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FIRST MOLECULAR DIAGNOSIS OF SOME DIFFERENT CESTODES OF FRESHWATER FISHES IN MISAN PROVINCE, SOUTH OF IRAQ.

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Abstract

The present study was carried out to identify the helminthic parasites of some freshwater fish species collected from Umm an Ni'aj Marsh at Misan Province. A total of 310 fishes specimens were collected belonging to two families, two species. The fish were (110) *Silurus triostegus* and (200) *Carasobarbus luteus.* The genetic diagnosis of the three species of helminthes parasites in fishes was described for the first time by using polymerase chain reaction (PCR) and DNA sequencing technology for ribosomal gene (*28S rDNA*) in Misan Province. Genetic analysis was give the true diagnosis of worms *Schyzocotyle acheilognathi* its synonym *Bothriocephalus acheilognathi* Based on present molecular study considered *B. acheilognathi* as synonyms of *S. acheilognathi. Khawia armeniaca* according to molecular supported the morphological analysis that 98% identity with *K. armeniaca*. Also molecular analysis Confirmed the morphological analysis of *Postgangesia inarmata* with 98% identity with *Postgangesia inarmata* from GenBank.

Key words : Molecular diagnosis, *28S*, Misan, Iraq.

Introduction

All animals in nature, including fishes uncovered to parasites, these infections in fishes are common, it is not sign of pollution, as long as fish live in normal conditions (price and Tom, 1995). But fishes are more susceptible to infection when exposed to Stress as a result of any defect in those circumstances, this Stress may lead to deaths of fishes and more bio losses (Barnham, 1998). The parasites of freshwater fishes especially in last years had a great deal and much attracted the attention of biologists in Iraq as a result of fish culture industry (Muhammad *et al.,* 2013). However, the importance of fish parasites is related directly to the importance of the fish that they may affected. Therefore, the past knowledge of fishes breeder for his farming about parasites and their affect it's important for economic point (Ali *et al.,* 1988). The fish parasites may directly affect the fish population and its nutritive value, with the increase in world population, and increasing the gap of protein supply, fish is of a higher nutritive value as compared to red meat (Davies, *et al.,* 2006). The world working on exploitation of further healthy fishes because the decline of food resources, large number of fish may become inedible due to parasitic diseases (Bauer, 1961). Freshwater fishes may be playing as intermediate or vectors host for many parasitic diseases. Therefore, Some of these parasites like nematodes, cestodes and trematodes may be transmitted from fishes or from fishes to bird to human, in this way these parasites called Zoonotic parasites such as *Diphyllobothrium latum*, *Clonorchis sinensis*, *Heterophyes heterophyes* and *Anisakis simplex* (Hoffman, 1998; Roberts and Janovy, 2009). The aim of present study, is to identify some parasites helminthes from the fishes collected from the Umm an Ni'aj marsh in Misan province. The investigation includes molecular identification by used ribosomal gen.

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Materials and Methods

Sample collection

The collection of fishes carried out during the period of June 2019 till Jon 2019 from Am Al-Naaj marsh at Miasn Province Iraq for 348 fishes Return to three species of fish were collected; (*Carasobarbus luteus*, *Silurus triostegus* and *Leuciscus vorax*) from central slaughterhouse of Amara city in Misan province

(31°35’35.2"N 47°37’50.8"E). After isolating helminthes washed with normal saline and then in distal water in prepared to DNA extraction. The specimens were preserved in labeled tubes with 70% ethanol and stored in deep freeze (-20°C) until used for DNA extraction.

DNA extraction

The DNA were extracted according manufacturer’s instructions of DNA extraction kit (gSYNC™ DNA Extraction Kit, geneaid, Korea). The purified DNA samples were frozen at -20°C until used in the polymerase chain reaction (PCR) technique.

PCR amplification and gel electrophoresis

The ribosomal DNA target sequences for *28S* gene were amplified by PCR with using the following protocol: PCR was carried out for the purified DNA samples with PCR preMix (BioNeer, Korea) in a 50ìl final volume of reaction. A portion of the 28S rDNA was amplified by PCR. The PCR primers were forward primer C1 (ACCCGCTGAATTTAAGCAT) and reverse primer C3 (CTCTTCAGAGTACTTTTCAAC) and expected to be specific to Platyhelminthes (Mollaret *et al.*, 2000). The *28S rDNA* amplification began with an initial denaturation at 94°C for 5 minutes, 40 cycles of 94°C for 45 seconds, 58°C for 35 seconds, and 72°C for 45 seconds, followed by a final extension at 72°C for 10 minutes. The amplification products were resolved by electrophoresis in a 1% agarose gel using TBE buffer 1X (at 65 V for 1 h.) A 100bp ladder (Bioneer, Korea) was included as molecular size marker. Gels were visualized by staining with ethidium bromide solution (0.5 µg/ml) and banding patterns were photographed over UV light using Gel documentation.

Sequencing

The PCR amplified *28S rDNA* gene specimens were automatically sequenced with using Applied Bio-system (genide, Korea) then The sequences of the helminthes were isolate was deposited to GenBank (http:// www.ncbi.nih.gov) where accession number required. And phylogentic tree created according to MOLE BLAST in NCBI.

Results

The results of PCR Amplicons electrophoresis with 1% agarose gel which visualized by ethidium bromide stain showed that PCR products sizes were 412bp (Fig. 1).

The results of *28S* gene sequences for isolates of The genetic diagnosis of the three cestodes species *Schyzocotyle acheilognathi*, *Khawia armeniaca* and

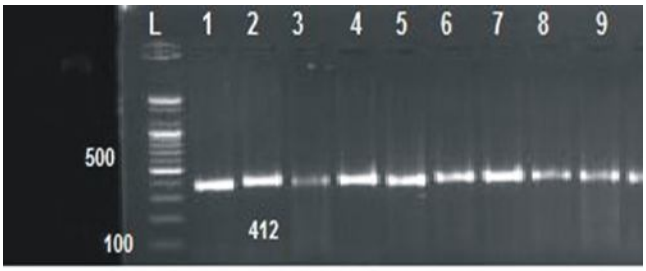

Fig. 1: Electrophoreses pattern of PCR product for *28S* gene 412 bp, 1% Agarose, 70 V, 60min, M: DNA marker ladder 100bp.

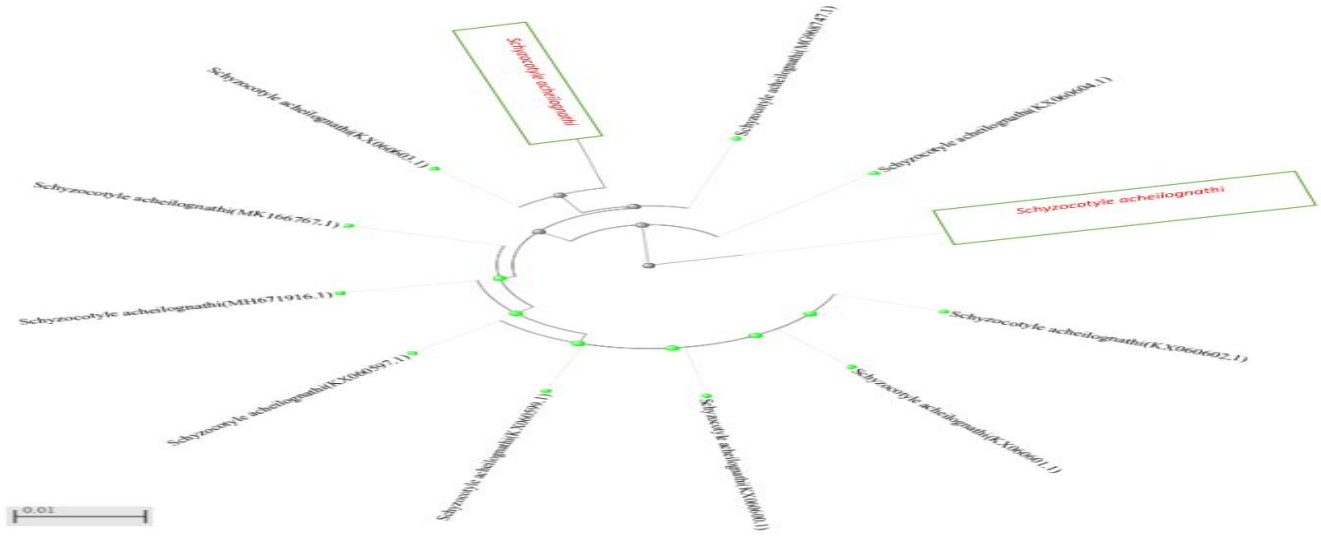

Fig. 2: Phylogenetic tree created of 28S rDNA of *Schyzocotyle acheilognathi* by MOE-BLAST current our compared with other helminthes show identity with different accession numbers tree created according to this parameters:
A. Tree method: neighboring methods. B. Max Seq Difference: 0.75. C. Sequence Label: taxonomic name (sequence ID)

Table 1: The species of worms and their hosts.

Species worms	No. of worms	Hosts
Schyzocotyle acheilognathi	8	*Cyprinidae*
Khawia armeniaca	4	*Carasobarbus luteus Heckel*, 1843
Postgangesia inarmata	3	*Siluridae*
		Silurus triostegus Heckel, 1843

Table 2: The species of worms and their some available accessions number.

Species worms	No of specimens	Accession numbers	Per identity
Schyzocotyle acheilognathi	4	MH671916.1MK166767.1MG968747.1	99%
Khawia armeniaca	2	JN004257.1	98%
Postgangesia inarmata	3	JX477442.11JX477441.1AM931032.1	98%

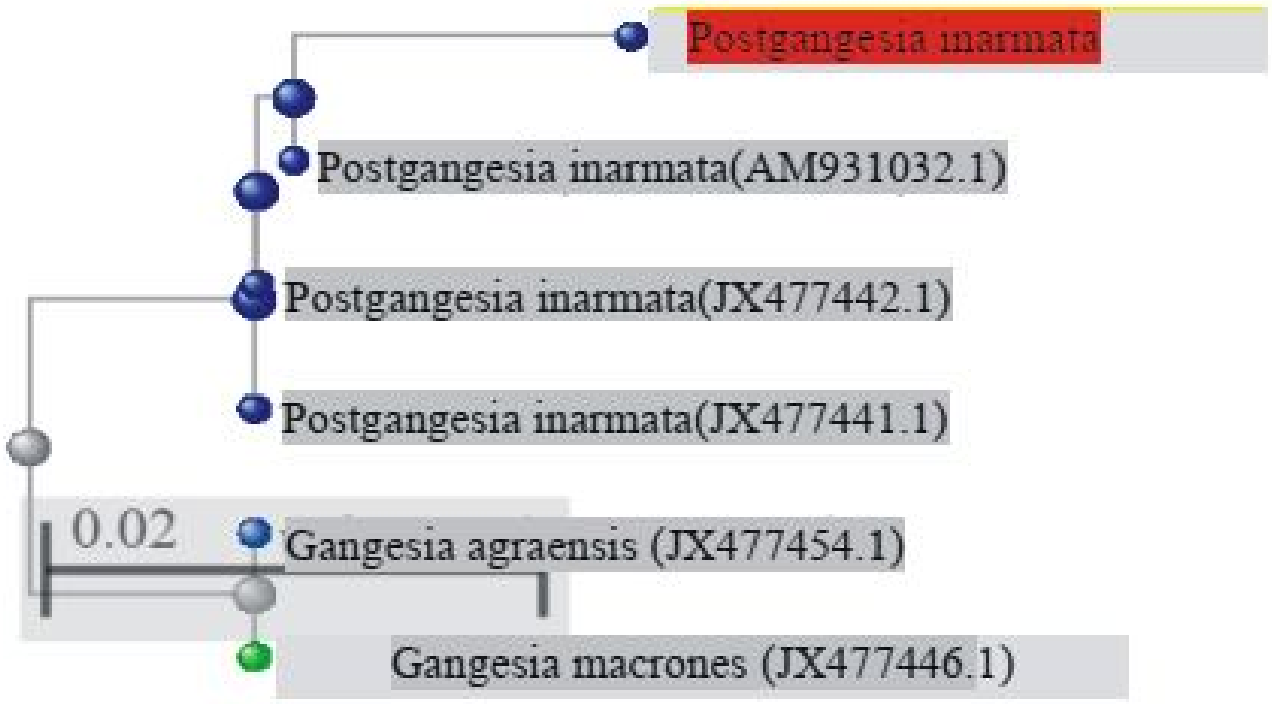

Fig. 3: Phylogenetic tree of 28S of *Postgangesia inarmata* created by MOLE BLAST our sequences with available helminths tree created according to these parameters: A. Tree method: fast minimum evaluation. B. Max Seq. Difference: 0.75. C. Sequence Label: taxonomic name (sequence ID).

Postgangesia inarmata of helminthes parasites that isolated from two different fish species table 1 was described by using polymerase chain reaction (PCR) and DNA sequencing technology for ribosomal 28S rDNA in Misan Province for first time. Genetic analysis was used to confirm the morphological analysis to give the he true diagnosis of worms. The species of worms show high identity with sequences from GenBank.

K. armeniaca 98%, *Schyzocotyle acheilognathi (99%), Postgangesia inarmata (98%)* identity with same species from GenBank. Their accession number showed in table 2. According to Phylogenetic tree created by MOE-BLAST for our specimens *Schyzocotyle acheilognathi* showed in fig. 2 taxonomic name is *Schyzocotyle acheilognathi* not *B. acheilognathi* and This is very clear from the abundant accessions numbers.

Discussion

Schyzocotyle acheilognathi

The newly reported of *Schyzocotyle acheilognathi* from *R. pentamaculata* in the USA (Boonthai *et al.*, 2017), and in Brazil (Souza *et al.*, 2018). In iraq this parasite was reported from different region, the first record of *S. acheilognathi* as *B. acheilognathi* was in

the intestine of *C. carpio* from unspecified fish farms (Khalifa, 1982) and also, *S. acheilognathi* was reported by another synonym, *B. gowkongensis*, from the intestine of *C. luteus* from Basrah fish market (Mhaisen, 1986). We should have noted that this cestode has been described under more than 23 names, the valid name for the species, until very recently, was *Bothriocephalus acheilognathi*, (Kuchta and Scholz, 2007). But present study showed 28S rDNA genes identity with *Schyzocotyle acheilognathi* and this agree with taxonomic action, the valid name of the species based on molecular studied is currently *S. acheilognathi* (Brabec., 2015) (syn. *B. acheilognathi* Yamaguti, 1934). The phylogenetic tree approved that cestoda is high distribution (Scholz *et al.,* 2012*;* yera *et al.,* 2013) and this truth supported by high accession numbers (Fig. 2). Also present study showed the identity with different contraries

Khawia armeniaca

This worm was isolated from intestine of *C. luteus*. In Iraq Rahemo and Mohammad. (2002), recorded this worm from the *C. luteus* as new species *Khawia barbi*. Also Chubb *et al*., 1997, who identified as *K. baltica* from Portugal. So that the Molecular analysis is a good tool to supported morphological study and conformed the classification status of some taxa, especially those described from China and Iraq that were not cleared (Scholz *et al.,* 2011). Present study based on molecular diagnosis conformed the classification status of *Khawia armeniaca* especially that described as *K. barbi* (Rahemo and Mohammad, 2002)and *K. lutei* (Al-Kalak and Rahemo, 2003) its synonyms to *K. armeniaca* according to (Scholz *et al.,* 2011) in Iraq.

Postgangesia inarmata

Postgangesia inarmata was isolated from Asian catfish (jiri) *Silurus triostegus*. This worm was isolated from *S. glanis* from Tigris River in Mosul city by de Chambrier *et al*., (2003), as new species in Iraq contrary. *P. inarmata* in present study by sequencing 28S rDNA identity with the sequences of *P. inarmata* from the genbank under accession number (AM931032.1, 1JX477441.1) from Iraq. This result is closely to study carried by Bilal and Abdullah. (2013), Showed that 99% resemblance with the sequences of *P. inarmata* by sequencing the same gen. we must discussion this cestoda. It may well be conspecific with *Postgangesia inarmata* described from the European catfish (*S. glanis*) in Iraq (Bilal and Abdullah, 2013). But present study depends on phylogenetic tree showed there is no evolutionary relationship. However the adult *P. inarmata* according to phylogenetic tree showed evolutionary relationship with *Gangesia agraensis* and *Gangesia macrones* form the same order. The molecular diagnosis in present study showed closely identity to *P. inarmata* far away from other species in this order (Fig. 3).

Recently we expected that the ribosomal 28S rDNA gene for this primer is good for cestodes identification from different orders and class.

Conclusion

The molecular study is good tool to supported the classification status of some taxa. The molecular investigated indicated the cestoda infected *Carasobarbus luteus* was *Khawia armeniaca* which identity with GeneBank BLAST search from NCBI but not with *K. barbi* or *K. lutei* that considered as synonym *K. armeniaca* according to the present molecular investigated found the cestoda that infected *Carasobarbus luteus* classified as *Schyzocotyle acheilognathi* instead of *Bothriocephalus acheilognathi* which considered as synonym for *B. acheilognathi*. So considered this new name *Schyzocotyle acheilognathi* a valid name in Iraq

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