SHORT COMMUNICATION

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First molecular report of *Hydatigera krepkogorski* (Schulz and Landa, 1934) in abdominal cavity of gerbil (*Rhombomys opimus*) in Iran

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Abstract

A polycephalic larva of Taeniidae family isolated from abdominal cavity of a great gerbil (*Rhombomys opimus*) from Golestan province, northern Iran, was subjected to molecular analysis. Genomic DNA from the larva was obtained using a DNA extraction tissue kit. Polymerase chain reaction was performed for amplification of the partial 12S rRNA, cytochrome *c* oxidase subunit 1 (cox1) and NADH dehydrogenase 1 (nad1) mitochondrial genes. BLAST analysis of DNA sequencing indicated 99.00% homology in 12S rRNA and cox1 genes and 98.00% homology in nad1 gene with *Hydatigera krepkogorski* (accession No. AB731762). The sequences of current isolate were deposited in GenBank by accession Nos. MF281971, MF281972 and MF281973 for 12 SrRNA, cox1 and nad1 genes, respectively. This study was the first report of molecular characterization of *H. krepkogorski* from Iran. Isolation and characterization of the adult stage from definitive host will help to better clarify incomplete life cycle and morphology data of this species in the world.

نخستین گزارش مولکولی *هیداتی‌ژرا کرپکوگوروسکی* (Schulz and Landa, 1934) در حفره شکمی موش بیابانی (*رومبومیس اوپیموس*) در ایران

چکیده

لاروی پلی‌سفالیک از خانواده تنیده جدا شده از حفره شکمی موش بیابانی (*Rhombomys opimus*) بزرگ از استان گلستان در شمال ایران تحت بررسی مولکولی قرار گرفت. DNA ژنومی لارو با استفاده از یک کیت بافتی استخراج DNA به دست آمد. واکنش زنجیره ای پلیمراز جهت فزون‌سازی ژن های میتوکندریایی S rRNA۱۲ ، سیتوکروم c اکسیداز زیر واحد ۱ (cox1) و ۱ دهیدروژناز(nad1) NADH انجام پذیرفت. ارزیابی بلاست توالی DNA میزان ۹۹/۰۰ درصد تجانس در ژن‌های 12S rRNA و cox1 و ۹۸/۰۰ درصد تجانس در ژنnad1 با *هیداتی‌ژرا کرپکوگوروسکی* (شماره دسترسی: AB731762) را نشان داد. توالی های مورد جدا شده مطالعه حاضر با شماره های دسترسیMF281971، MF281972 و MF281973 به ترتیب برای ژن‌هایrRNA12S، cox1 و nad1 در ژن‌بانک ثبت گردید. این مطالعه اولین گزارش شناسایی مولکولی *هیداتی‌ژرا کرپکوگوروسکی* از ایران بود. جداسازی و شناسایی مرحله بالغ از میزبان نهایی به روشن‌شدن بهتر چرخه زندگی ناکامل و داده های ریخت شناسی این گونه در جهان کمک خواهد نمود.

واژه های کلیدی: ایران، *رومبومیس اوپیموس*، *هیداتی‌ژرا کرپکوگوروسکی*

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Introduction

Hydatigera krepkogorski (syn. *Taenia krepkogorski*) is a platy helminth from Taeniidae family that its larvae occur in some species of rodents such as *Rhombomys opimus* and the adult worm is a parasite of the *Felis* spp. or red fox (*Vulpes vulpes*).[1] The metacestode is polycephalic and also strobilocrcus type with a prominent segmented strobila. Large rostellar hooksare is another character of *H. krepkogorski*.[2]

During the past decades, molecular methods have been applied as a useful method for precise identification of parasites originating from humans or animals. Mitochondrial markers have also been served as proper tools for genetic classification of Taeniidae.[3] To date, many studies have been performed on rodents parasites in Iran based on morphological characters.[4-6] Of these, only a few cases have been characterized using molecular analysis.[7] In the present study, a polycephalic larva of a cestode already obtained from great gerbil (*Rhombomys opimus*) and deposited in the Helminthology Laboratory of School of Public Health, Tehran University of Medical Sciences was subjected to molecular analysis for precise characterization of the parasite infecting rodents in Iran.

Materials and Methods

Collection of isolates. A polycephalic larva of a *Taenia* sp. available in the archive of Helminthological Laboratory of the School of Public Health, Tehran University of Medical Sciences was subjected to molecular analysis. The larva was found from abdominal cavity of a gerbil (*R. opimus*) during a study on helminth parasites of this particular rodent in a temperate rural area of Golestan province, northern Iran, bordering southeastern Caspian Sea in 2011.[4] The study was approved by Ethics Committee of the Tehran University of Medical Sciences with No. IR.TUMS.REC.1394.879.

Molecular analysis. Total genomic DNA was extracted from 20 mg of the strobilocercus, kept in 70.00% ethanol, using a DNeasy blood and tissue kit (Qiagen, Düsseldorf, Germany) according to the manufacturer's instructions for animal tissue. For better DNA extraction, the subject was squeezed completely in lysis buffer employing a micro pestle and incubated with proteinase K solution at 56.00 ˚C overnight.

Partial fragments of the cytochrome c oxidase subunit 1 (cox1), 12S rRNA and NADH dehydrogenase 1 (nad1) mitochondrial DNA genes were amplified by polymerase chain reaction (PCR) reactions. The forward 12SRF (5'-AGGGGATAGGACACAGTGCCAGC-3') and reverse 12SRR (5'-CGGTGTGTACATGAGCTAAAC-3') primers were used to amplify a fragment of 12SrRNA,[8] the forward JB3 (5'-TTTT TTGGGCATCCTGAGGTTTAT-3') and reverse JB4.5 (5'-TAAAGAAAGAACATAATGAAAATG-3') primers were used to amplify a fragment of cox1 and forward MS1 (5'-CGTAG GTATGTTGGTTTGTTTGGT-3') and reverse MS2 (5'-CCAT AATCAAATGGCGTACGAT-3') primers were employed for amplification of a fragment of nad1.[9]

All PCR reactions were performed in a 25.00 µL reaction mixture, containing 1.00 µL of template DNA, 25.00 pmol of each primer and 12.50 µL of PCR premix (2× Master Mix RED; Ampliqon, Odense, Denmark) which included 0.20 units per µL of Taq DNA polymerase, 0.40 mM of each deoxy nucleotide triphosphate and 2.00 mM $MgCl_2$.

The temperature condition of PCR was as follows: one cycle of 94.00 ˚C for 5 min (primary denaturation) followed by 35 cycles of 94.00 ˚C for 30 sec (denaturation), 60.00 ˚C (12SrRNA and nad1) and 55.00 ˚C (cox1) for 45 sec (annealing) and 72.00 ˚C for 30 sec (extension) and a final extension of 72.00 ˚C for 5 min. For each set of PCRs, a sample containing distilled water instead of DNA template was included as a negative control. No amplification was observed in the negative control reactions during the study.

The aliquots of PCR products (7.00 µL) were subjected to electrophoresis on a 1.50% agarose gel in TBE (Tris, Boric acid, EDTA) buffer at 80 V for 1 hr. Gels were stained with DNA safe stain (Sinaclon, Tehran, Iran) and the bands were visualized using a transilluminator. A 100 bp ladder (Fermentas, Vilnius, Lithuania) was employed as a DNA size marker.

Sequencing and phylogenetic analysis. The PCR products were purified and sequenced in both directions using the same primers as used in the PCRs. Sequence results were edited and analyzed by the BioEdit software (version 7.0.5; Ibis Therapeutics, Carlsbad, USA)[10] and the consensus sequences were compared with BLAST (Basic Local Alignment Search Tool) programs and databases.

For better understanding of relationship among current isolate and other *Taenia* spp., a phylogenetic analysis of concatenated 12S rDNA+ cox1 + nad1 was performed employing Bayesian Inference method (BI). The BI was conducted using MrBayes software (version 3.1.2; Florida State University, Tallahassee, USA). Posterior probabilities (pp) were obtained for 2000000 generations (ngen = 2000000; burnin = 20000) using the Monte Carlo Markov Chain method and four simultaneous tree-building chains (nchains = 4) with each 100th tree saved (samplefreq = 100). *Diplydium caninum* (accession No. AB732959) was employed as out-group.

Results

The PCR reactions using specific primers demonstrated bands about 500 bp for 12S rRNA and 400 bp for both cox1 and nad1 genes on agarose gel (Fig. 1). The assembling sequences of both forward and reverse directions revealed a consensus sequences of 468

nucleotides for 12S rRNA and 420 nucleotides for both cox1 and nad1 genes.

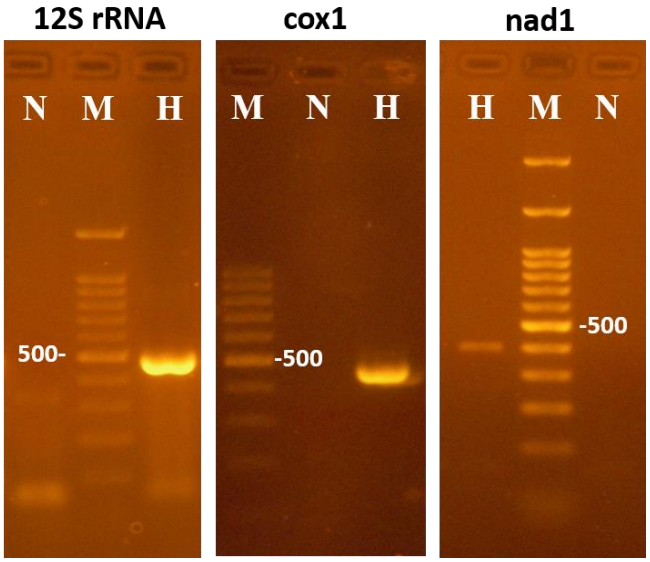

Fig. 1. Electrophoresis of PCR products of three mitochondrial genes including 12S rRNA, cox1 and nad1 relevant to larval stage of *Hydatigera krepkogoroski* from *Rhombomys opimus* in Golestan province, northern Iran. Lane M: 100 bp ladder DNA size marker; Lane H: *H. krepkogoroski*; Lane N: negative control.

The BLAST analysis of the isolate indicated 99.00% homology in 12S rRNA and cox1 genes and 98.00% homology in nad1 genes with *H. krepkogorski*. Thus, the polycephalic larva of current study was identified as *H. krepkogorski* and relevant nucleotide sequences were deposited in the GenBank/EMBL/DDBJ under accession numbers of MF281971, MF281972 and MF281973 for 12S rRNA, cox1 and nad1 genes, respectively.

A concatenated analysis of 468, 420 and 378 nucleotides of 12S rDNA + cox1 + nad1 genes, respectively revealed a consensus tree with two distinct clades having strong statistical supports (pp = 0.97 and 1): a clade (pp = 0.97) containing the isolate of the present study within a subgroup with *H. krepkogorski* from china (accession No. AB731762), (pp = 1.00) inside a group with *Taenia taeniaeformis* (pp = 1.00) and within a cluster with *H. parva* (pp = 0.97) and another clade (pp = 1.00) involving other *Taenia* spp. having two distinct cluster; a big cluster including *Taenia solium*, *Taenia ovis*, *Taenia saginata*, *Taenia asiatica* and *Taenia multiceps* placed in a distinct position sister to the other cluster containing only *Taenia hydatigena* (Fig. 2).

Discussion

There are several studies on helminth parasites of rodents from different regions in Iran,[4-6] mostly reporting prevalence of different species of parasites and one has identified species using molecular characterization.[7] In the present study, a polycephalic larval isolate of Teaniidae family from *R. opimus* in Iran was characterized as *H. krepkogorski*.

The taxonomy of *H. krepkogorski/ T. krepkogorski* has been on controversy along with some other *Taenia* species. Firstly, Schulz and Landa in 1934 described a strobilocercus larval stage of this worm in mesenteries of abdominal cavity of *R. opimus* from Kazakhstan and in *Meriones meridianous* from the northern Caucasus.[11] Later, the larval stage was found in *R. opimus* from Uzbekistan and Tajikestan and in *Meriones erythrourus* from Uzbekistan. The adult worm has been found in different felines.[12] Petrov and Potekhina reported the

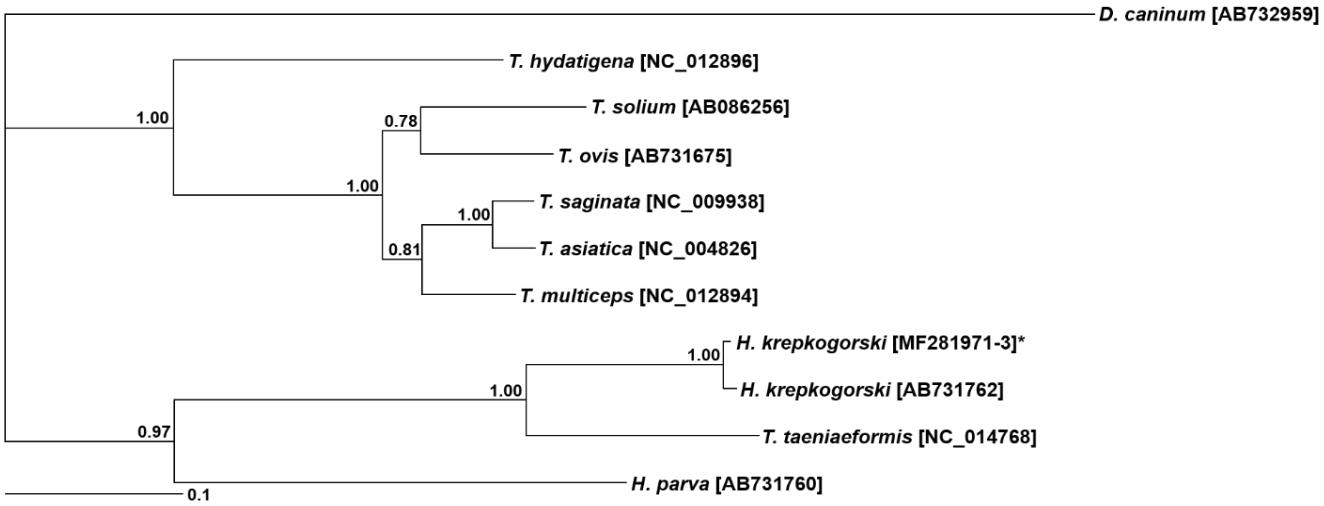

Fig. 2. Genetic relationships of *Hydatigera krepkogoroski* isolate from Iran (indicated with asterisk) with other Taeniidae family deposited in the GenBank. *Dipylidiu mcaninum* was used as out-group. The relationships were inferred by phylogenetic analysis of concatenated partial 12S rRNA + cox1 + nad1 sequence data using Bayesian Inference. The accession numbers of sequences are given in the square parentheses. The scale bar indicates distance. Nodal support is given as a *p* value.

adult stage of this worm from *Felis libyca caudate* in Tajikistan.[13] Sadikhov reported *T. Krepkogorski* from *V. vulpes* in 1954 and *Felis chaus*, *Felis silvestris.* and *V. vulpes* in 1962 from Azerbaijan.[14-16] Agapova and Sapozhenkov have isolated this parasite in *Felis libyca ocreata* and *Felis margarita* in Turkmenistan.[17]

Verster performed a comprehensive study on 70 *Taenia* species and presented a taxonomic revision of the genus *Taenia* in 1969. She reported 32 valid species and three subspecies of *Taenia* and inserted *T. krepkogorski* and some other *Taenia* spp. in the list of inquiry species. She supposed the possible equality with *T. taenieformis, T. macrocystis* or *T. endothoracicus*.[1] Verster believed that description of adult worm of *T. krepkogorski* by Petrov and Potekhina may be equal to *T. macrocystis* and cestodes isolated from *V. vulpes* by Pova and Sapozenkovas signing as *T. krepkogorski* possibly were *T. endothoracicus*.[1] Bray reported the adult worm of *T. krepkogorski* in Arabian sand cat (*Felis margarita*) in Bahrain. He believed that *T. krepkogorski* has a preference for felines as the definitive host and its strobilocercus stage occurs solely in gerbils and its distribution is limited to desert regions of southwestern central Asia.[12]

Loos-Frank wrote an up-date to Verster's after 31 years and described 44 valid species including *T. krepkogorski*.[18]

Recently, Nakao and colleagues performed a comprehensive molecular phylogeny of several isolates of Taeniidae family using phylogenetic analysis of mitochondrial and nuclear genes and suggested the resurrection of *Hydatigera* Lamarck, 1816 for *T. krepkogorski, T. parva*, and *T. taeniaeformis*. They assigned *Hydatigera* as a separate but close genus to *Taenia* in the Taeniidae family.[2] The genus *Hydatigera* is characterized by the strobilocercus type metacestode in rodents with large rostellar hooks.[2,19] Nakao and colleagues stated genus *Hydatigera* consists of three valid species namely *H. krepkogorski, H. parva*, and *H. taeniaeformis* and considering the strobilo cercus larva, they are in common with some *Taenia* species including *T. endothoracicus, T. macrocystis, T. rileyi, T. recracta* and *T. selousi*. Also, *T. endothoracicus, T. rileyi* and *T. selousi* resemble to *Hydatigera* spp. in terms of the intermediate host. Furthermore, there is a significant overlap in the length of rostellar hooks with *T. endothoracicus, T. macrocystis, T. laticollis,* and *T. pseudolaticollis*.[2] These authors have documented some mitochondrial and nuclear sequences of *T. krepkogorski* in GenBank for the first time. Unfortunately, there is not any sequence data of *T. endothoracicus, T. macrocystis, T. rileyi, T. recracta* and *T. selousi* in GenBank for better comparison with *Hydatigera* species.[2]

The present study is the first molecular identification of *H. krepkogorski* from Iran. Phylogenetic analysis of partial mitochondrial genes in the present study is in concordance with previous studies suggesting *Hydatigera* as a distinct genus having three species since *H. krepkogoroski*, *T. taeniaeformis* and *H. parva* placed in a distinct clade with a strong statistical support (pp = 0.97) compare to other *Taenia* spp. (Fig. 2).

Further molecular characterization of Taeniidae adult or larval isolates from felines and rodents, especially the species without any former accession numbers in the GenBank from different parts of the world will be helpful for better understanding of phylogenetic placements of *Hydatigera* and also clarification of incomplete life cycle and morphology data of this species.

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Conflict of interest

The authors declare no conflicts of interest.

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