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Two new species of microsporidia from the larvae of *Tramea limbata* (Odonata : Insecta)

C C NARASIMHAMURTI, S NAZEER AHAMED* and C KALAVATI

Department of Zoology, Andhra University, Waltair 530 003, India

* Present address : Department of Zoology, Government H S School, Pasighat, Arunachal Pradesh, India

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Abstract. Two new species of microsporidians, *Nosema limbata* n. sp. and *Thelohania limbata* n. sp. from the adipose tissue of the larvae of *Tramea limbata* collected from the dairy farm area, Visakhapatnam and the Andhra University Campus at Waltair respectively are described and their systematic position discussed.

Keywords. *Nosema limbata* n. sp. ; *Thelohania limbata* n. sp. ; *Tramea limbata.*

1. Introduction

Numerous species of microsporidians have been reported from insects (Sprague 1977) but so far only 4 species, *Glugea aeshnae and Gurleya aeshnae* from *Aeshna grandis* from Canada (Fantham *et al* 1941), *Microsporidium calopterygis* from *Calopteryx virgo* and *C. sp.* from Czechoslavakia (Weiser 1958) and *Toxoglugea tillargi* from *Tholymis tillarga* from India (Kalavati and Narasimhamurti 1978) are from Odonata. In the following account 2 new microsporidians, *Nosema limbata* n. sp. and *Thelohania limbata* n. sp. from the adipose tissue of the larvae of the odonate, *Tramea limbata* are described.

2. Materials and methods

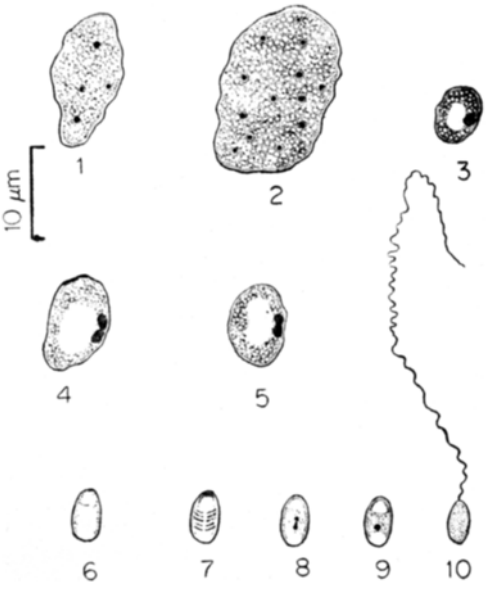
The larvae of the odonate, *Tramea limbata* (Odonata : Insecta) were collected from two different localities, specimens collected from the dairy farm area were infected with *Nosema limbata* n. sp. and others collected from the University area were infected with *Thelohania limbata* n. sp. The larvae were collected from the scum of the fresh water tanks and ponds, washed free of adhering debris and maintained in the laboratory in glass finger-bowls containing filtered water obtained from the same locality from where the larvae were collected. Smears of the infected adipose tissue were air-dried, fixed in methyl alcohol and stained with Giemsa. Spores were stained according to PAS technique to show the polar cap. The polar

filaments were released by the addition of a drop of hydrogen peroxide to the air-dried smears and exerting slight pressure. The spores were measured both in the fresh condition and fixed and stained condition.

3. Observations

Host : *Tramea limbata* (Odonata: Insecta).
Parasite: *Nosema limbata.*
Locality: Dairy farm area, Visakhapatnam.

Seven per cent of the larvae examined were infected with the parasite. Plasmodia measuring 15–20 μm, containing hyaline cytoplasm were seen in the adipose tissue cells. The number of nuclei varied from 4–13 depending on the size of the plasmodium (figures 1, 2). The cytoplasm did not have any inclusions. Sporonts could be identified by the presence of a large vacuole adjacent to the nucleus as in *Nosema fennicum* (Lom and Weiser 1969) (figure 3). They were ovoid measuring 5–8 × 3–6 μm. Successive stages in the transformation of the sporont to a spore were not observed. Stages showing 2 nuclei with a large vacuole lying by its side were seen and were probably Diplokarya (figures 4, 5). Spores occur singly in the adipose tissue cells. They were oval-pyriform measuring 5·4–6·4 × 1·6–3·0 μm in the fresh condition and 4·8–6·4 × 1·6–3·0 μm in fixed and stained



Figures 1–10. *Nosema limbata.* **1.** Plasmodium showing 4 nuclei. **2.** Plasmodium showing 13 nuclei. **3.** Sporont (Note vacuole adjacent to nucleus). **4.** and **5.** Diplokarya stages. **6.** Fresh spore showing anterior polaroplast. **7.** Spore stained with PAS showing polar cap. **8.** Spore stained with Feulgen. Note the nucleus in the form of two granules connected by a filamentous structure. **9.** Spore stained with Giemsa showing nucleus and sporoplasm. **10.** Spore with an extruded polar filament.

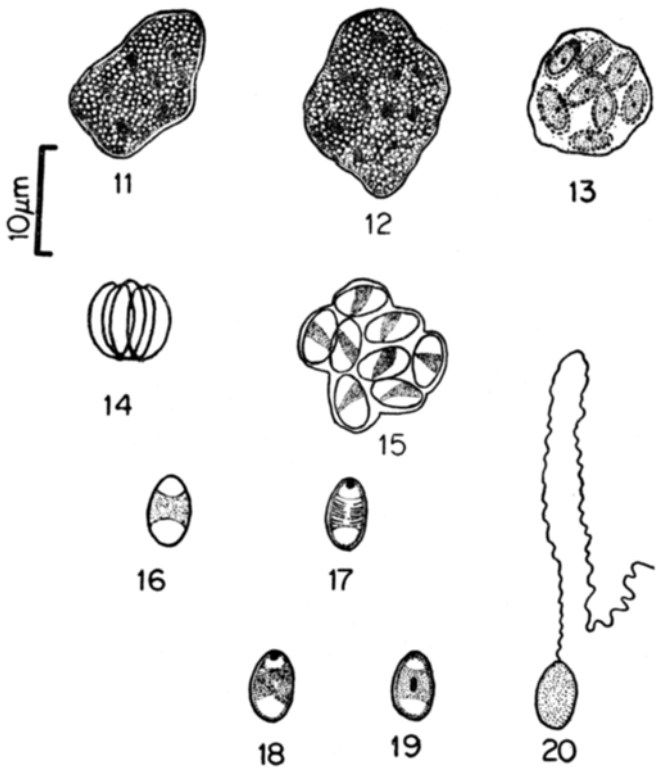
condition. Spores stained with Heidenhain's iron haematoxylin or Giemsa after an initial hydrolysis in 1 N HCl for 10 min showed the sporoplasm in the form of a band extending across the spore between the anterior polaroplast and posterior vacuole (figure 9). The deeply stained nucleus was placed at the centre of the sporoplasm. Spores stained according to Feulgen technique showed the nucleus to be in the form of two deeply stained granules connected with a faintly stained filamentous structure (figure 8). A crescent-shaped PAS positive polar cap was present in front of the polaroplast (figure 7). The polar filament which was released by the addition of a drop of hydrogen peroxide to the air-dried smears was in the form of a fine uncoiled spring and measured 80–150 μm (figure 10).

Remarks : In the present form each sporoblast gives rise to one spore and there is a diplokaryon stage and hence is placed in the genus *Nosema.* The only previous report of *Nosema* from the odonate host is *N. aeschanae* from the fat bodies of *Aeschna grandis* L. (Weiser 1951). The spores of *N. aeschnae* are oval and much bigger ranging in size from 5·9–7·4 × 3·4–4·6 μm and the polar filament measured 80 μm in length whereas in the present form the spores are oval-pyriform measuring 5·4–6·4 × 1·6–3·0 μm and the polar filament ranged from 80–150 μm. In addition to the differences in the size of the spore and polar filament the present form is from a different host and from a different geographical area. Since there is no previous record of a microsporidian from this host it has been named as *Nosema limbata* n. sp.

Host : *Tramea limbata* (Odonata: Insecta).
Parasite: *Thelohania limbata.*
Locality: University Campus, Waltair.

The adipose tissue adjoining the midgut region of 5–7% of the larvae of *Tramea limbata* showed conspicuous cysts measuring 1.0 — 1.5 mm in diameter enclosing several pansporoblasts. Earlier stages of development reaching a maximum size of 20 × 15 μm containing paired nuclei were observed in smears (figures 11, 12). The cytoplasm was hyaline and did not contain any inclusions. Pansporoblasts containing 8 nuclei with incipient demarcation of cytoplasm were seen in smears (figure 13). Fully formed sporoblasts measuring 14·4 × 13·2 μm covered with a thin, transparent tough outer membrane containing 8 spores were observed (figure 15). The spores were oval measuring 7·2 × 3·6 μm. An anterior small polaroplast and a large posterior vacuole with the sporoplasm extending in the form of a band are clearly seen in fresh spores (figure 16). Spores stained with Giemsa revealed an essentially same structure and in addition the polar filament could be seen along the sides (figure 17). In spores stained according to Feulgen technique the nucleus appeared as two deeply stained granules, placed one behind the other (figure 19). A deeply stained PAS positive polar granule was present in front of the polaroplast (figure 18). The polar filament which was released by the addition of a drop of hydrogen peroxide to air dried smears was uniformly thin and measured 120–140 μm in length (figure 20).

Remarks : The pansporoblast gives rise to 8 spores in the present case and hence is placed in the genus *Thelohania.* So far only four species of microsporidians, *Nosema aeschnae* from the fat body of *Aeschaena grandis, Gurleya aeschnae*



Figures 11–20. *Thelohania limbata.* **11.** An early plasmodium showing paired nuclei. **12.** A fully developed plasmodium showing 10 paired nuclei. **13.** A developing pansporoblast : Note incipient demarcation of cytoplasm. **14.** A fresh pansporoblast. **15.** A fully developed pansporoblast with 8 spores. **16.** A fresh spore showing anterior polaroplast and posterior vacuole. **17.** A spore stained with PAS showing PAS positive granule at anterior end. **18.** Spore stained with Giemsa showing nucleus and sporoplasm. **19.** Spore stained with Feulgen showing the nucleus in the form of 2 deeply stained granules. **20.** A spore with extruded polar filament.

from the *oenocytes of Aeschena grandis, Stempellia calopterygae* from the larval fat body of *Calopteryx virgo* and *Toxoglugea tillargi* from the oenocytes of *Tholymis tillarga* are reported from different parts of the world, the last named one being from India. The pansporoblasts in *Toxoglugea tillargi* are octosporous, but the spores are either bean- or kidney-shaped and occur in two size groups, the microspores measuring 3·5–4·0 × 1·8–2·0 and the macrospores measuring 4·8–5·4 × 1·8–2·0 μm and the polar filament measures 45–50 μm in length. In the present form although the pansporoblasts are octosporous, the spores are oval and measure 7·2 × 3·6 μm and the polar filament measures 120–140 μm in length. Since there is no previous report of any microsporidian belonging to the genus *Thelohania* from any odonate and since there is no previous report of a microsporidian from this host it is named *Thelohania limbata* n. sp.

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