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Short Communication

Molecular and Morphological Data Confirmed First Record of *Abbreviata kazakhstanica* Markov and Paraskiv, 1956 (Spirurida: Physalopteridea) in Iran

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Abstract

Background: The genus *Abbreviata* (Spirurida: Physalopteridea) currently contains 47 species. Physalopteridae nematodes infect a large number of vertebrates, including mammals, birds, reptiles and amphibians. The current study is a report of the first morphological and molecular identification of *A. kazakhstanica* (Spirurida: Physalopteridea) in *Pseudopus apodus* in Iran.

Methods: Eleven road-killed *P. apodus*, were collected from, Iran during 2016-2018. The nematodes were isolated from stomach. After morphological study, the genomic DNA of the parasites was extracted using CTAB method. The DNA was used for PCR amplification of cytochrome c oxidase subunit I (cox1). The PCR products were sequenced, the sequence data were analyzed and multiple alignments were conducted using the Clustal Omega.

Results: After detailed microscopic examination, the *A. kazakhstanica* was identified. The cox1 sequences confirmed the species of helminth. The new sequences of *A. kazakhstanica* were submitted to GenBank under the accession number MK578751-2.

Conclusion: Regarding the limited data on parasitological status of Iranian reptiles, more specific and comprehensive investigations are needed to identify the parasitic fauna.



Introduction

A broad range of pathogens especially those with public health concerns are associated with reptiles (1). Until now, 125 species of lizards with 36 genera from 8 families have been reported in Iran that *Pseudopus apodus* (Anguillidae) (Pallas, 1775) is one of them (2). This lizard species is distributed from southern Europe to Central Asia. They prey on rodents, lizards, and eggs and fledglings of ground-nesting birds (3). Despite that lizards are well-studied group in Iran (4), the data on their parasitic infections is limited. The reported parasites from lizard in Iran are 14 species as follows: 12 species of nematodes, *Abbreviata baltazardi* in *Phrynocephalus heliosopus* (Agamidae), and *Skrjabinodon pigmentatus*, *Spauligodon lacertae*, *Thelandros baylisi*, and *Oswaldo filaria chlamydorauri* in *Laudakia caucasia* (Agamidae), *Parapharyngodon* sp., *Thelandros* spp., and *Thubunea mobedii* from Large-scaled (Rock) Agama, *Laudakia nupta* (Agamidae), and *Spauligodon persiensis* from Rough bent-toed gecko, *Cyrtopodion scabrum* (Gekkonidae), and two species of cestode, *Nematotaenia dispar* from the desert monitor, *Varanus griseus* (Varanidae), and *Oochoristica tuberculata* from the snake-eyed lizard, *Ophisops elegans* (Lacertidae) (5-12). There is just one report of parasitic infection in European legless lizard, *P. apodus* in Iran that was the nematode *E. entomelas* (8).

The nematodes of Physalopteridae family can infect mammals, birds, reptiles and amphibians (13). As well, there are several reports of infection in humans in different regions of the world highlighting the zoonotic importance of these parasites (14). The genus *Abbreviata*, bisected from the genus *Physaloptera* by Travassos, 1920, contains 47 species (15). They are characterized through one external tooth, one internal tooth and two double submedian teeth on each pseudolabium. The number of uterus branches is the main factor for subgeneric classification (16).

Life cycles of *Abbreviata* species are poorly known and invertebrates as Coleopterans, Mantopterans, and Othopterans are probably suitable intermediate hosts (17), although the larval form has been observed in mammals, reptiles, and amphibians and it is difficult to determine whether they are definitive, intermediate, or paratenic hosts. Sometimes pseudoparasite may be found in an unusual host, which occurs when predator swallows definitive host as the prey with the helminthes inside its body (15). Nematodes in the genus *Abbreviata* spp. have a worldwide distribution and more than one nematode species may occur in more than one host species. Lizards are the most important hosts and among parasitic species for reptile, there are host-specific and euryxenous species (18).

Considering that no study in this regard has been done in Iran so far, this study was performed with the aim of molecular study on *A. kazakhstanica* isolated from *Pseudopus apodus*.

Materials and Methods

Sampling

Eleven road-killed *P. apodus*, were collected from Guilan Province in Iran during 2016-2018. At necropsy, the digestive tract, lungs, and body cavity of each specimen was examined for helminths using a dissecting microscope. All of the nematodes were isolated from stomach.

Parasitology test

Collected helminths were relaxed in water and preserved in 70% ethanol. Nematodes were placed on a glass slide in a drop of glycerol, a coverslip was added, and identification was made from these temporary wet mounts.

Molecular study

Genomic DNA was extracted using cetyltrimethylammonium bromide (CTAB) meth-

od (19). One ml of lysis buffer (8% Triton X-100, 0.25 M sucrose, 50mM Tris-HCl, 50mM EDTA, pH 7.5) and 50 µl of proteinase K (20 mg/ml) were added to 50 mg of homogenized tissue of worm. Then, it incubated at 65 °C for 1-2 h (until it becomes clear). DNA was precipitated by adding 1 ml of sterile 2% CTAB solution to the cleared lysate and centrifugation 1500×g for 5 min. Then, 0.5 ml of dissolving buffer 2.5 M NaCl, 10 mM EDTA, pH 7.7 and dilute with 1 ml of 40 mM Tris-HCl, 2 mM EDTA, pH: 7.7) added to pellet and then 1 ml of diluting buffer (40 mM Tris-HCl, 2 mM EDTA, pH: 7.7) was added to this solvent. Two volume of chloroform were add, mix gently and centrifuged at 12000 ×g for 5 min. The aqueous phase was transferred to a fresh tube, add 2 volume of ethanol (room temperature) and incubate the tube at -20 °C for overnight. Finally, pure DNA were precipitated by centrifugation at 12000 ×g for 10 min at room temperature.

The DNA was used for PCR amplification of cytochrome *c* oxidase subunit I (COX1), as is published (20). For PCR reaction mixtures were used Taq Polymerase master mix was used (AMPLIQON). The volumes of DNA and primer were set up based on concentration (50 ng DNA, 10 pM for each primers).

The PCR reaction was initiated by denaturation step at 94 °C for 5 min; and then the mixtures were subjected to 35 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec, and extension at 72 °C for 30 sec, with a final extension at 72 °C for 5 minutes. The PCR product was analyzed by electrophoresis

in a 1.5% agarose gel and was visualized after DNA safe staining.

Nucleotide sequence analysis

The PCR products were sequenced in Macrogen Inc., South Korea. Sequence data were analyzed and multiple alignment were conducted using the Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). Phylogenetic tree was created by MEGA7 software. The phylogenetic tree was drawn using sequences obtained in this study as well as published sequences available for *Spirurida* from GenBank. *Thelazia callipaeda* (AF182301) was applied as outgroup.

Results

Eleven adult *P. apodus* (8 male and 3 female) were collected during 2016-2018 in Guilan Province, Iran. After necropsy, 8 of 11 (72.7%) were infected by *Abbreviata* genus. Each of the eight hosts had a very severe infection (>10 worms in stomach). After detailed examination, we found genus *Abbreviata* and species *A. kazakhstanica*. In *Abbreviata*, oral opening surrounded by 2 large single-lobed lateral pseudolabia. Usually entire margin of pseudolabia dentate.

In males, tail with broad, lateral alae, which connect across the ventral surface in front of the cloac. Usually 4 pairs of pedunculated or stalked papillae supporting lateral alae, typically 2 pairs pre-cloacal and 2 pairs post cloacal. Ventral surface of tail also covered with longitudinal or broken rows of tubercle. Spicules equal, subequal, but generally unequal (Fig. 1 & Table 1).

Table 1: Body measurements of adult *Abbreviata kazakhstanica* isolated from *Pseudopus apodus* in Iran.

Male	Range	Mean ± SE
Length (mm)	15-17	16 ± 0.2
Width (µm)	386-403	394.5 ± 8.4
Width (µm)	386-403	394.5 ± 8.4
Eggs (µm)	50.1-51.4 x 30.2-32.0	50.3 x 31.2
Female		
Length (mm)	10-12	10.5 ± 0.4
Width (µm)	403-691	494.5 ± 65.4
Spicule Length (µm)	1700-1900	1840 ± 95

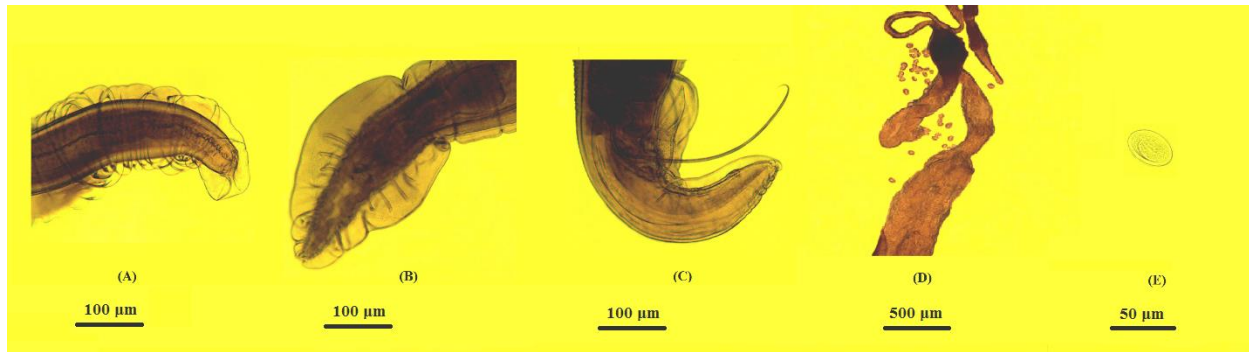


Fig. 1: *Abbreviata kazakhstanica* (A) Anterior part with pseudolabia dentat, (B) lateral alae in male with 4 pairs of pedunculated papillae, (C) unequal spicules, (D) uterus with 2 branches (didelphys), (E) embryonated egg

In Females vulva in first half of body, usually in anterior third; uterus with 2 (didelphys), 4 (tetradelphys), or more than 4 (polydelphys) branches. Eggs usually oval, smooth, thick-shelled, containing embryos when deposited. For basic species affiliation, the new *COI* sequences were checked via BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Up to now, the sequences of *A. kazakhstanica* were not presented in GenBank. The blast result revealed that the sequences of *A. ka-*

zakhstanica have highest percentage of identity (77%) with *Heliconema longissimum* of Physalopteroidea family. The new sequences of *A. kazakhstanica* were submitted to GenBank under the accession number MK578751-2. Based *COI* result of phylogenetic analysis revealed that two main cluster in Spirurida and 3 superfamily Physalopteroidea, Filarioidea and Habronematoidea fall in cluster 2 and the rest subfamily placed in cluster 1 (Fig. 2).

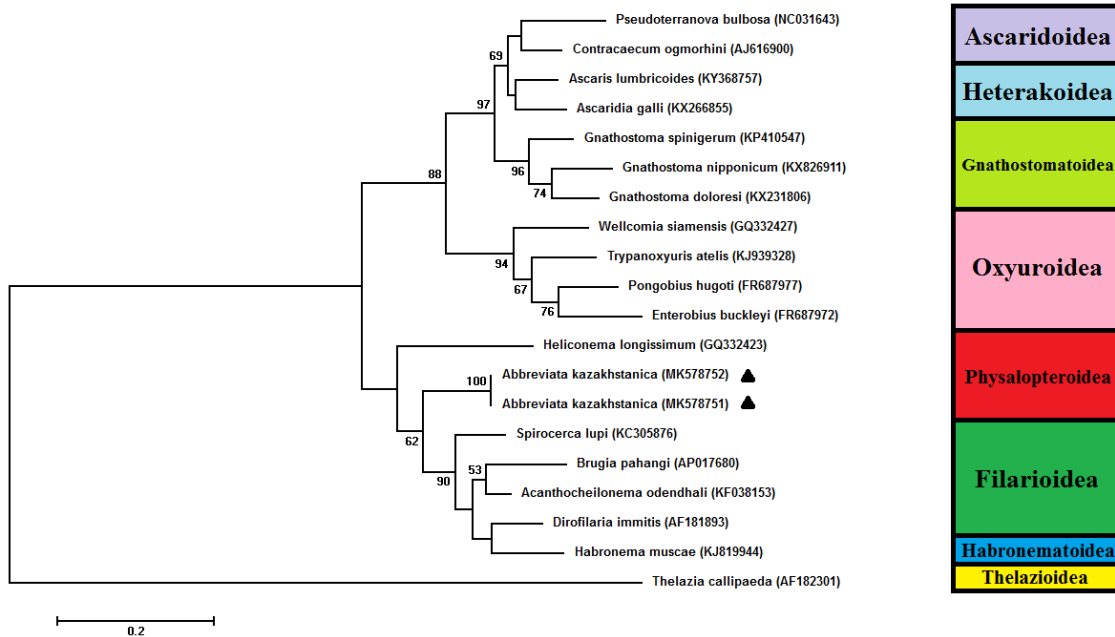


Fig. 2: Molecular phylogenetic analysis of *Abbreviata kazakhstanica* isolate from *Pseudopus apodus* by Maximum Likelihood method on partial mCOI 444 bp sequence. Sequences from *Theleazia callipaeda* (GenBank accession No.: AF182301) were employed as outgroup for phylogenetic analysis. The percentage of trees in which the associated taxa clustered together is shown next to the branches

Discussion

This is the first molecular identification of *A. kazakhstanica* in *P. apodus* in Iran. Glass lizard samples are rarely available for parasite examination, being often confined to dead carcasses in nature and road-killed animals. Previously, this genus from the Physalopteridae family has not been reported from any hosts in Iran.

In other study, the larvae and adult of *A. kazakhstanica* were reported from *P. apodus* (21-23). Until now, *A. kazakhstanica* was isolated from the Union of Soviet Socialist Republics (USSR), UzbSSR (21-24). Six species of tenebrionid beetles including *Gryllus bimaculatus*, *Decticus verrucivorus*, *Platycleis intermedia*, *Tettigonna viridissima*, *Emenoviana tamerlana*, *Pimelia verrucosa* and *Stalagmoptera confuse* play the role of intermediate hosts. Moreover, *Dila laevicollis*, *Prosodes nitida*, *Prosodes baeri*, and *Hierodula kazakhstanica* were determined as intermediate hosts (25).

Conclusion

Considering the limited parasitological data on reptiles in Iran, identifying the parasite fauna precise and comprehensive studies with the aim of and particularly the helminthes diversity are required.

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Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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