

An Introduction to Fish Parasitological Methods: Classical and Molecular Techniques



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Review

A review on the molecular characterization of digenean parasites using molecular markers with special reference to ITS region

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Summary

The rDNA region of eukaryotes has the immense potential to resolve the evolutionary and phylogeny problems using molecular markers. As evident from the present review, ITS region data is considered for interpretation of inter and intra-specific variations of 136 studies in 53 families including 78 genus and 114 species affecting individuals worldwide. Along with ITS-1 and ITS-2 region in 29 studies 18S region, in 38 studies 28S region and in 43 studies mitochondrial genes (COI and ND1) were also analyzed. Three new genera (*Alobitarzia* gen. nov., *Cavilaeus* gen. nov. and *Lafiferus* gen. nov.) and 49 new species were discovered. Only 47 studies expressed variations at intra-specific and inter-specific level in complete ITS region. ITS-1 and ITS-2 rDNA sequences due to differences in nucleotide positions. According to the findings ITS region is more reliable and precise marker for demarcation and identification of species in combination of other DNA markers. Major studies were involved around the parasites of families Fasciolidae, Schistosomatidae, Opisthorchiidae, Paragonimidae and Paramphistomidae, Clinostomidae, Diplostomidae, Hagloporidae, among others infecting humans, farm animals, birds, fishes, reptiles and amphibians on the clinical basis. In future, molecular and bioinformatics aspects based on genetic variations will lead to explore the untouched areas of trematodes.

Keywords: Internal transcribe spacer; Cytochrome c oxidase I; Inter and Intra-specific variations; phylogeny; Fasciolidae; Schistosomatidae; trematodes

Introduction

The ribosomes, intracellular and molecular machines, found in all living organisms play major role in protein synthesis and gene expression. The ribosomal RNA (rRNA) gene and spacer regions extensively provide the phylogenetic information in prokaryotes and eukaryotes, collectively known as ribosomal DNA (rDNA), composed of coding regions (18S, 5.8S and 28S) and non-coding region of 2 internal transcribed spacers (ITS-1, ITS-2) and one non-transcribed spacer (NTS) (Wei *et al.*, 2006) (Fig. 1). The coding regions are highly conserved sequences in living creatures and infer the phylogenetic relationship between major phyla, while the non-coding regions are highly variable and have great potential to study the relationships among closely related genera or species due to faster evolutionary rate (Chen *et al.*, 2004; Nolan

& Cribb, 2005). The ITS regions are extensively used as molecular markers for taxonomy and phylogenetic analyses (Porter & Collins, 1991). The preference of ITS over other non-coding regions are: (1) multiple copies of rRNA genes; (2) suitable for PCR amplification with several universal primers for different organisms; (3) average sequence length for sequencing; and (4) high degree of variations at the genetic and species level due to frequent nucleotide polymorphisms or insertions/deletions in sequences (Poccai & Hyvönen, 2010; Calonje *et al.*, 2009).

The digeneans form the group of flatworms and are most common and abundant among parasitic worms. They parasitize all the classes of vertebrates and inhabit nearly every body organ. Their life cycle involves at least two hosts, although few species include a second and even a third intermediate host. Thus, the complexity is reflected in involvement of many representatives in devel-

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