

Ultrastructural Data on a Microsporidian Infesting the Ovaries of an Araneid

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Oligosporidium nov. gen. arachnicolum (Codreanu-Bălcescu, Codreanu and Traciuc, 1978) is one of the more intensively studied microsporidians from an araneid. It develops into parasitophorous vacuoles formed in the oocytes of *Xysticus cambridgei* from Bucharest, Romania. The uninucleated schizogonic and sporogonic stages multiply through binary fission and the dense bordered sporoblasts give rise to isolated spores.

KEY WORDS: *Xysticus cambridgei*; microsporidian; *Oligosporidium nov. gen. arachnicolum*.

INTRODUCTION

In the last 20 years, some microsporidia have been described in different ticks, mites, and in an opilionid (Sprague, 1977). Only one species has been reported in spiders (Aranea); it has been described as having 4 μm oval spores situated in the muscles of *Epeira diadema* (Leydig, 1863). The rarity of microsporidia in spiders is puzzling since spiders are naturally exposed to infestation in sucking insects which often include microsporidia. During an ultrastructural study of the genital system in araneids we found a microsporidian developing in the oocytes and the ovarian pedicular cells.

MATERIALS AND METHODS

Among spiders collected from a grassland of our institute in Bucharest, near the Dîmbovita River, we found a few parasitized immature female *Xysticus cambridgei* (family Thomisidae).

The dissected ovaries were fixed with 2.5% glutaraldehyde in 0.1 M Na cacodylate buffer at pH 7.4 for 2 hr at 4°C. Following six washes in buffer with 0.25 M sucrose at 4°C for 3 hr, the pieces were post-fixed in 1% buffered osmium tetroxide, dehydrated in a graded ethanol series, and embedded in Epon 812. After polymerization for 24 hr at 45°C and 2 days at 60°C, the thin sections were stained with 2% al-

coholic uranyl acetate, contrasted with lead citrate, and examined with a JEM-7 electron microscope.

RESULTS

Within the 60- to 80- μm wide oocytes, various parasitic stages (about hundred on an ultrathin section) are closely grouped together inside a parasitophorous vacuole. Such a vacuole measures 25–45 μm in diameter and is devoid of a proper wall but is bounded by a dense reticulate zone which represents a reaction of the ovular cytoplasm (Figs. 1, 6). Between the parasites, the disintegrated vacuolar content gives rise to areas of sinuous microtubular products, each 25–35 nm in diameter, and scattered, large irregular multimembranous concentric bodies which measure 1.7–2.5 μm in diameter (Fig. 4). There are also large homogeneous, perhaps previtelline inclusions which measure 1–1.9 μm in diameter. These inclusions are more numerous toward the vacuolar border (Figs. 1, 6). The large polymorphic nuclei of the pedicular cells are compressed by the microsporidian vegetative stages. Most of the parasites are embedded into the host's cytoplasm, which may also react by forming a small vacuolar space and a mass of microtubular structures.

In the oocytes, some schizonts and sporonts may be in direct contact with the ovu-

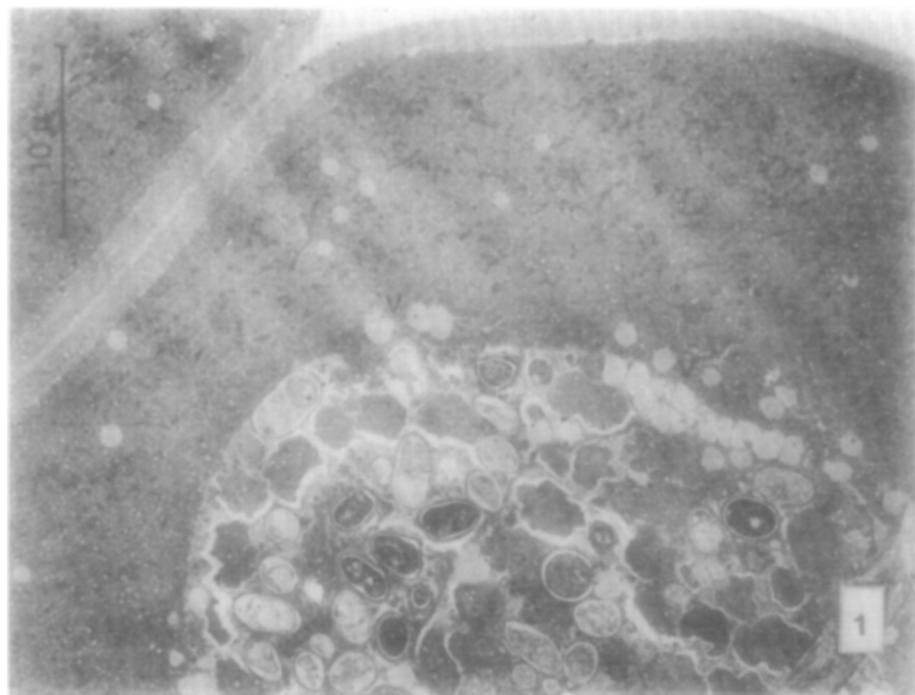


FIG. 1. *Oligosporidium arachnicolum*, developing stages in the parasitophorous vacuole of an araneid oocyte, $\times 2,500$. V, Previtelline inclusions.

FIG. 2. *Oligosporidium arachnicolum*, mature spore, $\times 38,000$. ad, Anchoring disc; el, ergastoplasmic lamellae; en, endospore; ex, exospore; N, spore nucleus; pf, polar filament coils; pt, polaroplast.

lar cytoplasm, which still includes mitochondria (Fig. 5). Cytoplasmic as well as intravacuolar schizogonic stages of the parasite have an irregular sinuous outline,

are amoeboid, measure 2.2–4.2 μm in diameter, and are limited by a unit membrane (Fig. 3). Their centrally located voluminous double-walled nucleus, 1.1–2.5 μm in di-

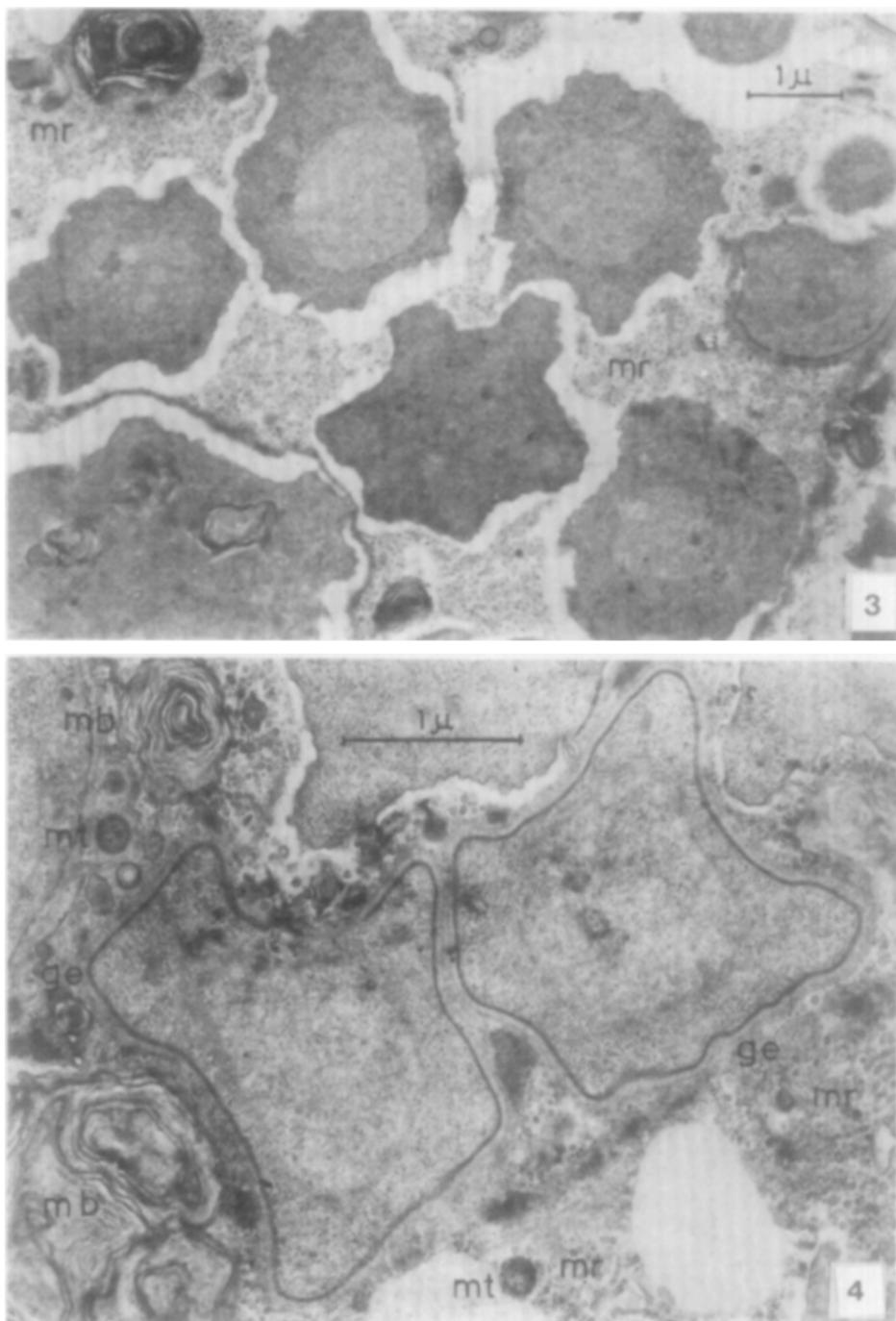


FIG. 3. *Oligosporidium arachnicolum*, schizogonic stages, $\times 12,920$. mr, Microtubular reactive products.

FIG. 4. *Oligosporidium arachnicolum*, young sporogonic stages, $\times 25,000$. ge, External edge; mb, Multimembranous bodies; mr, microtubular reactive products; mt, host mitochondria.

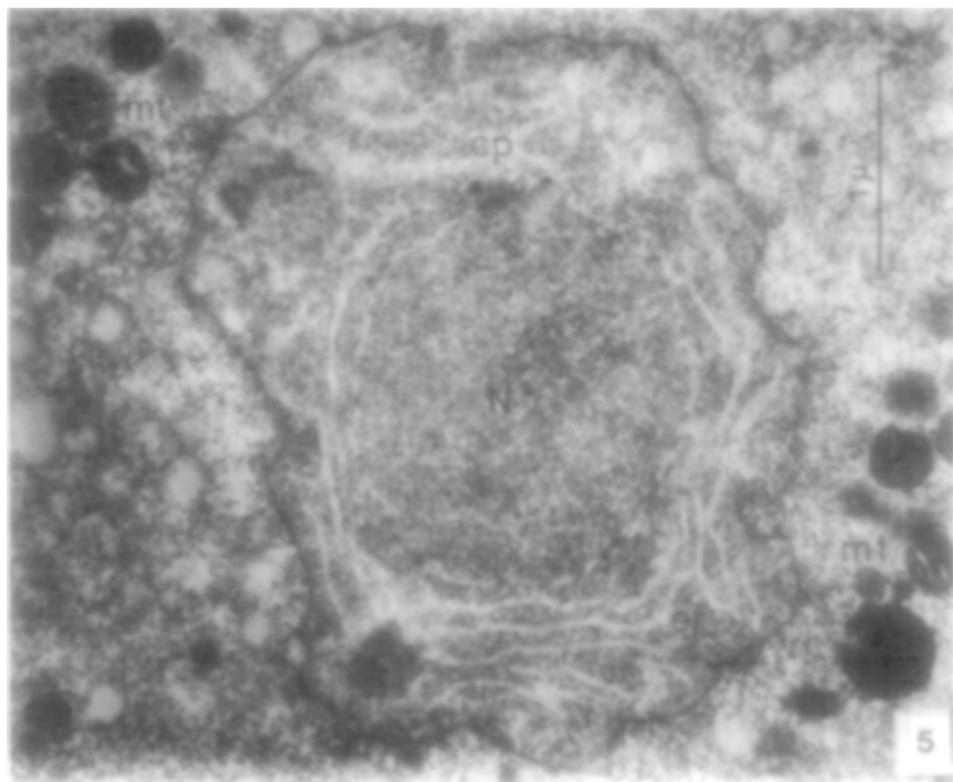


FIG. 5. *Oligosporidium arachnicolum*, sporont in oocyte cytoplasm, $\times 24,990$. cp, Centriolar plaque; er, endoplasmic reticulum; mt, host mitochondria; N, nucleus.

ameter, is surrounded by few ergastoplasmic lamellae in the cytoplasm crowded with ribosomes. Some elongate stages ($5.5 \mu\text{m}$) with two nuclei indicate that the schizonts multiply by binary fission.

In the $4 \times 3 \mu\text{m}$ large sporonts, the cytoplasm is deeply marked and a well-developed endoplasmic reticulum encircles the wide nucleus. Against the nuclear membrane, the presence of a dark centriolar plaque associated with external polar vesicles indicates sporont division (Fig. 5).

The sporogenesis begins with the fragmental deposition of an electron opaque material along the marginal membrane, which becomes continuously thickened and doubled in the sporoblasts by an outer clearer transversal striated edge, measuring $50-76 \text{ nm}$ in all (Fig. 4). Tending to lengthen, the sporoblasts reach $4.3 \mu\text{m}$ long. The nucleus measures $2.2 \mu\text{m}$ in diameter. A Golgi vesicular-reticulated complex precedes polar filament formation and

the retraction of the cytoplasmic content parallels the development of the electron lucid endosporal layer. The external finely granular envelope fuses with the dense sporoblastic wall to produce the compact exospore (Fig. 2).

Covered by a shell $0.3 \mu\text{m}$ broad, the mature oblong spores, averaging $3.6 \times 2 \mu\text{m}$, contain many condensed ergastoplasmic lamellae surrounding the centrally situated nucleus ($0.8-1.5 \mu\text{m}$ diameter) and the overlapped alveolar polaroplast. There is an apical anchoring disc on the polar filament, which forms seven or eight peripheral coils at the base of the spore (Fig. 2). Into the oocytic parasitophorous vacuole, several immature spores appear to exhibit abnormal structures (Fig. 1).

DISCUSSION

We first assigned this microsporidian into the genus *Unikaryon* Canning, Lai, and Lie, 1974 (Codreanu-Bălcescu et al., 1978)

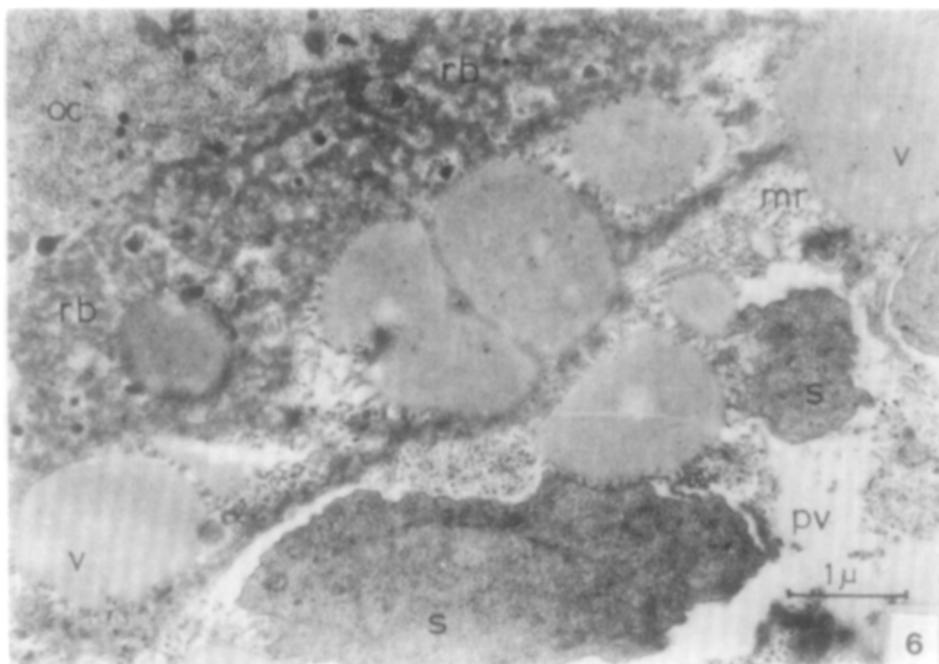


FIG. 6. Marginal zone of the parasitophorous vacuole, $\times 15,470$. mr, Microtubular reactive products; oc, oocyte cytoplasm; pv, parasitophorous vacuole; rb, thickened reaction border; s, schizogonic stages; v, previtelline inclusions.

belonging to the family Unikaryonidae Sprague, 1977. But it also shows affinities with the genus *Encephalitozoon* Levaditi, Nicolau, and Schoen, 1923, which belongs to the family Glugeidae Thélohan, 1892. The respective host groups, trematode larvae and mammals, are phylogenetically and ecologically so distant from araneids that is not possible to link their microsporidian in any evolutionary way. Therefore, we propose for our araneid microsporidian the following new genus.

Oligosporidium nov. gen.

Diagnosis. Disporous sporogony through sporont bipartition. Sporoblasts uninuclear developing separately into isolated spores within a parasitophorous vacuole formed in the host cell cytoplasm. Binary fission during schizogony.

Type species. *Oligosporidium arachnicolum* (Codreanu-Bălcescu, Codreanu, and Traciuc, 1978) nov. comb. by monotypy.

Remarks. *Oligosporidium* nov. gen. is provisionally assigned to the family Unikaryonidae Sprague, 1977, although spo-

rogony takes place in parasitophorous vacuoles like the representatives of the family Glugeidae Thélohan, 1892, and not in direct contact with the host cytoplasm. The chief differences from *Encephalitozoon* Levaditi, Nicolau, and Schoen, 1923, recently studied by electron microscopy (Sprague and Vernick, 1971; Pakes et al., 1975) are that *Oligosporidium* is a parasite of an invertebrate and fills compactly in large number a parasitophorous vacuole. It also lacks the sporoblastic rosette of *Nosemoides* as established by Vinckier (1975), and its spores do not develop in pairs as in *Perezia* Léger and Duboscq, 1909 resulting in vegetative plasmodia (Ormières, Loubès, and Maurand, 1977). The name *Oligosporidium* means a small spore number resulting from schizogonic and sporogonic multiplication.

Oligosporidium arachnicolum (Codreanu-Bălcescu, Codreanu, and Traciuc, 1978) nov. comb.

Synonym. *Unikaryon arachnicolum* Codreanu-Bălcescu et al., 1978.

Host and site. (Aranea) *Xysticus cambridgei*; in oocytes and ovarian pedicular cells, within parasitophorous vacuoles.

Vegetative stages. Amoeboid uninucleated schizonts undergoing binary fission.

Sporulation stages. Sporont bipartition leads to thick-bordered sporoblasts forming isolated spores.

Spore. Oblong, $3.6 \times 2 \mu\text{m}$, uninuclear, with seven or eight basal coils of the polar filament.

Locality. Bucharest, Romania.

REFERENCES

- CANNING, E. U., LAI, P. F., AND LIE, K. J. 1974. Microsporidian parasites of trematode larvae from aquatic snails in West Malaysia. *J. Protozool.*, **21**, 19–25.
- CODREANU-BĂLCESCU, D., CODREANU, R., AND TRACIUC, E. 1978. Ultrastructural aspects of a Microsporidian parasitizing the ovaries of an Araneid. *Abstr. 6th Int. Colloq. Invertebr. Pathol., Prague*, 21. *Idem*, 1979. In "Progress in Invertebrate Pathology," pp. 35–36. Prague.
- LEYDIG, F. 1863. Der Parasit in der neuen Krankheit der Seidenraupe noch einmal. *Arch. Anat. Physiol. Wiss. Med.*, 186–191.
- ORMIÈRES, R., LOUBÈS, C., AND MAURAND, J. 1977. Étude ultrastructurale de la microsporidie *Perezia lankesteriae* Léger & Duboscq, 1909, espèce type: Validation du genre *Perezia*. *Protistologica*, **13**, 101–112.
- PAKES, S. P., SHADDUCK, J. A., AND CALI, A. 1975. Fine structure of *Encephalitozoon cuniculi* from rabbits, mice and hamsters. *J. Protozool.*, **22**, 481–488.
- SPRAGUE, V. 1977. "Systematics of the Microsporidia". In "Comparative Pathobiology" (L. A. Bulla, Jr. and T. C. Cheng, eds.), Vol. 2. Plenum Press, New York.
- SPRAGUE, V., AND VERNICK, S. H. 1971. The ultrastructure of *Encephalitozoon cuniculi* (Microsporida, Nosematidae) and its taxonomic significance. *J. Protozool.*, **18**, 560–569.
- VINCKIER, D. 1975. *Nosemoides* gen. n. *N. vivieri* (Vinckier, Devauchelle, Prensier, 1970) comb. nov. (Microsp.), étude de la différenciation sporoblastique et genèse des différentes structures de la spore. *J. Protozool.*, **22**, 170–185.