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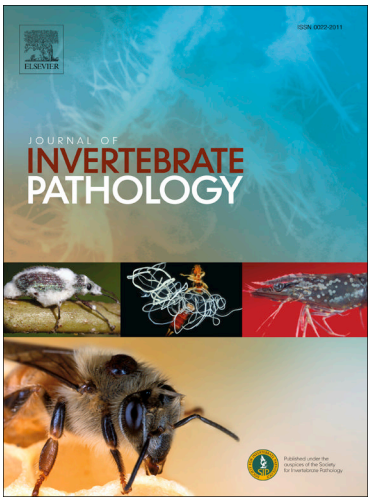

Ultrastructural and molecular characterization of *Vairimorpha austropotamobii* sp. nov. (Microsporidia: Burenellidae) and *Thelohania contejeani* (Microsporidia: Thelohaniidae), two parasites of the white-clawed crayfish, *Austropotamobius pallipes* complex (Decapoda: Astacidae)

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Ultrastructural and molecular characterization of *Vairimorpha austropotamobii* sp. nov. (Microsporidia: Burenellidae) and *Thelohania contejeani* (Microsporidia: Thelohaniidae), two parasites of the white-clawed crayfish, *Austropotamobius pallipes* complex (Decapoda: Astacidae)

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Highlights:

- A new microsporidia *Vairimorpha austropotamobii* is described in *Austropotamobius pallipes* complex
- Two different microsporidia cause comparable muscle lesions in white-clawed crayfish
- *V. austropotamobii* has a monomorphic octosporoblastic sporogony within SPV
- Phylogenetic analyses refer this new species to the *Nosema*/*Vairimorpha* clade
- Microsporidian tropism is restricted to the skeletal muscles not affecting the heart and gut musculature.

Abstract

The microsporidiosis of the endangered white-clawed crayfish *Austropotamobius pallipes* complex has generally been attributed to only one species, *Thelohania contejeani*, the agent of porcelain disease. Species identification was mostly assessed by macroscopic examination or microscopic evaluation of muscle samples rather than by molecular or ultrastructural analyses. A survey conducted on *A. pallipes* complex populations in Northern Italy highlighted the presence of two different microsporidia causing similar muscular lesions, *T. contejeani* and an undescribed octosporoblastic species *Vairimorpha austropotamobii* sp. nov. Mature spores and earlier developmental stages of *V. austropotamobii* sp. nov. were found within striated muscle cells of the thorax, abdomen, and appendages of the crayfish. Only octosporoblastic sporogony within sporophorous vesicles (SPVs) was observed. Diplokaryotic sporonts separated into two uninucleate daughter cells, which gave rise to a rosette-shaped plasmodium, and eight uninucleate spores were produced within the persistent SPV. Ultrastructural features of stages in the octosporoblastic sequence were similar to those described for *Vairimorpha necatrix*, the type species. Mature spores were pyriform in shape and an average of 3.9 x 2.2 µm in size. The polar filament was coiled 11-14 times, lateral to the posterior vacuole. The small subunit ribosomal RNA gene (SSU rRNA) and the large subunit RNA polymerase II gene (RPB1) of *V. austropotamobii* sp. nov. were sequenced and compared with other microsporidia. The highest sequence identity of SSU rRNA (99%) and RPB1 (74%) genes was with the amphipod parasite *Nosema granulosis* and subsequently with *V. cheracis*, which infects the Australian yabby *Cherax destructor*. In our work we discuss about the reasons for placing

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