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# Ribosomal DNA as molecular markers and their applications in the identification of fish parasites (Platyhelminthes: Monogenea) from India

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## KEYWORDS

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This is a very short review. This reviewer believes that it can serve a bigger purpose if the authors could provide a clear and in-depth overview for all has been done in this area outside of India, so that the Indian researchers (or the outsiders) in this will have a clear picture of what is missing in this country and what needs to be done as pure science or for the fish industry is proven to be a problem.

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## ABSTRACT

The development of molecular techniques for taxonomic analysis of monogenean parasites has led to a great increase for proper identification and factualness. These molecular techniques, in particular the use of molecular markers, have been used to identify and validate the monogenean parasites. Although, improvements in marker detection systems particularly of elements of rDNA like 18S, ITS and 28S used in monogeneans parasites have enabled great advances to be made in recent years in India. However, the molecular sequence analysis and phylogenetic relationships among the parasitic helminthes is unconventional in India. Many workers have been always questioned the validity of Indian species of monogeneans and emphasized the need to ascertain the status of species from Indian fish. Here we would like to provide additional resolution for the interpretation of use of molecular markers in study of monogeneans in India. This review provides an overview of current stage of studies in India that have been used in applying molecular techniques to monogenean.

Monogenea, Ribosomal DNA, Molecular markers, Fish parasites, India

## 1. Introduction

Class Monogenoidea Bychowsky of phylum Platyhelminthes is one of the largest class that includes a group of parasites commonly found on the gills, skin, and fins of fishes, lower aquatic invertebrates and some other amphibious vertebrates[1]. There are more than 5000 known species of Monogenea and all of them are reported as parasitic[2]. Among the known species, at least 95% of the genera have been reported from fishes and the remaining 5% are reported from mouth, urinary bladder of

amphibious vertebrates. However, some of them have also been abstracted from nasopharynx of aquatic mammals, one (*Oculotrema hippopotami*) infecting mammal, parasitizing the eye of the hippopotamus. There are about 50 families and thousands of described species. Approximately 112 genera belonging to 28 families on Monogenoidea have been abstracted from India[3].

According to Bauer, among parasites infecting fishes, the monogeneans constitute a group, which plays an important role as pathogens of severe diseases[4]. This is because the monogeneans affect those organs and tissues which are vital

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to normal functioning such as gills and skin, the organs of respiration<sup>[5,6]</sup>. The negative effect of monogeneans on the fish host may vary between species. Also bacterial or viral organisms may get entry into the host through the injuries inflicted by monogenean anchors and feeding apparatus<sup>[7,8]</sup>. Fish gills infested by monogeneans start copious production of mucous which in turn leads to decrease in the respiratory efficiency, as a result, the host becomes vulnerable to low oxygen concentration of water. In most cases, monogeneans cause a dual type of injury to their hosts. Through their hooks and other organs of attachment, they injure the tissue at the site of attachment, break its continuity and cause localized haemorrhages. At the same time, they feed on the cells of ruptured tissues and as some studies have shown, on the blood<sup>[9,10]</sup>.

Control and treatment of parasitic infection depends upon proper and quick identification of related parasites. The morphological species perception is based on the observed discontinuities, and it is non-dimensional, without good information about the fine-scale (species level) evolution<sup>[11]</sup>. Taxonomy underpins all biological research, with implications for many scientific and applied fields but this comes at a time when taxonomy is facing a crisis, because fewer specialists are available. By exploring the possibility of using DNA based methodology to overcome these taxonomic problems, the utility of DNA sequences for taxonomic purposes is well established. DNA sequencing provides a valuable approach for the genetic characterization of a parasite species<sup>[12,13]</sup>. Hence, systematic workers are extremely interested in new technologies that could make this procedure more efficient.

## 2. Molecular markers use in monogenean systematics

In Monogenea taxonomy, molecular marker technology offers such a possibility by adopting a wide range of novel approaches to improve the systematics of these parasites. However, the problems in morphological taxonomy are enough for extension of the molecular approach to all life. In fact, there are a growing number of cases in which DNA-based identification systems have been applied to higher organisms<sup>[14–17]</sup>. Genomic tools exploit diversity among DNA sequences to identify organisms<sup>[18,19]</sup>. Although in recent years, the use of molecular biological tools in monogenean systematics is carried out from foreign lands such as ribosomal and mitochondrial DNA<sup>[20]</sup>. According to Pandey and Agrawal, there are about 50 families and thousands of described species, approximately 112 genera belonging to 28 families on monogeneoidea have been abstracted from India<sup>[3]</sup>. But in India, monogenean molecular identification is in infancy and very few works have been done by Indian workers. If we search the GenBank database of NCBI for Indian monogeneans, we can find that only 17 sequences of 28S ribosomal DNA (rDNA); 6 sequences of 18S rDNA and

3 sequences of internal transcribed spacer (ITS) of rDNA were found submitted by Indian workers. Besides this, only 1 sequence of COI subunit of mitochondria of Indian monogenea has been found which was submitted on GenBank database by foreign workers. These results showed the negligible amount in comparison of the diversity of these parasites in India.

## 3. Conclusion

Without doubt, we can say that a lot of work should be done in molecular of Indian monogeneans. DNA barcoding approaches can therefore be recommended to assess the biodiversity of largely unknown and problematic taxa, such as monogeneans. To some extent, DNA barcodes provide important information for taxonomy, systematics, biogeography, ecology and evolutionary biology<sup>[21,22]</sup>. However, for a meaningful delimitation of relevant taxonomic units that facilitate the management of parasite induced diseases in fishes, a purely DNA based molecular classification system is satisfactory. As such, from the point of view of monogenean validation and identification, DNA taxonomy and barcoding by the use of nucleotide sequence data to achieve comprehensive species descriptions that facilitate reliable species diagnostics of biodiversity of these parasites. These methods are of great importance for parasitologists and such molecular approaches are in need to apply to the monogeneans.

## Conflict of interest statement

The authors declare that there are no conflicts of interest.

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## Comments

### Background

Morbidity and mortality in cultured fish caused by parasites are associated with crowding, insanitation and deterioration of water quality. Monogeneans are common on wild fish, but they are rarely a direct etiology of disease or death in free-ranging species. This review attempts

to provide an overview of the current status of studying employing molecular systematics in India.

### Research frontiers

Like similar research conducted in various species, the progress of molecular systematics in fish flatworms relies on the availability of usable gene sequences. This is particular the case in India where many species of the flatworms are either unknown or just starting being researched. This review has some value in providing the general overview of such an endeavor for an emerging field in India.

### Related reports

A lot of work in fish flatworms have been done outside of India. Very little has been done in those species in India.

### Innovations & breakthroughs

This may be important to the policy makers with regard to cultured fish industry where the parasites may become an economic problem.

### Peer review

This is a very short review. This reviewer believes that it can serve a bigger purpose if the authors could provide a clear and in-depth overview for all has been done in this area outside of India, so that the Indian researchers (or the outsiders) in this will have a clear picture of what is missing in this country and what needs to be done as pure since or for the fish industry is proven to be a problem.

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