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The secondary structure of the unusually long 18S ribosomal RNA of the myxozoan *Sphaerospora truttae* and structural evolutionary trends in the Myxozoa ☆

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Abstract

The nearly complete 18S rRNA sequence of the myxozoan parasite *Sphaerospora truttae* shows an extraordinary length (2,552 bp) in comparison with other myxozoans and with metazoans in general (average 1,800–1,900 bp). The sequence shows nucleotide insertions in most variable regions of the 18S rRNA (V2, V4, V5 and V7), with especially large expansion segments in V4 and V7. In the myxozoans, nucleotide insertions and specific secondary structures in these regions of the gene were found to be strongly related to large scale phylogenetic clustering and thus with the invertebrate host type. Whereas expansion segments were generally found to be absent in the malacasporeans and the clade of primary marine myxozoan species, they occur in all taxa of the clade containing freshwater species, where they showed a consistent secondary structure throughout. The longest expansion segments occur in *S. truttae, Sphaerospora elegans* and *Leptotheca ranae*, which represent a clade that has emerged after the malacosporeans and before the radiation of all other myxozoan genera. These three species demonstrate structural links to the malacosporeans as well as other unique features. A smaller number of nucleotide insertions in different subhelices and specific secondary structures appear to have evolved independently in two marine genera, i.e. *Ceratomyxa* and *Parvicapsula.* The secondary structural elements of V4 and V7 of the myxozoan 18S rRNAs were found to be highly informative and revealed evolutionary trends of various regions of the gene hitherto unknown, since previous analyses have been based on primary sequence data excluding these regions. Furthermore, the unique features of the V4 region in *S. truttae* allowed for the design of a highly specific PCR assay for this species.
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Keywords: *Sphaerospora truttae*; Myxozoa; 18S rRNA secondary structure; Molecular phylogeny; Specific PCR

1. Introduction

The myxozoan *Sphaerospora truttae* Fischer-Scherl, El-Matbouli & Hoffmann 1986 is a significant pathogen in some Scottish freshwater salmon hatcheries (McGeorge et al., 1997; Özer and Wootten, 2000; Holzer et al., 2003). Study of the 18S rRNA gene region of *S. truttae* revealed its unusual length (Holzer et al., 2003) and resulted in phylogenetic positioning of the species in a clade of marine myxozoans (Holzer et al., 2004). Recently, Fiala (2006) conducted a comprehensive phylogenetic analysis of a considerably larger dataset of myxozoan 18S rRNA sequences which allocated *S. truttae*, with high bootstrap support, to a separate clade, basal to the marine myxozoans. The outcome of phylogenetic analyses is highly dependent on the alignment of primary sequence data, and rRNAs are often hard to align due to frequent base changes and extensive insertions in the so-called variable regions (Neefs and De Wachter, 1990). This characteristic of the rRNA suggests that, for phylogenetic reconstruction, these molecules should benefit from a higher order structure of rRNA

☆ Nucleotide sequence data reported in this paper is available in the GenBank[TM], EMBL and DDBJ databases under the Accession No. AM410773.

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sequences, i.e. their secondary and, if possible, tertiary structure. The general secondary structure model of the 18S rRNA in eukaryotes (Fig. 1) contains six variable regions whose secondary structure can aid considerably in alignment and improve the reliability of subsequent phylogenetic analyses (e.g. Kjer, 1995, 2004; Titus and Frost, 1996). Robust phylogenies are especially difficult in the phylum Myxozoa, which shows extremely high sequence variability between most species, probably due to the "fast clock" characteristic (Kent et al., 1996) of the myxozoan 18S rRNA chronometer. Thus, information on 18S rRNA secondary structure might be of special importance in determining myxozoan phylogeny. However, even if primary sequence data based on secondary structure cannot be compared due to large differences between some species, common structural evolutionary changes in the gene might reveal phylogenetic relationships between these taxa. Taxon-specific rRNA morphologies have been reported from various groups of the animal kingdom and can be phylogenetically indicative (e.g. Crease and Taylor, 1998; Lydeard et al., 2000; Gillespie et al., 2005; Misof et al., 2006). The current study aimed to identify regions in the 18S rRNA of *S. truttae* and other myxozoans that show frequent base changes and nucleotide insertions, to determine their secondary structure and to compare these structural elements in order to reconstruct the evolution of the 18S rRNA structure in the phylum. This could then be used to confirm the phylogenetic positioning of taxa based on primary sequence data only and to optimise future alignment procedures. This is of particular importance for the positioning of unusual sequences such as that of *S. truttae*.

Furthermore, the identification of unique inserts in the 18S rRNA sequence of *S. truttae* has allowed the development of a specific PCR assay, which enables early detection of low levels of the parasite in the fish host and offers the possibility of identifying extrapiscine life cycle stages and thus the alternate host of *S. truttae*, which is so far unknown.

2. Materials and methods

2.1. DNA extraction, cloning and sequencing

In order to avoid inaccuracy in the primary sequence of *S. truttae*, the 18S rDNA gene of *S. truttae* was resequenced from a cloned insert. A previously obtained sample of DNA from isolated *S. truttae* spores (Holzer et al., 2003) was used. These spores were derived from infected 1-year-old Atlantic salmon reared in a freshwater hatchery on the north coast of Scotland, which shows annually recurring *S. truttae* infections with 100%

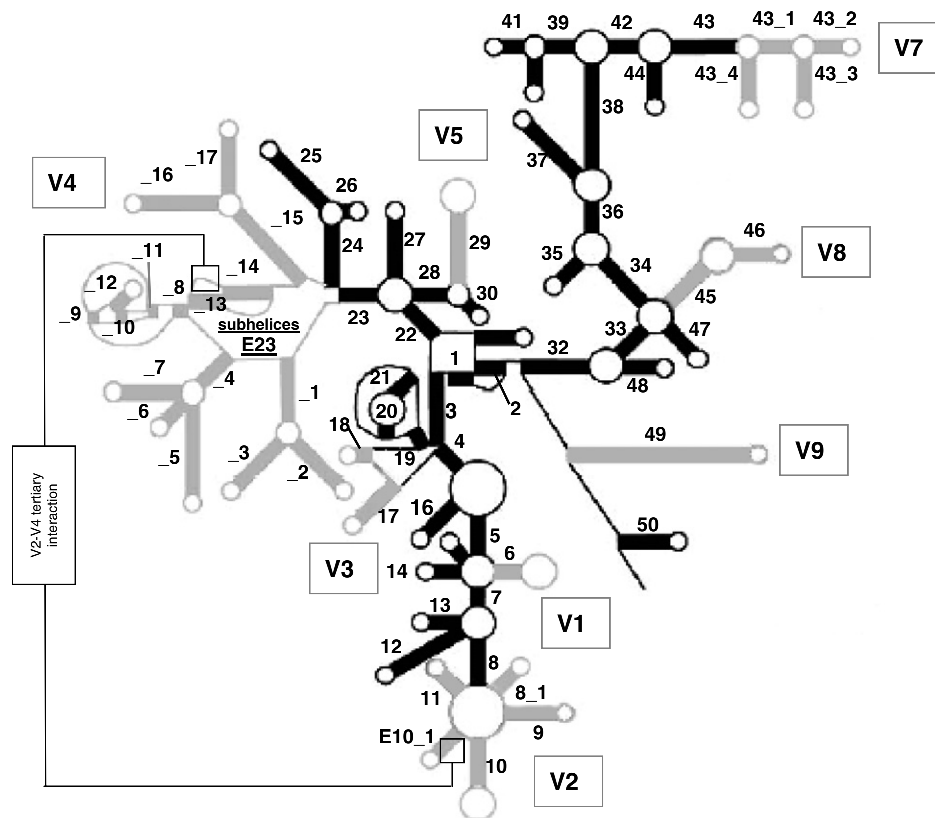

Fig. 1. Generalised model of the 18S ribosomal RNA gene in eukaryotes with all possible helices composing the variable regions V1–V9 indicated in grey (V6 is formed by helix 37 which is variable only in prokaryotic small subunit rRNA where this helix is branched), simplified after Wuyts et al. (2001). Helix numbering clockwise from 5′- to 3′-terminus. Note presence of four pseudoknots in V4 and tertiary interaction between helix E10_1 and one of the pseudoknots formed by helices E23_13 and E23_14 proposed by Alkemar and Nygård (2003, 2004).

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