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Short Communication

# *Vairimorpha ephestiae* is a synonym of *Vairimorpha necatrix* (Opisthosporidia: Microsporidia) based on multilocus sequence analysis

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## Abstract

An isolate of the microsporidium *Vairimorpha ephestiae* (originally isolated from *Ephestia kühniella*) from collection of Prof. J. Weiser was propagated in a laboratory culture of *Galleria mellonella.* Only disporoblastic sporogony was observed and formation of octospores, characteristic of the genus *Vairimorpha*, never occurred. A partial nucleotide sequence of the small subunit rRNA gene (1247 bp) for this microsporidium showed 100% identity to the homologous sequences of *Vairimorpha (Nosema) necatrix* (Genbank accession # U11051 and # DQ996241), a microsporidium with a broad host range within the Lepidoptera. Sequence similarity of protein-coding genes (RPB1, HSP70 and actin) between *V. ephestiae* and *V. necatrix* was about 98–100%. The level of genetic polymorphism in the RPB1 locus between these two species was essentially the same as between isolates of *V. necatrix.* It is therefore concluded that *V. ephestiae* is in fact an isolate of *V. necatrix* and the former species should be synonymized with the latter. Though described later, *V. necatrix* has prevailing usage and its precedence over *V. ephestiae* is proposed to conserve stability and avoid confusion.



***Keywords:*** Microsporidia; Protein coding genes; Ribosomal DNA; Sequence similarity; Species identification

## Introduction

Correct species identification is essential for many types of biological studies and this is especially important in cases of applied research such as the biological control of harmful organisms. Microsporidia of the genus *Vairimorpha* are common parasites of Lepidoptera including major agricultural and forest pests. Certain species, such as *Vairimorpha necatrix* and *Vairimorpha lymantriae*, are regarded as potential biocontrol agents (Solter and Becnel 2007). The microsporidium *Thelohania ephestiae* was described from *Ephestia kühniella* by Mattes (1928). Later a new genus, *Vairimorpha*, was established with the type species *V. (Nosema) necatrix* (Pilley 1976) after it became evident that *N. necatrix* and *Thelohania diazoma* described from *Pseudaletia unipuncta* (Kramer 1965) represent two morphologically distinct developmental patterns of a single species (Maddox 1966). Meanwhile, *T. ephestiae* was reisolated from *E. kühniella* populations (Purrini 1976). Observation of two

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sporogonial sequences in its life cycle allowed for a redefinition of this taxon as *Vairimorpha ephestiae* (Weiser 1978), later supplemented with ultrastructural studies (Weiser and Purrini 1985).

Molecular genetic tools are widely used for detection and identification of various biological organisms including insect pathogens (Sim et al. 2009). For microsporidia the main molecular genetic marker for taxonomic identification is the small subunit ribosomal RNA (SSU rRNA) while several protein-coding gene loci are considered for multilocus sequence analysis of closely related species. In *V. necatrix*, nucleotide sequencing is available for three protein-coding genes. The largest subunit of RNA polymerase II (RPB1) is represented by a single copy in the parasite's genome (Hirt et al. 1999) and as many as three distinct haplotypes are known from one host *Lacanobia oleracea* (Ironside 2013). The other two loci are for heat shock protein 70 (HSP70) and actin, both are represented by a single sequence in Genbank.

In this paper we used these molecular genetic markers to compare *V. necatrix* and *V. ephestiae* and to show that these two taxa are sympatric and should be synonymized.

## Material and Methods

The long-term culture of *Galleria mellonella* was maintained at the Laboratory of Insect Pathology, Institute of Systematics and Ecology of Animals (Novosibirsk, Russia). The infective spores of the type isolate of *V. ephestiae* from the collection of Prof. Dr. Jaroslav Weiser were kindly provided by Dr. Tomas Tonka (Institute of Entomology, ASCR, Ceske Budejovice, Czech Republic). The spore suspension ($10^4$–$10^5$ spores per larva) in distilled water was applied onto diet used to feed *G. mellonella* II–III instar larvae. After the spore-contaminated diet was consumed, the larvae were reared on microsporidia-free diet in the dark either at 20–22 °C or at 24–26 °C. The larvae were dissected and examined using phase contrast light microscopy 20–30 days post infection for the presence of spores. The microsporidia-infected culture of *G. mellonella* was maintained between 2000 and 2003. Continuous culture was discontinued and dry cadavers of the host larvae heavily infected with the *V. ephestiae* spores were stored for about 10 years at RT. For the molecular phylogenetic study, two individual specimens of infected *G. mellonella* larvae were subjected to DNA extraction, amplification and direct sequencing (Tokarev et al. 2010). A partial SSU rRNA gene sequence was amplified with 18f:1492r primers (Weiss and Vossbrinck 1999). Three protein-coding genes of *V. necatrix* from *Helicoverpa zea* were used to design species-specific primers. The primers VnACT1F (5′-GCACCTTTGAATCCTAACCAG-3′) and VnACT1R (5′-CCTATCCAAACACTATACTTCCT-3′) were designed to amplify a 704 bp long fragment of actin based upon a Genbank entry under accession # AF031818. The primers VnHSP1F (5′-TGGTCTTGATAAATCGGCTA-3′) and VnHSP1R (5′-TCTTGACTAACTTCCTGACGT-3′) were designed to amplify a 512 bp long fragment of HSP70 based upon an entry # AF008215. The primers VnRPB1F (5′-GTGAAATTGGGGAAAATGGG-3′) and VnRPB1R (5′-TAATTACAGACCTGGCACTG-3′) were designed to amplify a 559 bp long fragment of RPB1 based upon an entry # AF060234. The latter locus was found to be polymorphic and was subjected to molecular cloning. The sequences were compared to Genbank entries obtained using the BLAST utility (blast.ncbi.nlm.nih.gov/Blast.cgi?), aligned and analyzed in BioEdit Sequence Alignment Editor (Hall 1999). The sequences of RPB1 obtained in the present study and downloaded from Genbank were subjected to molecular phylogenetic reconstruction using the Maximum Likelihood (ML) method based on GTR + G model with 100 replicates in MEGA 7 (Kumar et al. 2015).

## Results and Discussion

The partial SSU rRNA gene, 1247 bp long, obtained for *V. ephestiae*, was identical in both specimens and showed 100% identity to the Genbank entries for *V. necatrix* (Genbank accession # DQ996241) and *N. necatrix* ATCC 30460 (# U11051), which is the primary taxon name for *V. necatrix* (Kramer 1965). On the other hand, a Genbank entry # Y00266 for *V. necatrix* SSU rRNA gene is slightly different (Fig. 1A). It contains two point substitutions (a transition and a transversion at positions 755 and 901 of the sequence # U11051, respectively), as well as a triplet deletion (positions 816–819) and a triplet substitution (positions 831–833). The overall sequence similarity of the two haplotypes is 99.3%. The sequence similarity of these two haplotypes to the other species of the *Vairimorpha* genus is below 97%.

The Genbank entry # Y00266 is one of the earliest sequences obtained for microsporidia (Vossbrinck et al. 1987). The sequence differences could be due to multiple reasons, including amplifying or sequencing artifact, using a different (though closely related) species of Microsporidia etc. Meanwhile, uniformity between the other sequences, including that of *N. necatrix* (# U11051) deposited in the American Type Culture Collection, prompts us to consider the respective haplotype as the type for *V. necatrix*.

The sequence of actin derived from *V. ephestiae* (# MG808088) was 98.6% similar to a homologous sequence from *V. necatrix* (# AF031818). Similarly, the HSP70 sequences from *V. ephestiae* (# MG808089) and *V. necatrix* (# AF008215) were 98.7% similar to each other. As for RPB1, among six molecular clones derived from *V. ephestiae*, five belonged to one variant (# MG808086) and one to another (# MG808087), displaying 99.0% similarity to each other. The first haplotype was 98.3–99.0% similar to Genbank-accessible sequences of *V. necatrix* from *Heliothis zea* (#AF060234), *P. unipuncta* (# DQ996236) and three isolates (Vnec1-Vnec3, ## JX213796-JX213798) from *L. oleracea*.