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Phylogenetic Placement of Egyptian *Taenia saginata* and *Cysticercus bovis*

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Abstract: *Taenia saginata* (*T. saginata*) is a tapeworms that causes taeniasis in humans and cysticercosis in intermediate host animals as cattle. Taeniases remain an important public health concerns in the world. Molecular diagnostic methods using PCR assays have been developed for rapid and accurate detection of human and animal infecting taeniid tapeworms, including the use of sequence-specific DNA primers PCR amplifications using 7 samples (4 of bovine cysticercus and 3 of human adult worm) for HDP2 gene with the molecular size of about 599bp. Sequencing of the fragments was performed and the sequence obtained (Accession number JX265977) was compared with the published sequences for taeniids and the identity of the samples was determined. The objectives of the present study were molecular diagnose and characterization of cysticercus bovis and adult *T. saginata* samples by PCR, sequencing and phylogenetic relationship of Egyptian *T. saginata*.

Key words: Phylogenetic · *Taenia saginata* · *Cysticercus bovis* · Sequencing

INTRODUCTION

Human taeniasis results from intestinal infection with parasitic tapeworms of the genus Taenia. The most common are *Taenia saginata* and *Taenia solium (T. solium)*, but another species, *T. saginata asiatica (T. asiatica)*, has been relatively recently described [1].

T. saginata is a medically and economically important cestode parasite. Infection with the cysticercus larval stage in cattle causes economic loss in the beef meat industry. The life cycle of human tapeworm, *T. saginata* involves humans as definitive host of *T. saginata* and cattle as the IH for larval stage [4].

Until recently genetic variation within the species *T. saginata* was a virtually unknown phenomenon, though there has been increased interest in this area. DNA analysis of *T. saginata* isolates from different areas of the world indicates variation between two broad geographic regions, Asia and Africa/Latin America [4]. More is known about its inter-specific variation, which has been used to differentiate *T. solium* from *T. saginata* and *T. asiatica*. DNA analysis has proven to have more discriminatory power than morphological characteristics [5].

More recently, the value of the different PCR protocols derived from *T. saginata* HDP2 DNA sequence (HDP2- PCR) [6] in the rapid, sensitive and specific identification of *T. saginata* and *T. solium* infection, in patients from both endemic and non-endemic geographical areas, was demonstrated [7].

However, morphology based phylogenetic studies on parasites provided an ‘‘Out of Africa’’ hypothesis in which human-Taenia evolved from carnivore infecting ancestors in Africa and dispersed globally [8-10]. The morphology-based phylogeny of Taenia suggested that *T. saginata* is sister to *T. asiatica* and that *T. solium* is distantly related to them [8]. The close genetic relatedness between *T. saginata* and *T. asiatica* and the distinctiveness of *T. solium*, were supported by molecular phylogenies based on mitochondrial DNA (mtDNA) and

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nuclear DNA (nDNA) [11]. Positions of these human parasites in a morphology based phylogeny suggested that host switching from carnivores to humans has occurred at least twice [10].

The present endeavor points to the usefulness of molecular biology tools for diagnosis of bovine cysticercosis and adult *T. saginata*. This study was undertaken to partial sequence of ribosomal DNA non transcribed repeats (NTR) region and its applicability to diagnosis of *T. saginata.* and *C. bovis*

MATERIALS AND METHODS

Parasite and DNA Extraction: *Cysticercus bovis* were dissected from naturally infected cattle, washed with 0.01 M Tris-HCl (pH 8.0) and then cut to drain out the cyst fluid. The cyst tissue was again washed and stored at 0°C until needed. Tapeworm, collected from patients treated at Assuit educational hospital. Three samples were identified by morphology. Genomic DNA was isolated from adult tapeworms by homogenization and treated by QIAamp Kits (Qiagen) according to the manufacturer's recommendations.

DNA Extraction and PCR Amplification: Primer designation based on the sequence of HDP2 gene as in Table (1) according to Gonza´lez *et al.,* Gonza´lez *et al*. and Harrison *et al.,* [6,7,12].

DNA amplification was carried out in a Biorad Mini Thermocycler in a final volume of 25µl as recorded by Gonza´lez *et al.* [6] as follows: cycling conditions were: 1 cycle of initial denaturing of 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 1min, annealing at 56°C for 1 min, extension at 74°C for 1 min and final extension at 74°C for 7 min. Electrophoresis of the amplicon in agarose gel stained with ethidium bromide and illumination under short-wave length ultraviolet radiation [13] against100 bp Plus Ladder (Fermintas).

Sequencing of *Taenia saginata*, IGS, Isolate Assuit, Egypt: The amplicons were extracted from mature segment of *T. saginata* and purified from low melting agarose gel by QIAquick Gel Extraction Kits (Qiagen). The same primers with the same PCR protocol were used in sequencing of PCR amplicon by automatically analyzed using fluorescence-base label sequencing (BigDyes chemistry, Applied Biosystems).The sequencing of the fragment was performed in one direction, with the specific primers [7].

Phylogentic Analysis: The sequences were analyzed and compared with previous described related genomic sequences in GenBank and were carried out with Megalign software packages (DNASTAR), FASTA and BLAST programs.

RESULTS

The sizes of PCR fragments of HDP2 gene of Egyptian *T. saginata* are presented in Fig. (1) with the Molecular expected size of about 599bp. In the present study, a band specific to *T. saginata* was obtained in all PCR reactions using *T. saginata*-HDP2 specific primers.

Sequencing and Phylogenetic Analyses: The specificity of PCR products was confirmed by sequencing of DNA amplicon.

Amplification of this segment with primer produced a fragment of about 599 bp, against all sequences in the GenBank database revealed the possibility of amplification. Comparison of the alignment of the local Egyptian *T. saginata* sequence fragment with other Taeniid worm showed a single or double mismatches or substitution revealed that at position 282, there is a letter G instead of A, at position 320 and 321 there are a substitutions of letters GG instead of CA Fig. (2).

Phylogenetic tree analysis of aligned HDP2 sequences of Egyptian *T. saginata* with the GenBank Accession number JX265977.The GenBank revealed close ancestral genetic relation among these Taenidea. The sequence identity was recorded with the reference *T. saginata* intergenic spacer, IGS Fig. (2). *T. saginata* was identified based on the similarity of nucleotide sequences and phylogenetic relationships with those of *T. asiatica* IGS, isolate 1 (GenBank No: FM212953.1) to IGS,isolate19 (GenBank No: FM212964.1).

Table 1: Oligonucleotide primers of HDP2for *Taenia Saginata*

Primer design	Oligonucleotide sequence	gene	Size of amplicons
Pts7S35 (F)	5´-CAGTGGCATAGCAGAGGA-GGAA- 3´		
PTs7S35(R)	5´- GGACGAAGAATGGAGTTGAAGGT -3´	HDP2	599bp

(F): Forward. (R): Reverse bp: base pair.

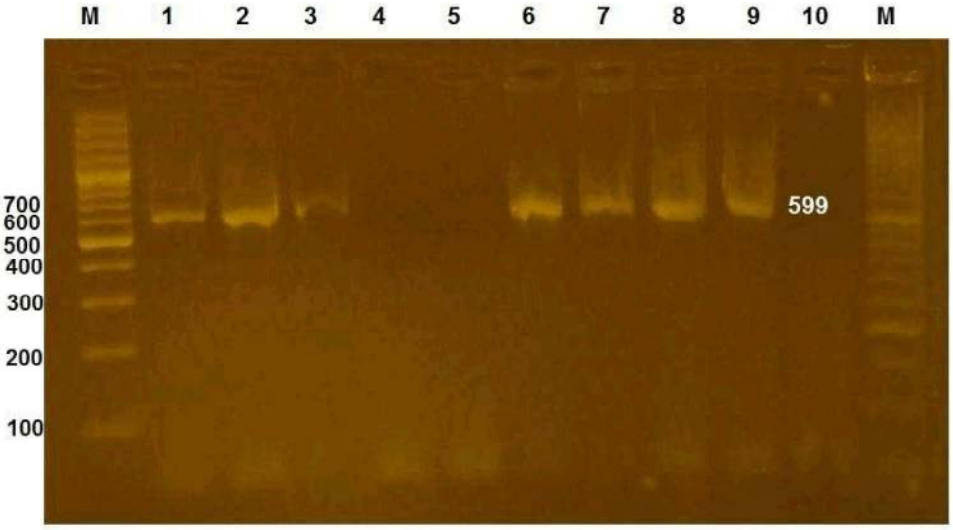

Fig. 1: Amplification of HDP2 genesegment of *T. saginata* by 1.5% agarose gel electrophoresis and stained with ethidium bromide against Lane: (M)100 bp DNA ladder consists of repeats of 100 bp fragment size, (Fermintas), (Lanes 1-3) PCR amplicons of local Egyptian *T. saginata* adult worms, (Lanes 4-5): Negative control, (Lanes 6-9):Amplicons from Egyptian isolates of *T. saginata* From bovine origin cysticercus, are at the size of approximately 599 bp.

Fig. 2: Continued

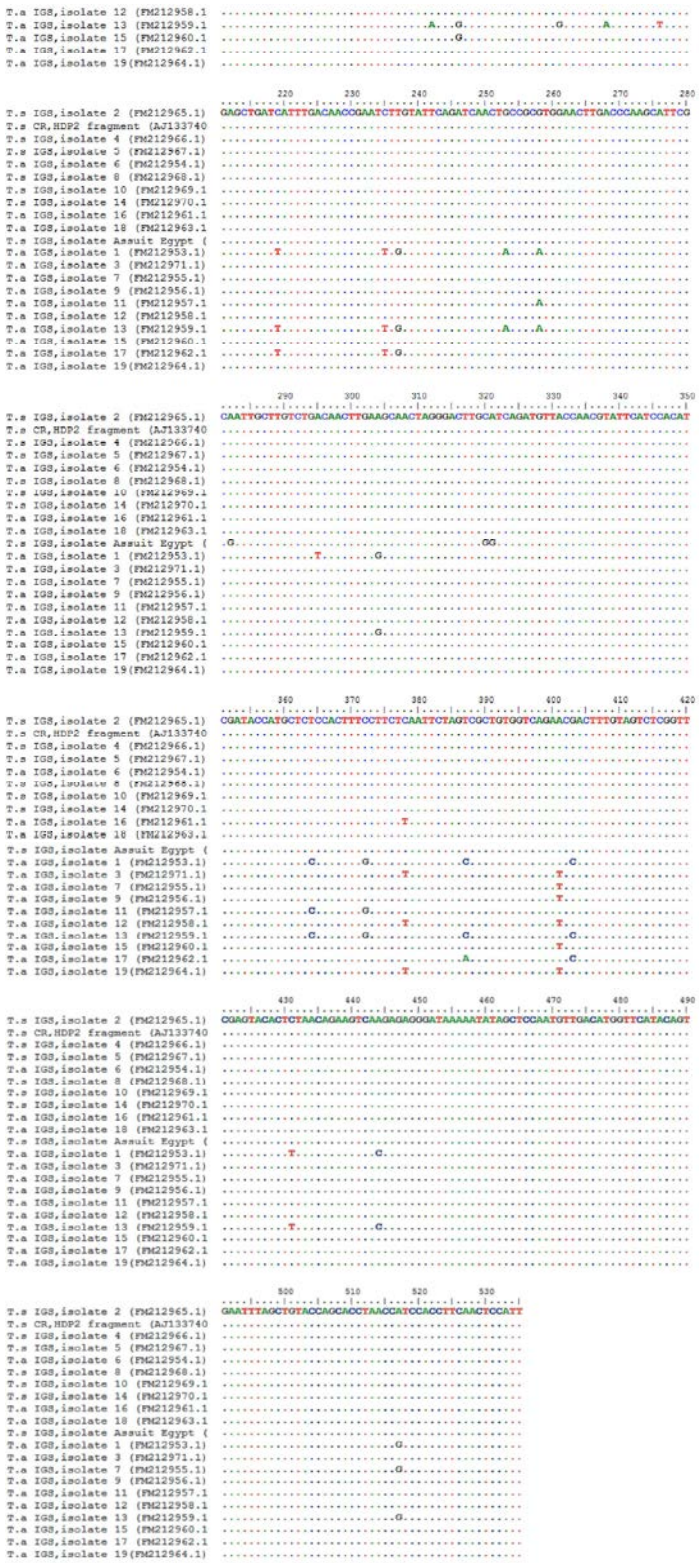

Fig. 2: Nucleotide sequences alignment of related Taeniid family genomes: local Egyptian *T. saginata* and other isolates. Numbers of the sequence indicate nucleotide positions in the HDP2 gene relative to the GenBank data for each isolate.

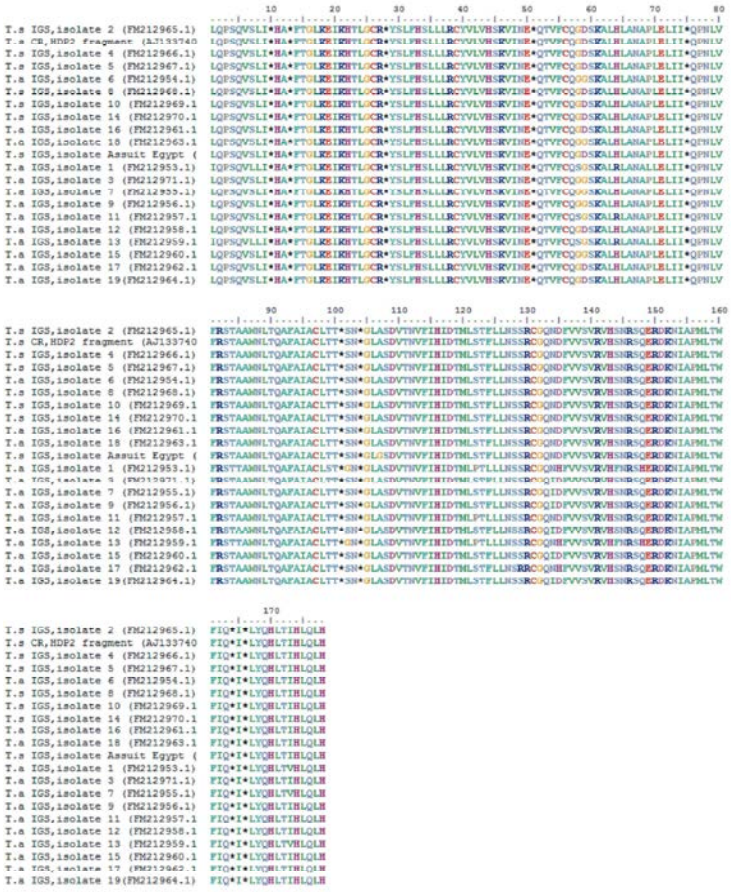

Fig. 3: Protein translation of multiple alignments of the deduced amino acid sequences of the local Egyptian *T. saginata*.

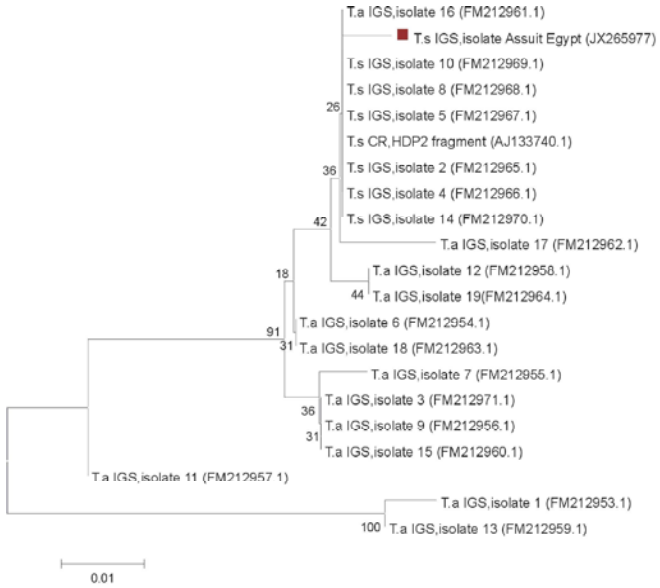

Fig. 4: Phylogenetic tree of the Egyptian *T. saginata* isolate with GenBank accession number JX265977 and other related Taeniid family with their accession No. generated from nucleotide sequences encoding for HDP2 gene of the analyzed Taeniid genomes. The sequences were first aligned using Clustal W (1.82) program and the phylogenetic analyses were performed using MEGA5.

Translation of the sequence to protein Fig. (3) includes a comparison of the amino acid sequence deduced for the HDP2 gene of the taeniid isolates studied here with the homologues described for *T. saginata* at GenBank, revealed that at position 107 amino acid G instead of A. The neighbor-joining tree was constructed under the parameter model by MEGA program version 5.2 to figure phylogenetic relationships Fig. (4).

DISCUSSION

Taenia solium, *T. saginata* and *T. asiatica* are taeniid tapeworms that cause taeniasis in humans and cysticercosis in intermediate host animals. Taeniases remain an important public health concerns in the world [14,15].

The PCR-based methodology was performed in this study in order to diagnosis *T. saginata* through amplification of host DNA fragments of about 599 bp Fig. (1).

Molecular diagnostic methods as PCR have been developed for the rapid and accurate detection of species and differential diagnosis of *T. saginata* and *T. asiatica* have been developed by Jeon *et al.* [16]. The application of the multiplex PCR would be useful not only for surveillance of taeniasis and cysticercosis control but also for the molecular epidemiological survey of these cestode infections [17]. The same result of HDP2-PCR was performed with oligonucleotide primers resulting in amplification of 600 bp DNA fragment specific for *T. saginata* by Nunes *et al.* [18]. Also [19] described *T. saginata* HDP2 DNA sequence, a 4-kb polymorphic fragment, was previously used as the basis for developing PCR diagnostic protocols for the species-specific discrimination of *T. saginata* from *T. solium* and *T. asiatica*.

The advent of the PCR has provided a highly sensitive approach that is now widely used to target mtDNA sequences for Echinococcus and *Taenia* identification purposes, including discrimination of eggs [20]. Performed PCR-RFLP and gene sequencing of the mit. *cox1* to identify the species of *T. saginata*. Application of molecular approaches and phylogenetic relationships are improved to be methods for diagnosis of taeniid [21].

In this result diagnosis of *T. saginata* is based on DNA sequence information and in the definition of evolutionary and phylogenetic relationships (Figure, 2 and 4).

The sequences were assembled and aligned by using Clustal W and BioEdit (BIOSOFT CO.) programs. There are substitutions of letters GG instead of CA Fig. (2).

The sequenced regions were identified by comparing those using BLAST searches with those of Platy helminthes that had been deposited in the GenBank database.

While there is significant DNA sequence information for taeniids of socioeconomic importance, there are limited data for the lesser-known species, particularly those from Africa (reviewed in [22]).

DNA approaches are now being used routinely for accurate identification of Echinococcus and *Taenia species*, subspecies and strains and in molecular epidemiological surveys of echinococcosis/taeniasis in different geographical settings and host assemblages [20].

Genome sequencing, based on the use of the polymerase chain reaction (PCR) have found broader applicability, mainly because their sensitivity permits the analysis of particular genes from tiny amounts of DNA from fresh, frozen or even ethanol fixed parasite material [20,23].

DNA sequencing revealed that the two nucleotides at positions 320 and 321 was guanine Fig. (2). The nucleotide sequence data reported in this paper are available in the EMBL/GenBank databases under the following Accession No. JX265977. Multiple alignments of the deduced amino acid sequences of the *T. saginata* were obtained in Fig. (3). In current study, for phylogentic relationship contrast with cladistic analyses which indicated that local Egyptian *T. saginata* appeared in a subclade separate from both *T. asiatica* IGS isolate 16 (Gen Bank no:FM212961.1) and *T. saginata* isolate 10 (Gen Bank no: FM212969.1) Fig. (4).

One of the molecular tools often used for phylogenetic studies is sequence fragment analysis based on PCR synthesis of the DNA of these Taeniid [24]. The corresponding sequences from all taeniid isolates were PCR-amplified with specific primers and then sequenced by Gonzalez *et al.* [25].

Phylogenetic studies involving morphological and molecular analyses, which have indicated that *T. asiatica* is indeed a distinct species, but one that is closely related to *T. saginata* [26]. The distance data of each aligned sequence were tested with a non-approximating decomposition method considering the significance of grouping according to a distinct branching order using the splits program. With the development of molecular technologies, PCR-based techniques have been developed for the identification and differentiation of *Taenia species* including *T. solium* or *T. asiatica* [27].

The conclusion was that Molecular genotyping reflects phenotypic differences through variation in sequence that may have important consequences in epidemiology and control of taeniasis.

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