

Isometric Viruslike Particles in Spores of Two Microsporidia Belonging to the Thelohaniidae

Two microsporidia, isolated from midge larvae of an unidentified species of the *notata* group of the genus *Macropelopia* (Diptera, Chironomidae, Tanypodinae) collected in the small stream Klingavälsån in the south of Sweden, were found to be infected with particles of suspected viral nature. Both microsporidia are undescribed species of the family Thelohaniidae. Microsporidium sp. 1 had rod-shaped spores with rounded ends (Slide Series No. 850819-E RL). Microsporidium sp. 2 had rod-like to pyriform spores of variable shape and dimensions (Slide Series No. 850819-K RL).

Microsporidia-filled adipose tissue of the two hosts was fixed in 2.5% (v/v) glutaraldehyde in 0.2 M sodium cacodylate buffer pH 7.2, at 4°C for 48 hr. After washing in buffer and post-fixation in 2% (w/v) osmium tetroxide in cacodylate buffer for 1 hr at 4°C, the pieces were dehydrated in an ascending series of buffer–acetone solutions to absolute acetone and embedded in Epon. Sections were stained with uranyl acetate and lead citrate.

Morphology, dimensions, localization, and prevalence of the particles were identical in the two microsporidia. The electron-dense particles were isometric, 20–24 nm wide (Fig. 1). They developed in the nucleus and were associated with wide areas of electron-dense material (Figs. 1,2). Paracrystalline arrays were not observed. The tissue samples were heavily infected, with thousands of spores, and particles were present in almost all spores. In a part of the spores, particles were also found outside the nucleus, most prominently in the proximity of the polaroplast (Fig. 1). The particles were only present in mature

spores and had no observable pathogenic effect. They were not observed in the host tissues.

The morphology and dimensions of the viruslike particles suggest two positions in the classification of viruses: Picornaviridae, with spherical virus particles, 22–30 nm in diameter, containing RNA, and Parvoviridae, with isometric particles, 18–26 nm wide, containing DNA (R. E. F. Matthews, *Intervirology* 12, 129–296, 1979). Viruses of the Picornaviridae develop in the cytoplasm; viruses of the Parvoviridae develop in the nucleus. The viruslike particles of the microsporidia seem to be related to the Parvoviridae. The morphology and association with wide electron-dense areas in the nucleoplasm resemble the agent of the insect disease densoinfection (E. Kurstak, P. Tijssen, and S. Garzon, in "The Atlas of Insect and Plant Viruses," K. Maramorosch, Ed., pp. 67–91, Academic Press, New York, 1977).

Viruslike particles have been observed several times in nuclei of protozoa other than microsporidia and have, for example, been reported from the flagellate *Leishmania hertigi* (S. L. Croft, *Protistologica* 15, 103–110, 1979), the amoebophilic flagellate *Naegleria gruberi* (F. L. Schuster, *J. Protozool.* 16, 724–727, 1969), and the amoeba *Entamoeba histolytica* (C. R. Gicquaud, *Biol. Cell.* 35, 305–312, 1979). The particles of *N. gruberi* (ca. 100 nm in diameter) and *E. histolytica* (ca. 35 nm) are considerably larger than the particles associated with the microsporidia, while the particles of *L. hertigi* are slightly smaller (15–17 nm wide). These particles from the sarcomastigophoreans were more or less clearly ar-

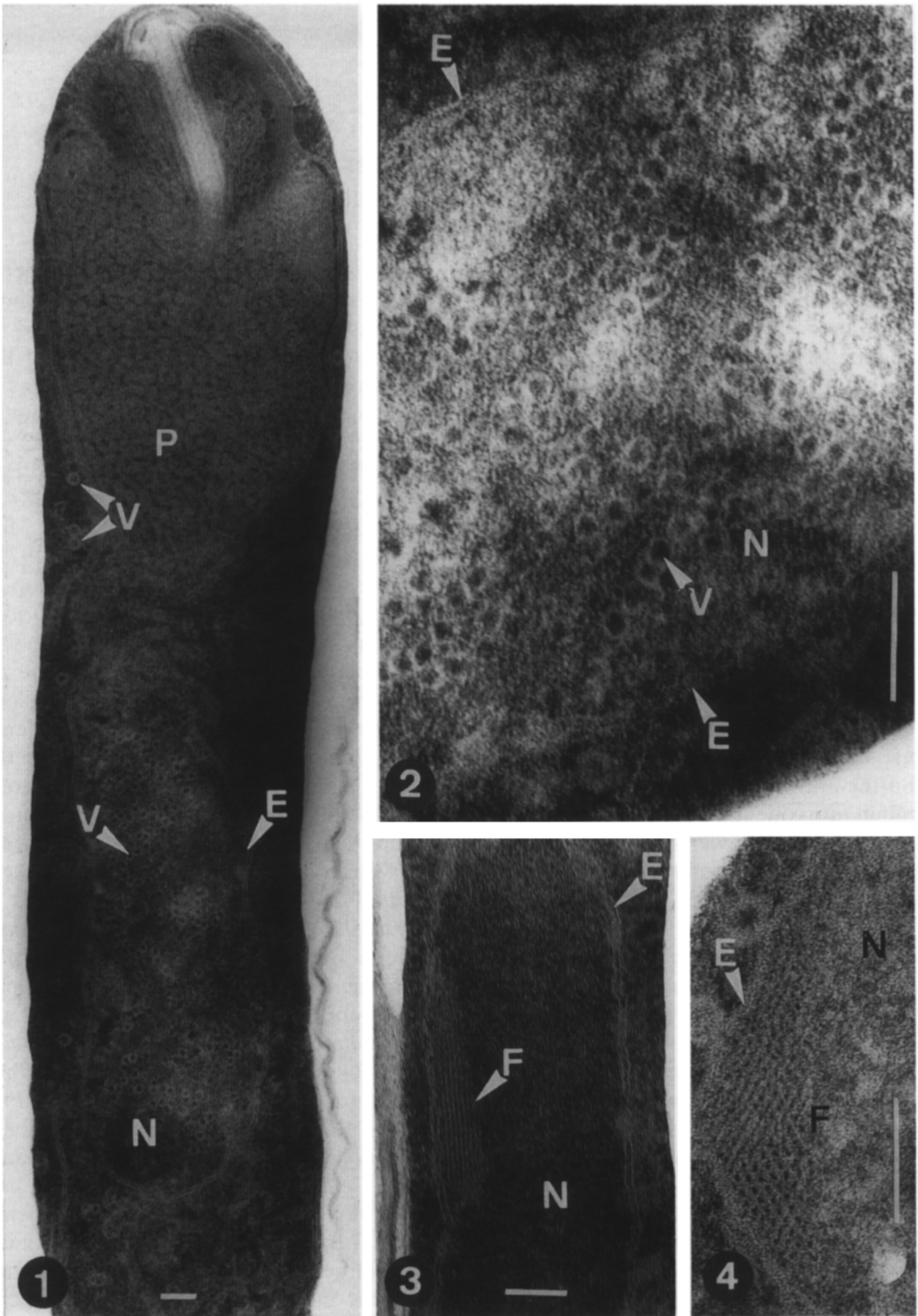


FIG. 1. Longitudinally sectioned mature spore of microsporidium No. 850819-K RL, with numerous viruslike particles in the nucleus and a smaller number in the cytoplasm.

FIG. 2. Detail of a nucleus with viruslike particles, associated with electron-dense areas.

FIGS. 3,4 Longitudinally and transversely sectioned parts of nuclei with fibrillar inclusions (*Cylindrospora fasciculata*, mature spores). (Scale bars v 100 nm. E, nuclear envelope; F, fibrils; N, nucleus; P, polaroplast; V, Viruslike particles).

ranged in strands or paracrystalline aggregates.

There is one report of viruslike particles in a microsporidium (T. P. Liu, *J. Invertebr. Pathol.* **44**, 103–105, 1984). The spherical particles, 15–38 nm in diameter, were present in lysed spores of the honey bee parasite *Nosema apis* from Canada. The particles were found in the cytoplasm; however, the localization seems to be uncertain as it was based on observations of lysed spores. Infected spores were larger than uninfected ones and were suggested to be immature. The micrographs show lysed spores with a well-developed endospore layer which therefore are mature. The particles resembled honey bee virus particles (T. P. Liu, *loc. cit.* 1984). The viruslike particles of the Swedish microsporidia appear morphologically similar to the particles of *N. apis*, but the effects on the hosts are strikingly different.

A third microsporidium belonging to the Thelohaniidae, *Cylindrospora fasciculata* (J. I. R. Larsson, *Protistologica* **22**, 379–398, 1986), collected from the same

host at the same time and processed in an identical way, had intranuclear particles of a different type. Transverse sections could erroneously give the impression of virus particles in paracrystalline arrangement (Fig. 4). However, the inclusions were fibrillar, ca. 5 nm wide and up to 430 nm long, arranged in a tight bundle close to the nuclear envelope in the anterior half of the nucleus (Fig. 3). These particles are not suggested to be of viral nature.

KEY WORDS: Viruslike particles, Parvoviridae; nuclei; microsporidia; Thelohaniidae; spores.

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