring 5 μm in diameter (fig. 9). Little by little, the sporoblast stretches out, showing one flat and one big-bellied side (figs. 10, 11, and 19–22); the single nucleus produced by the fusion of two nuclei in copula contains a well visible karyosome, where the copular partition is immediately absorbed. The elongated sporoblast reaches the size 8 μm in length and 4 μm in width, and the residues of the nuclei are still visible in the cytoplasm. Gradually, the sporoblast is transformed into a sporocyst, while the multiplying nucleus in the sporoblast produces 8 sporozoits (figs. 12, 13). The cytoplasm of the exospore disappears and the sporocyst becomes free. The sporocysts measuring 8.5–9 μm in length and 4–5 μm in width, are characterized by a particularly curved form ("half-moon like" form) having two very thick parts in each pole (figs. 14, 15, and 19–22). This is a resistent stage which can become the beginning of a new infection if it is swallowed by the new host.

Pleistophora xyloteri n. sp. (Plate II, figs. 15–16)
Host: *Xyloterus domesticus* L.
Tissue: gut wall, oenocytes
Locality: Einbeck, Lower Saxony (Federal Republic of Germany), 1978

Infection is spread in cells of the posterior part of the midgut. The infection also invaded oenocytes on the surface of the gut and the infected elements formed spherical pseudocysts with masses of spores (figs. 15, 16). Only spores were present in the examined animals. They were broad, oval, in dimensions varying from 2.5 × 2.0 μm to 4.0 × 2.3 μm, with the most common size of 3.0 × 2.0 μm. Such variability in the size of spores is quite usual in microsporidian parasites of bark beetles (table). A single spherical nucleus was staining in the second third of the spore length.

Nosema dryocoetesi n. sp. (Plate II, figs. 1–13)
Host: *Dryocoetes autographus* Ratz.
Tissue: Fat body, lymphocytes, oenocytes
Locality: Rosengarten, Lower Saxony (Federal Republic of Germany), 1979

Fat body, lymphocytes and oenocytes with masses of spores were observed in dissected adults (figs. 12–14). In young infections, vegetative stages are distributed throughout the cells of the fat body and the cytoplasm of lymphocytes (fig. 12), later growing and dividing so much that pseudocysts with several hundred spores are formed (figs. 13, 14). The earliest stages of the parasite found in some Giemsa stained smeares were binucleate (figs. 1, 2) and tetranucleate schizonts (figs. 3–5), spherical or oval in shape, measuring 2–7 μm in diameter; stages "diplokaryon like" measuring 2.5–5 μm in diameter were also observed (fig. 6). Sporonts were oval or round cells with light and dark areas of cytoplasm (figs. 7–10). They measured 3–12 μm in diameter. Sporonts were uninucleate, binucleate or tetranucleate and produces by nuclear and cytoplasmic division to binucleate sporoblasts (fig. 11) measuring 3–4.5 × 2.0 μm. They gave rise directly to spores. In Giemsa stained prepara-

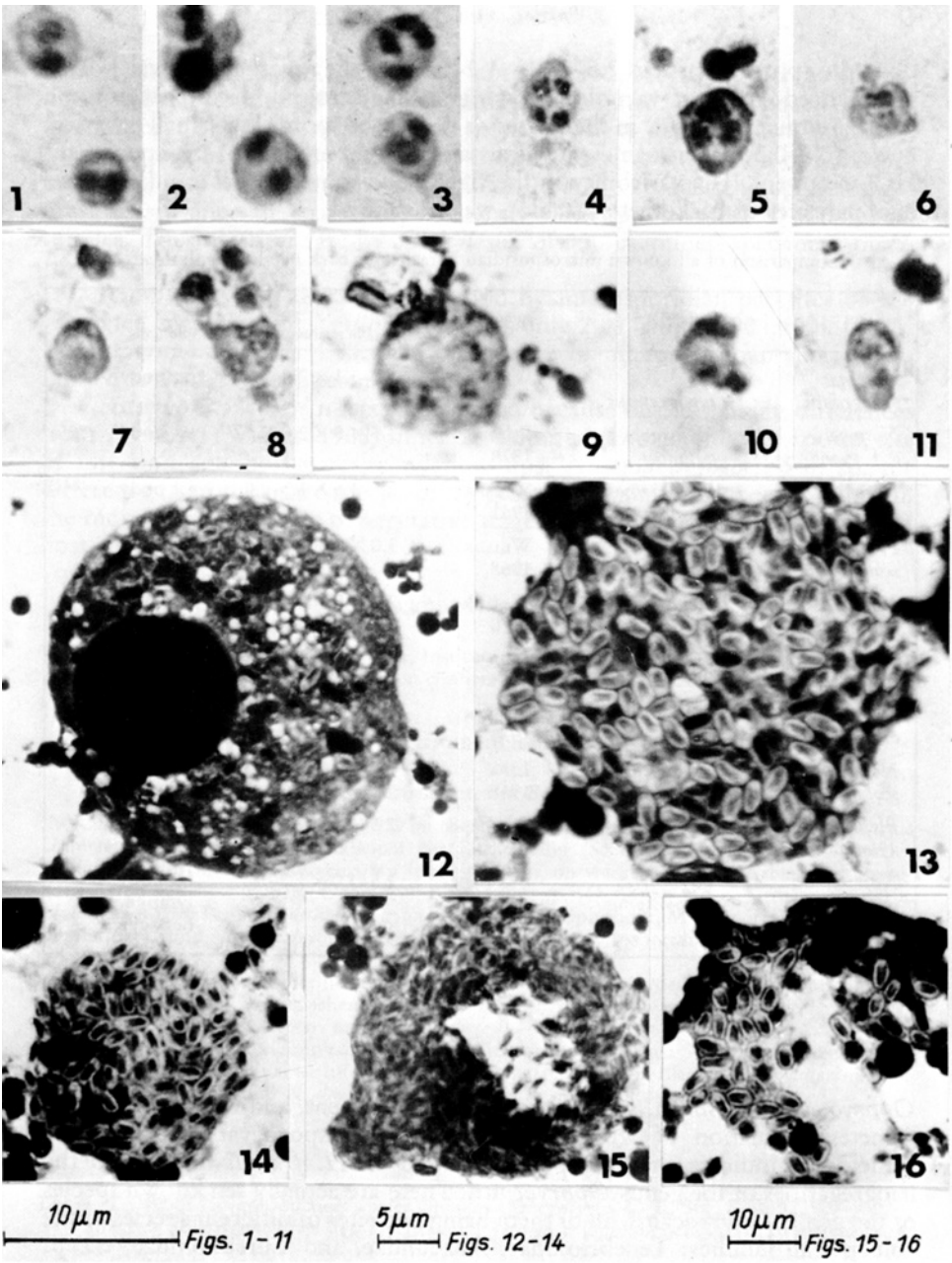

Plate II. Figs. 1–14: Nosema dryocoetesi n. sp. (figs. 1–14: Giemsa stain, 1–11: ca 5000 ×; 12, 13: ca 4000 ×, 14: ca 2500 ×). – 1, 2 = binucleate schizonts; 3–5 = tetranucleate schizonts; 6 = diplokaryon; 7 = uninucleate sporont; 8 = binucleate sporont during division; 8 = tetranucleate sporont; 10 = dividing sporont; 11 = sporoblast; 12 = lymphocyte, cytoplasm with masses of young stages and spores is visible; 13 = pseudocyst (from lymphocyte) full of mature spores; 14 = pseudocyst (from oenocyte) full of mature spores. – *Figs. 15–16: Pleistophora xyloteri* n. sp. (figs. 15, 16: Giemsa stain, ca 2000 ×). – 15, 16 = two pseudocysts (from oenocytes) full of mature spores, in fig. 16 the spores are dispersed (scattered)

tions the spores measured 2.5–3.0 × 1.2–1.5 μm; they were long, oval, with a pronounced anterior vacuole, showing a small round dark posterosome (0.4–0.5 μm in diameter) in the posterior end of the spore. Two small spherical nuclei (0.2–0.3 μm in diameter) were stained in the middle of the spore after HCl treatment. The variability in the size of the spores, usual in microsporidian parasites of bark beetles (table), was not found.

Comparison of all known microsporidian parasites of bark beetles (Scolytidae)

parasites	host	author	spore sizes in microns (μm)	site of infection
Nosema typographi	*Ips typographus*	WEISER 1955	3.6–5.3 × 2.0–3.5	fat body
Nosema c. f. *typographi*	*Hylurgops palliatus*	PURRINI 1978	3.5–4.5 × 2.0–2.5	fat body
Nosema curvidentis	*Pityokteines curvidens*	WEISER 1961	2.5–3.6 × 1.5–2.0	fat body
Pleistophora scolyti	*Scolytus scolytus*	WEISER 1968	3.0 × 2.0	midgut
Nosema dendroctoni	*Dendroctonus pseudotsugae*	WEISER 1970	2.0–3.0 × 2.1	midgut
Stempellia scolyti	*S. scolytus* *S. multistriatus*	LIPA 1968	2.6–3.6 × 1.0–2.0	midgut
Stempellia c. f. *scolyti*	*S. scolytus*	PURRINI 1975	2.0–3.6 × 1.0–1.5	midgut
Nosema scolyti	*S. scolytus* *S. multistriatus*	LIPA 1968	4.0–5.0 × 2.0–3.3	Malpighian tubules
Pleistophora xyloteri n. sp.	*Xyloterus domesticus*	this paper	2.5 × 2.0 to 4.0 × 2.2	gut wall, oenocytes
Nosema dryocoetesi n. sp.	*Dryocoetes autographus*	this paper	2.5–3.0 × 1.2–1.5	fat body, lymphocytes, oenocytes

4 Discussion

Ophryocystis hylesini n. sp. The habitat, development, and formation of two gametes copulation of which produces a single sporocyst from a single gametocyst indicate that the parasite found in *H. fraxini* belongs to the neogregarines of the genus *Ophryocystis*. There are actually ten known species of the genus *Ophryocystis*, all of them being parasites of different species of the coleopteran families: Tenebrionidae, Alleculidae, and Curculionidae, except *O. dendroctoni* Weiser 1970, which is associated with a scolytid beetle, *Dendroctonus pseudotsugae* (Scolytidae). *Ophryocystis hylesini*, the second *Ophryocystis* parasitizing a scolytid host, differs from the other species of this genus by the shape of the sporocyst. In all earlier described species the sporocyst is more or less big-bellied and elongated, but always uniform, while the "half-moon like" form of the sporocyst of *O. hylesini* is always evident. As rhizoid stages have been observed neither by WEISER (1970) nor by us, it is suggested that they may be absent from the life cycle of the genus *Ophryocystis*

in Scolytidae. Schizogonic stages were not observed, because sexual multiplication had been terminated in the host-animals examined. It is supposed that the development of *O. hylesini* occurs in the same way as described by ORMIÈRES, MASSOT, and MASSOT (1978), i.e. by means of a simple division of a mother individual into many daughter individuals. In any case, the schizogonic stages must exist, since the parasite persistently develops in infected Malpighian tubules. The infection rate by *O. hylesini* of 350 examined specimens of *H. fraxini* was only 2 %.

Microsporidians. The rate of infection by microsporidian parasites of 480 examined specimens of *X. domesticus* was only 2 %, and in 690 individuals of *D. autographus* it was 4 %. The occurrence of microsporidian species was limited to 2 of 11 localities investigated.

Pleistophora xyloteri n. sp. is proposed because of the striking differences from *P. scolyti* (WEISER 1968) in its development, morphology of spores, site of infection and host-parasite relationships. *Nosema dryocoetesi* n. sp. for difference from the other species of the genus *Nosema* in bark beetles (table) in the morphology and size of vegetative stages and spores, site of infection, and host-parasite relationships.

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We are indebted to Dr. Jaroslav Weiser, Institute of Entomology ČSAV, Department of Insect Pathology, Prague, Czechoslovakia, for confirmation of the identification of microsporidian parasites.

Zusammenfassung

Über drei neue parasitische Sporozoen-Arten in Borkenkäfern (Scolytidae, Coleoptera)

Im Rahmen der in den Jahren 1978–1979 im Institut für Forstzoologie, Göttingen, durchgeführten Untersuchungen über die Krankheiten der Borkenkäfer (Scolytidae) Niedersachsens wurden Infektionen durch neue Pathogenarten festgestellt. Davon werden in dieser Arbeit drei Sporozoen-Arten neu beschrieben: *Ophryocystis hylesini* n. sp. (Ophryocystidae, Neogregarinida) in *Hylesinus fraxini* Panz., *Pleistophora xyloteri* n. sp. (Pleistophoridae, Microsporidia) in *Xyloterus domesticus* L. und *Nosema dryocoetesi* n. sp. (Nosematidae, Microsporidia) in *Dryocoetes autographus* Ratz.

Der Lebenszyklus der hier beschriebenen Sporozoen-Arten wurde lichtmikroskopisch untersucht. Sie befallen die Malpigischen Gefäße *(O. hylesini),* die Darmwand, Fettkörper, Lymphocyten und Oenocyten (*P. xyloteri* und *N. dryocoetesi*).

Von den insgesamt 11 untersuchten Lokalitäten waren die neuen Sporozoen-Arten je einer Lokalität: *O. hylesini* mit einer Infektionsrate von 2 % (N = 350) in Bleckede, *P. xyloteri* mit 2 % (n = 480) in Einbeck und *N. dryocoetesi* mit 4 % (n = 690) im Rosengarten vertreten.

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Jahreszeitliche Qualitätsschwankungen des Fichtenbastes (*Picea excelsa* Link) als Brutsubstrat für den Borkenkäfer *Pityogenes chalcographus* L. (Col., Scolytidae)

Von E. FÜHRER

Abstract

Seasonal quality fluctuations of Norway spruce phloem (Picea excelsa Link) as a brood material of the bark beetle Pityogenes chalcographus L. (Col., Scolytidae)

Food quality of Norway spruce phloem for breeding *Pityogenes chalcographus* females and for their progeny is indicated by the density of egg niches, number of progeny produced per time unit, number of adult progeny, the time needed for its development, and by adult body weight, respectively. By means of these parameters it is demonstrated that food quality changes during the year, particularly in September/October, when breeding success is at a pronounced minimum.

1 Einleitung

Rindenbrütende Borkenkäfer gelten als mehr oder weniger ausgeprägte Nahrungsspezialisten, nicht nur wegen ihrer meist engen Bindung an wenige, nahe verwandte Brutbaumarten, sondern auch wegen ihrer spezifischen Ansprüche an deren physiologischen Zustand. Ein Baum gilt als befallsdisponiert, wenn

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