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Cloning and Sequencing of Its-1 Region of the Parasite *Tylodelphys clavata* (Von Nordman 1832)

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Abstract: Parasites morphologically consistent with Tylodelphys clavata collected from the vitreous humor of Chondrostoma regium one of native fish species inhabits in Choghakhor lagoon in Chaharmahal & Bakhtyari province. Samples examined by PCR of the ITS-1 region to identify the parasite using one pair Forward and Reverse Primers. The expected amplification band (560-bp) was observed on agarose gel after PCR operation. To assess accuracy of result and also to confirm obtained data, the amplified sample were cloned by T/A cloning and then sequenced. The blast result and comparing the results with other sequences were reported previously in GenBank confirmed that amplified product is corresponding to *Tylodelphys clavata* without any pseudo data could be generated by false priming/amplification or carry over contamination. It should be noted that the homologues sequences were found by blast search were not the same with those previously had deposited in GenBank by Ananden *et.al* that might indicates the heterogeneity of studied gene among different variants of this parasite in Iran.

Key words: *Tylodelphys clavata* · ITS-1 · Fish parasites · Iran

INTRODUCTION

Tylodelphys clavata belongs to Diplostomatidae family targets the eye of fish which settles and feeds from vitreous humor in larva phase [1]. Metacercarial stage targets fish as the second host infecting it which accompanies some damages in the eye of fish [2]. This parasite in hand is capable of infecting various species of Cyprinidae family members. This parasite is various in kind globe because it settles in many fish-eating birds [3-5]. Regarding the fact that morphologic diagnosis of Metacercarial stage always accompanies with some problems due to the physical similarities of this kind of parasite, using gene of parasite can be a good way to solve the problem. ITS-1 region has been selected with an eye on this problem using PCR. ITS-1 region is the commonest region of genomic DNA to realize continuation in most creatures. Distinctly this region is the most advantages genomic region to classify creatures to species or sub-species level [6-7]. The aim of this study is sequencing ITS-1 region of parasite gene and surveying the mentioned region's sequencing differences in the case in hand and the reported cases.

MATERIALS AND METHODS

Firstly the specimens of Tylodelphys clavata were collected from the eye of Chondrostoma regium fish caught from the Choghakhor lagoon located in Chaharmahal & Bakhtyari province in Southwest of Iran. The parasite specimens were identified by the use of identification key [1] regarding to morphologic and morphometric characteristics such as length and weight of body, sucker location and the diameter of suckers [1,8]. The specimens after being sorted out and identifying were kept in alcohol 70 percent to the time of PCR operation. To investigate molecular operation and regarding to reported genomic sequence for the parasite Tylodelphys sp in

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GenBank (Accession number AY380164) a pair of primers with following sequence were designed to identify the parasite by the use of Gen runner software.

F: 5'- TTG CAG TCT AGC CCA GGT TG -3'
R: 5'- AGT CCC TAT CTG AAA CTT ATC G -3'

It is necessary to mention that the primers were blasted in ncbi as a GenBank reference in the case of probable similarities with host fish gene. PCR test was carried out to gain ITS-1 under these circumstances: Twenty nano grams DNA in 25 µl, 2 mili molar Mgcl2, 25 picomol of each primer, one unit Taq polymerase enzyme and 20 Micro molar dNTP. The temperature program was set out as below respectively: 94°C for 1 minute, 57°C for 1 minute, 72°C for 1 minute and a final stage 72°C for 5 minutes [2]. Ultimately 10 µl of PCR product with 2 µl loading buffer were transmitted to agarose gel 1.5 % containing Ethidium bromide color and were observed under UV and then was shot. The amplified part for ITS-1 region has a length of 560 bp. The PCR product was extracted after electrophoresis on agarose 1.5 % gel using DNA extraction kit made by BioNeer and was cloned by the use of T/A cloning in Topo-invitrogen and were transformed in E.coli Top10F. The recombinant TOPO vector of ITS-1 region was sequenced to approve appropriate cloning and nucleotide sequence. Therefore 20 µl of each mentioned plasmid with related primers were cloned to the gene and were observed to find its sequence. The result was compared to the recorded ones in GenBank [9].

RESULTS AND DISCUSSION

After PCR and electrophoresis 560 bp band related to ITS-1 region was gained so the molecular identification of the investigated species was approved (Figure 1). Sequencing the mentioned part and comparing it to the recorded genes in GenBank is a proof to the appropriation of PCR product. It is to say that the parasite nucleotide sequence in first phase is consistent with Tylodelphys nucleotide sequence reported by Ananden et.al with accession number AY380164 in GenBank. Although there exist some slight differences of nucleotide in the studied case with the one in GenBank which can show the polymorphism of the case in hand and reported ones.

Principally diagnosing larval phase or metacercaria of digenean parasites is not an easy job due to their similarities. Moreover despite or organ specifity of these species of parasites and their inclination of replacement

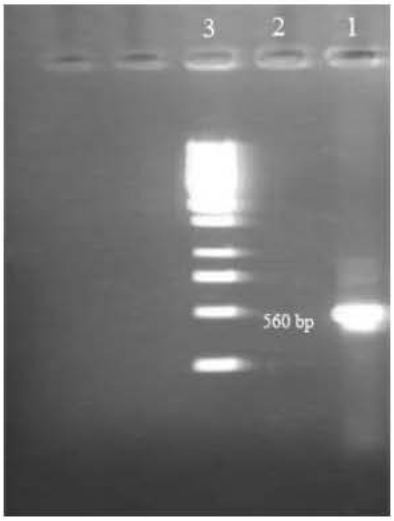

Fig. 1: PCR Result:
1.DNA of parasite which is positive in PCR
2. Negative control 3. Marker 1kb

in a specific organ, lots of them may target or move to different organs of the body, which will lead to some problems specifying them. This is the case reported in Diplostomum and Ornithodiplostomum [10-11]. Beside of the vast damages resulting of this parasite infection paying attention to this fact that diplostomatids and specifically Tylodelphys are poly host settlers highlight their importance of infection in aquatic ecosystems. So that the mature parasite lives in fish eater birds of grebe [5,12] and after lying, miracidium enters to the intermediate host snail (Lymnea) and targets the fish as the next host [2]. The definitive hosts of parasite which are grebes are the native birds which every kind of damages to them will be irreparable, on the other hand their permanent presence in the lagoon will lead to complete life cycle of parasite. The intermediate host snails have abundant Cercaria of parasite in their Hepatopancreas which leads to the death of snail. Paying attention to the damages of parasites in other creatures including birds, fish and snail can highlight the importance of the problem [2-3]. The parasite in hand was reported first in 2007 and secondly in 2008 from the Choghakhor and Gandoman lagoons. This parasite had infected 21 % of the fishes in Choghakhor lagoon and 26.2 % of native fishes [10,13]. Regarding to the fact that this parasite is new for the fresh water fishes in Iran and the pathogenesis importance of the parasite, molecular solution based on ITS-1 region is presented in this article. This study is first in its kind. Using ITS-1 region in identifying and classifying creatures to species level is more prevalent due to its various changes to the

other parts of rDNA. This study in hand and many others are a proof for using this gene in molecular diagnosis. Therefore the utilized primers were designed and used based on this gene region. Ultimately after PCR and observing the expected band with 560 bp molecular weight the diagnosis of parasite were approved. The mentioned band were sequenced which showed the appropriation of PCR. The present study in hand showed not only a molecular solution for diagnosing Tylodelphys clavata but also shows the differences in sequences of ITS-1 region of this parasite and the reported case from Scotland showing the existence of polymorphism in two cases.

REFERENCES

1. Gussev, A.V., 1987. Digenea. In: Key to parasites of freshwater fishes of the soviet USSR. Nauka publications.
2. Shahryari, F., 2002. Polymerase chain reaction. Imam Reza University Publications.
3. Barzegar, M., M. Raissy and B. Jalali, 2008. Parasites of the eyes of brakish and freshwater fishes in Iran. Journal of Veterinary Research and science, 9(3): 256-261.
4. Hoglund, J., 1999. Diplostomum spathaceum larvae (Diplostomosis) (Digenea) in fish. ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish, in the proceedings of International Council for the Exploration of the Sea, pp: 55.
5. Moody, J. and E. Gaten, 1982. The population dynamics of eyeflukes Diplostomum spathaceum and Tylodelphys clavata (Digenea: Diplostomatidae) in rainbow and brown trout in Rutland Water: 1974–1978. Hydrobiologia, 8(8): 207-209.
6. Liu, J.S. and C.L. Schardl, 1994. A conserved sequence in internal transcribed spacer 1 of plant nuclear rRNA genes. Plant Molecular Biology, 26(2): 775-778.
7. Niewiadomska, K. and Z. Laskowski, 2002. Systematic relationships among six species of Diplostomum Nordmann, 1832 (Digenea) based on morphological and molecular data. Acta Parasitol., 47(2): 20-28.
8. Jalali, B., 1998. Parasites and parasitic diseases of fresh water fishes of Iran. Fisheries institution of Iran Publications.
9. Ranga M.M., 2003. Fish Biotechnology. Agrobis Publications.
10. Raissy, M., F. Fadaeifard and A. Lashkari, 2008. Parasites of Aphanius vladykovi (Coad 1988) in Gandoman Lagoon, Iran. In the proceedings of 29th World Veterinary Congress, pp: 312-313.
11. Siegmund, I., R. Franjola and P. Torres, 1997. Diplostomatid metacercaria in the brain of silversides from Lake Rinihue, Chile. Journal of Wildlife Disease, 33(2): 362-364.
12. Reusch, T.B.H. and G. Rauch, 2004. Polymorphic microsatellite loci for the trematode Diplostomum pseudospathaceum. Molecular Ecology Notes, 4(4): 577-579.
13. Raissy, M., M. Barzegar, E. Rahimi and B. Jalali, 2007. Parasites of fishes in Choghakhor Lake, Iran. In the Proceedings of the 21st International conference of the World Association for the Advancements of Veterinary Parasitology, pp: 462.