# Nosema raphidiae sp.n. (Microsporida, Nosematidae): A Microsporidian Pathogen of the Predatory Snake-fly Raphidia ophiopsis (Raphidioptera: Raphidiidae)

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# *Nosema raphidiae* sp.n. (Microsporida, Nosematidae): A Microsporidian Pathogen of the Predatory Snake-fly *Raphidia ophiopsis* (Raphidioptera: Raphidiidae)

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**Summary.** The microsporidium *Nosema raphidiae* sp.n., a new pathogen of the predatory snake-fly *Raphidia ophiopsis* is described based on light microscopic and ultrastructural characteristics. It infects the gut of *R. ophiopsis*. All development stages are in direct contact with the host cell cytoplasm. Meronts with one diplokaryon are spherical or ovoid and are 3.8 to 6.2 µm. Sporonts are spherical to elongate and measure 4.2 to 6.2 µm. Diplokaryotic sporonts divide once to produce two sporoblasts which mature into spores. Sporoblasts are elongated and measure 3.2 to 5.2 µm in length. Fresh spores with nuclei arranged in a diplokaryon are oval and measure 4.13 ± 0.25 µm in length and 2.26 ± 0.19 µm in width. Spores stained with Giemsa's stain measure 3.80 ± 0.25 µm in length and 2.18 ± 0.17 µm in width. Spores have an isofilar polar filament with six or seven coils. All morphological and ultrastructural features indicate that the described microsporidium belongs to the genus *Nosema*. This species has been named *Nosema raphidiae* sp. n. after its host's genus.

**Key words:** Microsporidia, *Nosema*, snake-fly, *Raphidia ophiopsis*, Raphidiidae.

## INTRODUCTION

Recent concerns about the potential negative impacts of bark beetles on forests has heightened interest in the predators of these species. *Rhizophagus* spp. (Coleoptera: Rhizophagidae), *Thanasimus formicarius* (Coleoptera: Cleridae) and *Raphidia ophiopsis* (Raphidioptera: Raphidiidae) are among the important predators regulating bark beetle populations (Tosun 1975, Herard and Mercadier 1996, Ünal 1998).

One of these predators, the snake-fly *Raphidia ophiopsis*, is one of the most effective predators. All species of Raphidiidae are entomophagous and can be fed with any arthropod in captivity (Aspöck 2002). Potential prey may include eggs and larvae of any insect, particularly of Lepidoptera, Hymenoptera and Coleoptera (Aspöck *et al.* 1991, Kovarik *et al.* 1991). Therefore, this predator would be a good candidate for a potential biological control agent and could play a significant role in integrated pest control (Aspöck 2002). Therefore, any disease in *R. ophiopsis* populations is an un-

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desirable situation. The parasites and pathogens of the snake-flies belonging to the order Raphidioptera studied so far include eugregarines, mermithids and parasitoids but no microsporidia (Weber 1939, Batko 1974, Aspöck 2002). This paper is the first report of a microsporidian pathogen of *R. ophiopsis* in Turkey, i.e., *Nosema raphidiae* sp.n.

## MATERIALS AND METHODS

### Insect samples

Adults and larvae of *R. ophiopsis* were collected from bark beetle galleries in the bark of pine trees in Kastamonu (Turkey) in October and November 2007 and February 2008. The insects were put into sterilized glass bottles to prevent possible contamination. They were brought to the laboratory and dissected as soon as possible.

### Light Microscopy

Each larva and adult was dissected in Ringer's solution. The gut, Malpighian tubules, gonads, and parts of the fat body were placed in a drop of Ringer's solution on a slide, and wet smears were examined under a light microscope at a magnification of 400–1000×. In cases of infection, the slides were air-dried and fixed in methanol for two to five minutes. The slides were then washed with distilled water and stained for approximately ten hours in freshly prepared 5% solution of Giemsa stain. Afterwards the slides were washed in running tap water, air-dried and re-examined under the microscope (Toguebaye *et al.* 1988, Undeen and Vavra 1997). Detected vegetative stages and spores were measured and photographed using an Olympus BX51 microscope with a DP-25 digital camera and a DP2-BSW Soft Imaging System. Fresh spores were also measured, and the combined data of fresh and stained spores were presented as the mean value ± standard deviation.

### Transmission Electron Microscopy

Samples for transmission electron microscopy were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 h, rinsed in cacodylate buffer, postfixed in $OsO_4$ for 2 h, and rinsed in cacodylate buffer. After dehydration in an increasing ethanol series, the infected beetles were embedded in Spurr's resin (Spurr 1969). Ultra-thin sections were mounted on Pioloform-coated copper grids which were stained with saturated uranyl acetate and Reynold's lead citrate (Reynolds 1963). They were examined with a Philips EM 208 transmission electron microscope.

## RESULTS

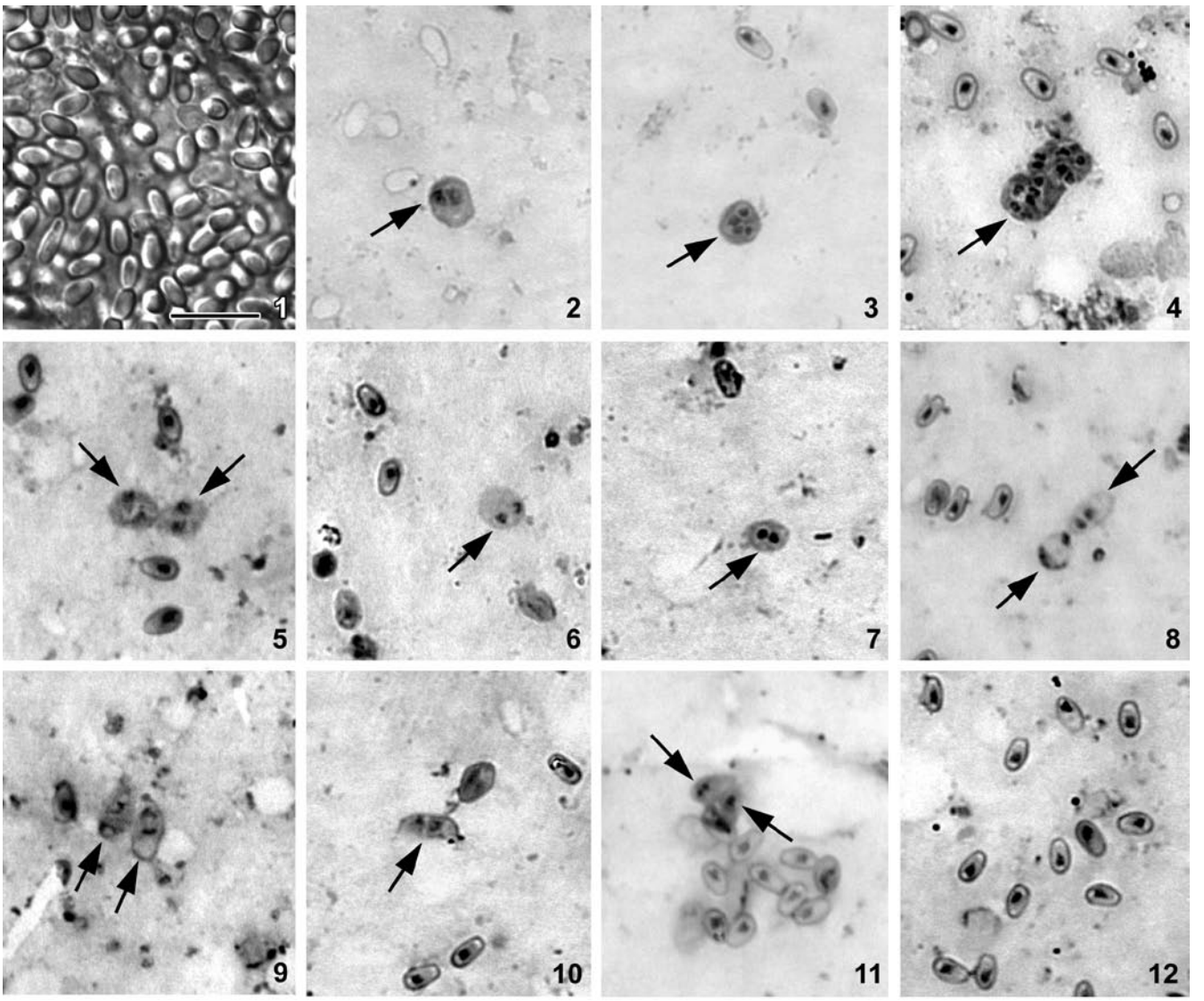
A microsporidian infection was only found in the larvae of *R. ophiopsis* collected from the Kastamonu region (Turkey). Two of 24 examined larvae were infected. Light microscopic examinations of the parasitized individuals revealed the presence of the pathogen in the gut. The gut was wholly filled by the spores of the pathogen (Fig. 1).

### Developmental stages observed with light microscopy

Several life stages of the pathogen were observed in the smears (Figs 1–12). Meronts with one diplokaryon were spherical or ovoid and measured 3.8 to 6.2 µm (Figs 2–5); some of them were dividing forms (Fig. 5). Both binary and multiple merogony were observed (Figs 4, 5). Sporonts were spherical to elongate and measured 4.2 to 6.2 µm (Figs 6, 7). Diplokaryotic sporonts divided once to produce two sporoblasts which mature into spores. Sporoblasts were elongated and measured 3.2 to 5.2 µm (Figs 8–10). The fresh spores were oval with only a minimum diversity in shape and size, measuring 4.13 ± 0.25 (3.38–4.82) (n = 60) µm in length and 2.26 ± 0.19 (1.83–2.82) (n = 60) µm in width (Fig. 1). Spores stained with Giemsa's stain measured 3.80 ± 0.25 (3.25–4.60) (n = 60) µm in length and 2.18 ± 0.17 (1.65–2.61) (n = 60) in width. On dry smears stained with Giemsa's stain, the typical cone-shaped internal structure is readily visible within the sporoplasm (Figs 11, 12).

### Spore ultrastructure

Electron microscopic observations confirmed that the oval spores contain a diplokaryon (Figs 13, 14). Regular meshes of endoplasmic reticulum are arranged on both sides of the diplokaryon (Fig. 15). The spore wall is quite thick (200 to 280 nm) and consists of a clear endospore (150 to 200 nm) and an electron-dense exospore (50 to 80 nm). Both endospore and exospore are thick (Figs 13–16). The polar filament is isofilar and has six or seven coils (Fig. 16). Mature coils measure 140 nm in diameter. The well-developed polaroplast covers half of the spore and has a lamellated structure with thin lamellae (anterior) and thick lamellae (posterior) with irregularly arranged membranes (Fig. 15). An anterior anchoring disc of the polar filament could clearly be seen (Figs 14, 15). The spore has a large, prominent posterior vacuole filled with the remaining material from the polar filament formation (Fig. 17). A sporophorous vesicle was not observed during the light and electron microscopical observations. All stages lay in direct contact to the host cell cytoplasm.

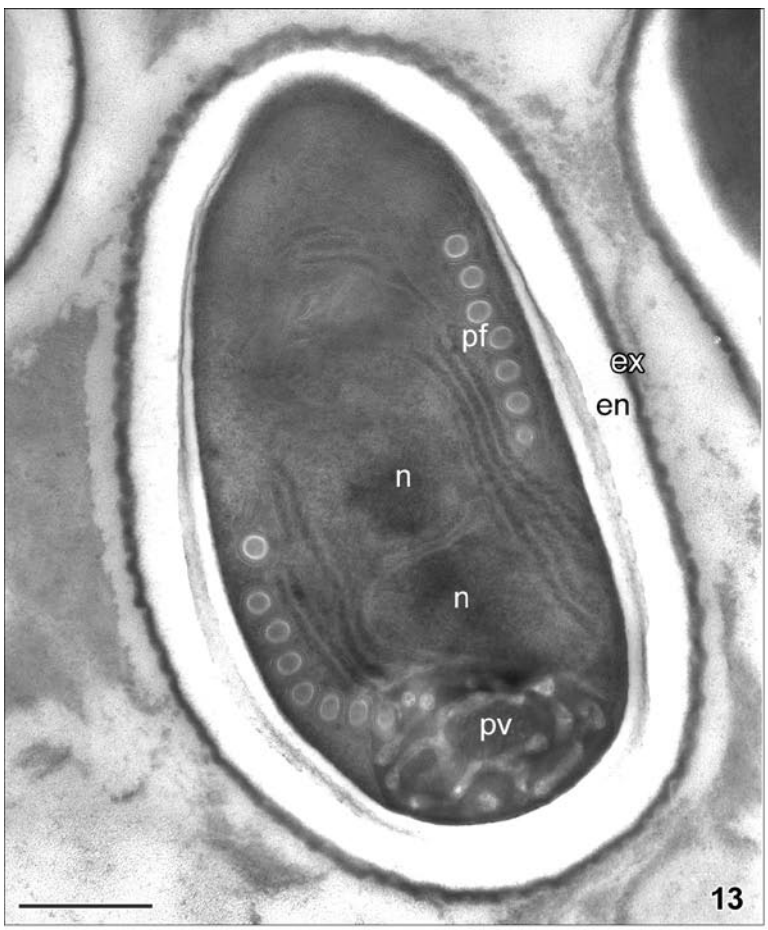



**Figs 1–12.** Light micrographs of the life stages of *Nosema raphidiae* from *Raphidia ophiopsis* in wet mount (1) and Giemsa-stained smears (2–12). Bar: 10 μm. **1** – fresh spores in the gut; **2** – diplokaryotic meront; **3** – meronts undergoing karyokinesis with four small nuclei; **4** – meront with twelve horseshoe-like diplokayotic nuclei; **5** – dividing meronts; **6** and **7** – diplokaryotic sporonts; **8** and **9** – sporoblasts just divided; **10** – early sporoblast; **11** –young spores with two nuclei (arrows); **12** – mature spores.

## DISCUSSION

The ultrastructure and the development cycle of the microsporidian pathogen of *Raphidia ophiopsis* showed the typical characteristics of the genus *Nosema*. These are: diplokaryotic stages throughout the life cycle (including spores), uniform exospore, the thickness of the spore wall, a large, prominent posterior vacuole filled with the remaining material, missing sporophorous vesicle, binary or multiple merogony, and disporoblastic sporogony (Toguebaye and Bouix 1989, Becnel and Andreadis 1999, Larsson 1999, Canning and Vavra 2000). Therefore, we conclude that it belongs to the genus *Nosema* Naegeli, 1857.

*R. ophiopsis* is an important predator regulating bark beetle populations. Up until this point, there has been no microsporidian record of this predator. However, four microsporidian species from the genus *Nosema*, i.e., *N. typographi* (Weiser 1955), *N. curvidentis* (Weiser 1961), *Nosema scolyti* (Lipa 1968), and *N. dendroc-*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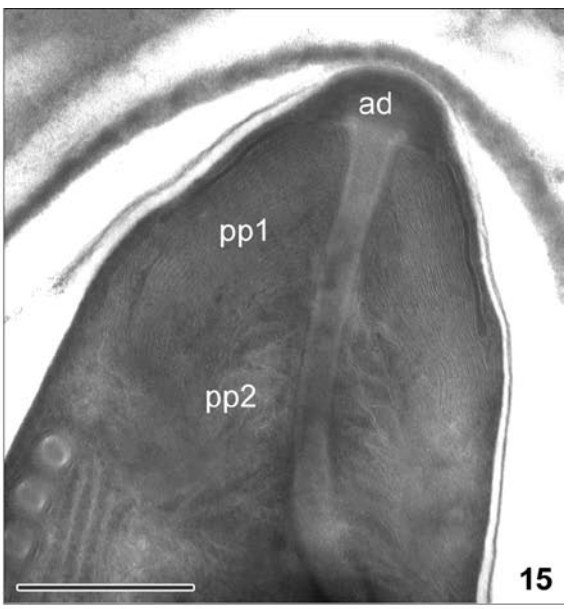
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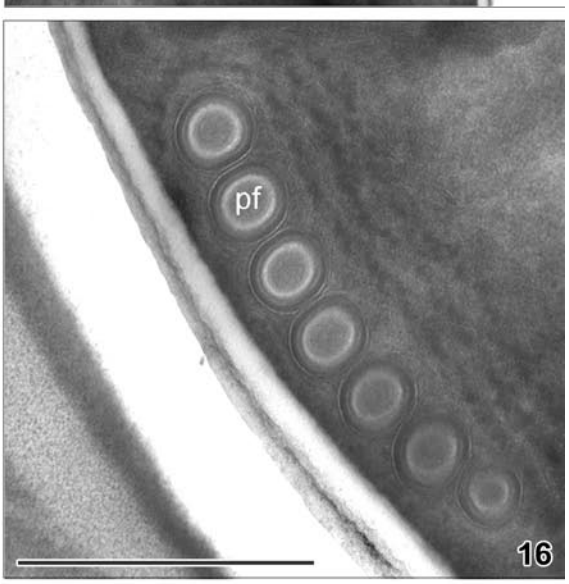
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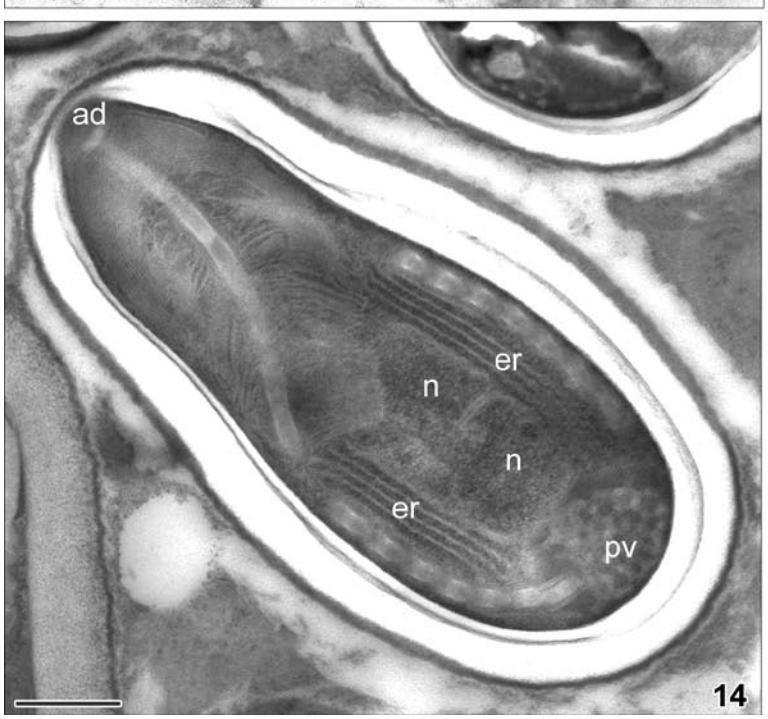
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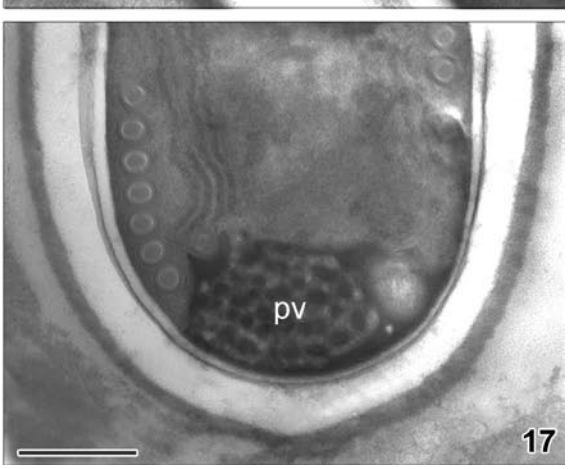
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*toni* (Weiser 1970) have been found to parasitize bark beetles. The distinctive characteristics of the *Nosema* species from the bark beetles are shown in Table 1. The spore dimension is one good morphological character for distinguishing these pathogens. The host affinity and the site of host infections are also generally recognized as valid taxonomic characters, at least in microsporidia which infect insects (Sprague *et al.* 1992). Our *Nosema* isolate differs from the other species in more than one of these characteristics (Table 1). Besides parasitizing different host species, three of the four *Nosema* species of bark beetles do not infect the gut, but rather other organs (i.e., fat body, Malpighian tubules). Only *Nosema scolyti* is found in Malpighian tubules and in the gut. However, the size of its spores is generally greater than that of *N. raphidiae*.

*R. ophiopsis* is a natural predator of *Ips* species in Turkey (Tosun 1975, Ünal 1998). The spore size of our isolate coincides with that of *N. typographi* Weiser, 1955 from *Ips typographus*. However, there is no any microsporidium record from *I. typographus* in Turkey and its ultrastructural characteristics are clearly different from this pathogen. It is the sole strain possessing only six or seven polar filament coils in contrast to as many as 16 or 17 coils in *N. typographi* (Weiser *et al.* 1997). Further differences exist in the details of the spore ultrastructure, the geographic location and the systematic position of the host (Weiser *et al.* 1997). Therefore, we consider this microsporidium to be a new species and propose the name *Nosema raphidiae* after the genus name of the host, *Raphidia ophiopsis*.

## Taxonomic summary

***Nosema raphidiae* n. sp.**

**Type host:** *Raphidia ophiopsis* L. (Raphidioptera: Raphidiidae).

**Host locality:** Bark beetle galeries in *Pinus sylvestris*, Taţköprü-Saraycýk/Kastamonu, 1400 m (N 45°79′ 56″, E 36°61′43″), Turkey.

**Site of infection:** Gut.

**Diagnosis:** Fresh spores are oval, 4.13 ± 0.25 (3.38–4.82) µm in length and 2.26 ± 0.19 (1.83–2.82) µm in width. Spores stained with Giemsa's stain measure 3.80 ± 0.25 (3.25–4.60) µm in length and 2.18 ± 0.17 (1.65–2.61) in width. The polar filament is isofilar and has six or seven coils. Mature coils measure 140 nm in

**Table 1.** *Nosema* species described in bark beetles and the crane-fly *Raphidia ophiopsis*. Not all authors mentioned whether fresh or fixed spores were measured.

| Nosema species | Spore size | Infected organ | Host |
|---|---|---|---|
| *Nosema typographi* Weiser, 1955 | 3.6–5.3 × 2–3.5 µm in fixed spores | Fat body | *Ips typographus* (Coleoptera: Scolytinae) |
| *Nosema curvidentis* Weiser, 1961 | 2.5–3.6 × 1.5–2 µm in fresh spores | Fat body | *Pityocteines curvidens* (Coleoptera: Scolytinae) |
| *Nosema scolyti* Lipa, 1968 | 4–5 × 2–3.3 µm in fresh spores | Malpighian tubules, midgut | *Scolytus scolytus* (Coleoptera: Scolytinae) |
| *Nosema dendroctoni* Weiser, 1970 | 2–3 × 1–2 µm in fixed spores | Malpighian tubules, fat body | *Dendroctonus pseudotsugae* (Coleoptera: Scolytinae) |
| *Nosema raphidae* Present work | 4.13 × 2.26 µm in fresh spores and 3.80 × 2.18 µm in fixed spores | Gut | *Raphidia ophiopsis* (Raphidioptera: Raphidiidae) |

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**Figs 13–17.** Transmission electron micrographs of spores of *Nosema raphidiae* in *Raphidia ophiopsis*. Bars: 0.5 µm. **13** – longitudinal section of a diplokaryotic microsporidian spore with a thick wall consisting of a thin exospore (ex) and a thick, electron-lucent endospore (en), showing seven coils of the polar filament (pf) and a clearly visible diplokaryon (n). pv posterior vacuole; **14** – microsporidian spore with an anchoring disc (ad) at the apical pole and a posterior vacuole (pv) at the posterior pole and a clearly visible diplokaryon (n). Regular meshes of endoplasmic reticulum (er) are arranged on the both side of diplokaryon; **15** – section of the anterior portion of a spore showing an anchoring disc (ad) attenuated apically to the endospore and a well-developed, lamellae-type polaroplast with thin lamellae anteriorly (pp1) and thick lamellae posteriorly (pp2); **16** – cross-section of the isofilar polar filament (pf) with seven coils; **17** – posterior end of a mature spore with a posterior vacuole (pv) including the remaining material from the polar filament formation. Note that the empty space, visible in all micrographs between plasma membrane and cytoplasm, is an artifact.

diameter. The well-developed polaroplast covers half of the spore and has a lamellated structure with thin lamellae anteriorly and thick lamellae posteriorly with irregularly arranged membranes.

**Type material:** Giemsa-stained microscopic slides (MY-05, 06 and 07) and grids for electron microscopy are stored at the Department of Biology, Karadeniz Technical University, Trabzon, Turkey.

**Etymology:** The name of the species refers to the genus name of the host, *Raphidia*.

The discovery of a microsporidian pathogen infecting a member of the insect order Raphidioptera is not only exciting from a scientific viewpoint, but may also become important for pest control. *R. ophiopsis* is an effective predator of bark beetles under natural conditions. Aspöck (2002) recorded that all larval stages of all species of both families of the order Raphidioptera, and at least the adults of the Raphidiidae, feed on mainly soft-bodied arthropods and could therefore play a significant role in integrated pest control. Rearing of snake-flies is possible; the procedure usually commences with a female collected in the field that is used for ovipositioning. In this case it is very important to use non-infected insects for rearing, as any infection can decrease the efficiency of the predator. Thus, to find this new microsporidium in the populations of *R. ophiopsis* is also very important in this respect.

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