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Article

Argas hermanni Audouin (Acari: Argasidae), a new member of Iranian tick fauna

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ABSTRACT

Argas reflexus group includes argasid ticks associated with bird species. We found adults of an *Argas* species within the houses near to domestic pigeon nests in Lorestan province, western Iran. Primarily, the specimens were recognized as *A. hermanni* Audouin, 1827 based on described morphological characters. The traditional taxonomic decision was supported by BLAST analysis of the mitochondrial nucleotide sequences. DNA barcoding approach can verify morphological identification of tick species. This study is the first Iranian record of *A. hermanni*, supported by DNA sequence evidences.

KEY WORDS: DNA barcoding; Lorestan province; pigeon-related ticks; phylogenetic tree; *reflexus* group.

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INTRODUCTION

The tick species *Argas hermanni* Audouin, 1827 (Acari: Argasidae) belongs with about 11 species to the *reflexus* species group (Hoogstraal *et al.* 1979). This bird-feeding ectoparasite was described in 1827 by Audouin from Egyptian collections (Audouin 1827). He stated that this species resembles *A. reflexus* Fabricius, 1794 in the shape of its body. It was firstly mentioned as a subspecies of *A. reflexus hermanni* Lamontellerie, 1960 (Camicas *et al.* 1998). This species is common in the Palearctic region, i.e., Northwest Africa to Nepal (Hoogstraal *et al.* 1979). It can be assumed that *A. hermanni* will subsequently be discovered in the south of Central Asia, as well as in Western Asia (Chunikhin and Filippova 1970). Pigeons are the preferred hosts of *A. hermanni* (El Kammah *et al.* 2002), but some populations of species have been reported infesting little owl (*Athene noctua* Scopoli, 1769), lanner falcon (*Falco biarmicus* Temminck, 1825), hooded crow (*Corvus cornix* Linnaeus, 1758), brown-necked ravens (*C. corax* Linnaeus, 1758) and some migrating birds (Hoogstraal and Kohls 1960). *Argas hermanni* has been found to harbor different arboviruses such as *Flavivirus*, *Nairovirus*, *Orthonairovirus*, Tunis, Quaranfil, Chenuda, and West Nile viruses (Schmidt and Said 1964; Taylor *et al.* 1966; Hoogstraal 1985; Chastel *et al.* 1994; Labuda and Nuttall 2008), as well as *Borrelia anserina* Sakharoff, 1891, the agent of avian borreliosis (Zaher *et al.* 1977). Following an increase of an *Argas* tick population in the colonies of the rock dove, *Columba livia* Gmelin, 1789 near to the

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human buildings in western Iran, we aimed to identify it to the species level, both morphologically and molecularly. Following the previous ambiguous, incomplete and poorly defined records of *A. hermanni* from Iran without any molecular data (Maghami 1968; Mazlum 1971; Khalil and Metwally 1974; Kamali *et al.* 2001; Labuda and Nuttall 2008), we decided to re-examine this species by morphological identification as well as throughmolecular methods. Initially, we believed in dealing with *A. reflexus* tick but since we found out no reports of any bites in residents of the urban houses adjacent to the pigeon's nests, the present study was designed to reveal the identity of pigeon-related ticks (*reflexus* group) in a locality in western Iran.

MATERIAL AND METHODS

Sample collection, DNA extraction and PCR

This study was conducted during 2018–2019. 150 adult ticks were collected from different locations of residential houses located in Khorramabad city in western Iran (33° 28' 57" N, 48° 24' 28" E and 1331 m a.s.l.). Tick specimens were identified to species level under a stereomicroscope (Wild-Heerbrugg M8Model) according to the morphological characters described by Hoogstraal and Kohls (1960) and Filippova (1966). To identify and prepare the ticks, photos of body parts (capitulum, lateral margin (suture), legs, Haller's organ and dorsal integumental texture) and total body shape were captured using a digital camera (Nikon® Coolpix S7000). Voucher specimens are retained in the Razi Herbal Medicine Research Center, Lorestan University of Medical Sciences. Genomic DNA of two representative specimens was extracted using CTAB method according to Doyle and Doyle (1987) with minor modifications. A 728 bp fragment of the cytochrome oxidase subunit I (*COI*) and 351 bp fragment of 16S ribosomal RNA (16S rRNA according to GB Accession L34322), were amplified by polymerase chain reaction (PCR) using the primers For*COI*: 3'- GCC ATT TTA CCG CGA TG -5', Ror*COI*: 3'- ACY TCT GGR TGA CCA AAA AAT C -5'(*COI*) and F16SHAema: 3'- CTG TRG TAT TTT GAC TAT ACA AAG G -5', R16Sor: 3'- CAT CGA GGT CGC AAA C -5' (16S rRNA). PCR for each 25 µl final volume reaction was performed using 12.5 µl RedMaster PCR 2X (Sinaclon®, Iran), 1 µl of each primer (10 pM), 4 µl gDNA template (100 ng/µl), and 6.5 µl ddH₂O. PCR reactions were carried out in a thermocycler (Corbett®, Australia) based on a touchdown temperature profile: 3 minutes at 94 °C, 11x [45 s at 94 °C, 50 s at 60 °C, 60 s at 72 °C], followed by 24x [45 s at 94 °C, 50 s at 50 °C, 60 s at 72 °C], 3 minutes at 72 °C. The PCR products were visualized using 1% agarose gel electrophoresis, and the desired bands were purified using the GF-1 Gel DNA Recovery Kit (Vivantis®, Malaysia). Finally, the purified PCR products were submitted to a third-party service provider (Codon Genetic Group®, Iran) for sequencing using Applied bioSystems-ABI, 3130XL.

Phylogenetic analysis

The DNA sequences were manually checked using FinchTV® software (www.geospiza.com) to correct any sources of error or ambiguities if present. Homologies with the available sequence data in GenBank were checked using BLAST option. Finally, sequences were submitted to GenBank under accession numbers MK318147 (*COI*) and MN538361 (16S rRNA). Then, the sequences were aligned using SeaView4 software (Gouy *et al.* 2010). The genetic distances among and between sequences were calculated using Maximum Composite Likelihood (MCL) modelled in MEGA7 (Kumar *et al.* 2016). To construct the phylogenetic tree, both 432 bp (*COI*) and 288 bp (16S rRNA) alignment sheets were analysed using BEAST® (Ver. 2.6.0) (Bouckaert *et al.* 2014) based on the Bayesian Inference (BI) method. We selected an appropriate substitution model using the FINDMODEL program (<http://hiv.lanl.gov/content/sequence/findmodel/findmodel.html>) (Posada and Crandall 1998), which identified Jukes-Cantor (JC69) as the appropriate tree model with a -lnL score of 5665.76 and 2113.36 for *COI* and 16S rRNA data sheets, respectively. BI employs Markov Chain Monte Carlo (MCMC) algorithms and infers a most credible tree given the posterior

probabilities of alternative tree topologies. For this purpose, 33 *COI* and 60 16S rRNA sequences including the sequences of present study (Accessions: MK318147 & MN538361), as well as the comparable data sequences of the argasid tick species *Argas*, *Ornithodoros*, *Antricola*, and *Carios* were used. Sequences were selected according to the similarity revealed by the BLAST algorithm. The out-groups were chosen according to Smith (1994) and Wenzel (2002). They suggested that out-groups could be selected from sister groups as well as successively more distant lineages. Thus, the genera *Ornithodoros*, *Antricola* and *Carios* were examined as out-groups. The phylogenetic trees were summarized and visualized using TreeAnnotator and FigTree (Ver. 1.4.4.), respectively.

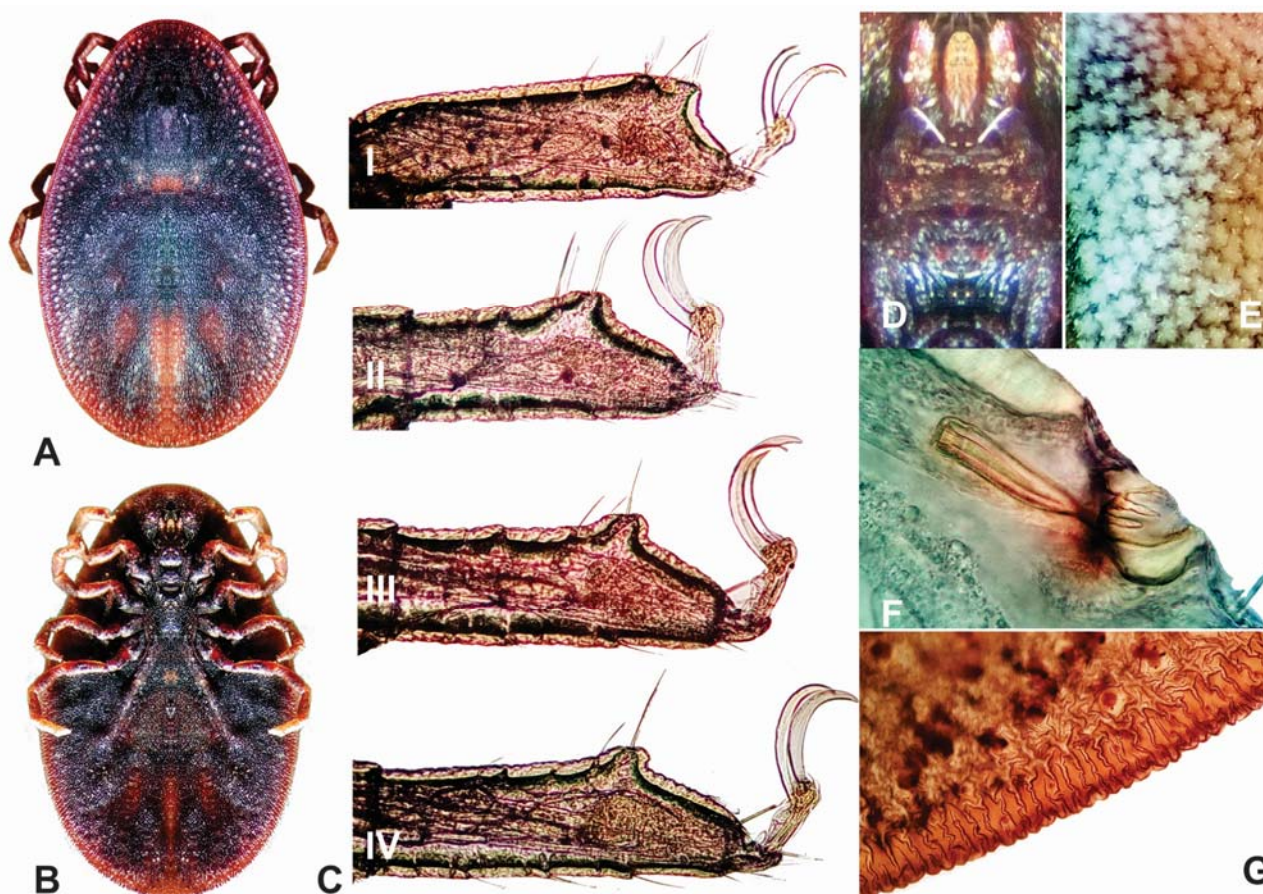


Figure 1. Diagnostic morphological characteristics of adult *Argas hermanni* – General body shape from dorsal (A) and ventral (B) views, legs I-IV (C), capitulum from ventral view (D), dorsal integumental texture (E), Haller's organ and its structure including cell or bottle-shaped sensillum, capsule and internal sensillum or inner chamber (F), body lateral margin (G).

RESULTS

Tick collection, identification and BLAST analysis

In total, 150 adult specimens of soft ticks were collected indoors throughout the years 2018–2019 at houses located in the countryside near Khorramabad city, Lorestan province. Then, the specimens were recognized as *Argas hermanni* based on described morphological characters, including the presence of lateral fringes or ridges as parallel without rectangular discs (Fig. 1G), leg dorsal tubercle pattern (Fig. 1C), post-hypostomal hair (Fig. 1D), dorsal integumental texture (Fig. 1E). Haller's organ shows a pattern like to the members of *A. hermanni* complex (i.e. *A. macrostigmatus* Filippova, 1961, *A. vulgaris* Filippova, 1961, *A. latus* Filippova, 1961) as the presence of “cell” or bottle-shaped sensillum exterior to Haller's organ capsule; moreover the internal sensillum (inner chamber) do not

exceed the capsule limit (Fig. 1F). BLAST analysis of the *COI* and 16S rRNA nucleotide sequence showed 86% and 91% sequence identity to *COI* and 16S rRNA sequence of *A. africanus* Hoogstraal, Kaiser, Walker, Ledger, Converse & Rice, 1975 (a member of *A. reflexus* group). Moreover, we found 84% identity between a 16S rRNA sequence of this study with *A. reflexus* 16S ribosomal RNA sequences (AF001401 and L34322). No further *COI* sequence related to *A. reflexus* was found in GenBank.

Phylogenetic analysis

Phylogenetic trees were constructed based on partial *COI* and 16S rRNA sequence data (Fig. 2). The *COI* phylogeny of genus *Argas* is rooted with clades of selected argasid genera (*Ornithodoros*, *Antricola* and *Carios*) as well as 16S rRNA phylogeny with a member of genus *Ornithodoros*. In *COI* phylogeny the out-groups I-II fall outside the main *Argas* clade but two internal out-groups exist inside one. Both *COI* and 16S rRNA phylogenetic trees show two main clades of *A. reflexus* group and *A. persicus* group. The genetic distance range between different species of the *A. reflexus* group is 12–14% and 4–28%, for *COI* and 16S rRNA, respectively.

DISCUSSION

We found adults of *Argas hermanni* within the houses near to domestic pigeon nests in Lorestan province, western Iran. Then, the specimens were identified as *A. hermanni* according to the morphological characteristics. To date only three *Argas* species have been reported from Iran including *A. (Persicargas) persicus* Oken, 1818, *A. (Argas) reflexus* Hoogstraal & Kaiser, 1973 and *A. vespertilionis* (Latreille, 1796) (Hosseini-Chegeni and Tavakoli 2013). The identity of *A. hermanni* and the *reflexus* group including *A. (A.) vulgaris* may be debatable in Iran. In previous reports, *A. hermanni* was found in adjacent countries, Saudi Arabia (Hoogstraal *et al.* 1981), Afghanistan (Buck *et al.* 1972) and former USSR (Chunikhin and Filippova 1970); in other countries only *A. reflexus* was recorded (Hoogstraal and Kaiser 1958; Bursali *et al.* 2012). Walker *et al.* (2007) believed that information on the distribution of *A. hermanni* was sparse but it appeared to be limited to Egypt and Ethiopia in Africa. The pigeon tick, *A. reflexus* (and, perhaps, other species from the *A. reflexus* group) also participates in transmission of diseases (Uspensky 2008). However, we did not find any reports of human bites by this species. The authors have also been in close contact with tick colonies inside human buildings (adjacent the pigeon's nests) for over two years, but no cases of tick bites were observed. Thus, we concluded it to be other than *A. reflexus*. The ticks of the *reflexus* group include *A. reflexus*, *A. hermanni*, *A. africanus*, *A. macrostigmatus*, *A. vulgaris*, *A. polonicus* Siuda, Hoogstraal, Clifford & Wassef, 1979, *A. latus*, *A. tridentatus* Filippova, 1961, *A. himalayensis* Hoogstraal & Kaiser, 1973, *A. brevipes* Banks, 1908, *A. dalei* Clifford, Keirans, Hoogstraal & Corwin, 1976. Morphological differentiation of some species of the *reflexus* group can be difficult and needs molecular approaches (Dabert *et al.* 1999). Several varieties (subspecies) were described from *A. hermanni* (*sensu lato*) namely; *A. hermanni* (*sensu stricto*), *A. h. macrostigmatus* Filippova 1966, *A. h. latus* Filippova 1966 and *A. h. vulgaris* Balashov & Filippova, 1964 (Filippova 1966). These varieties were later upgraded to species level (Horak *et al.* 2002). In the present study, the traditional taxonomy was supported by BLAST analysis of the mitochondrial nucleotide sequences including *COI* and 16S rRNA. BLAST analysis of the *COI* and 16S rRNA nucleotide sequences showed 86% and 91% sequence identity to *COI* and 16S rRNA sequence of *A. africanus* (a member of *A. reflexus* group), respectively. The life cycle of some members of the *reflexus* group such as *A. africanus* and *A. hermanni* is practically identical with regards to larval and nymphal cycles (Hoogstraal 1985).

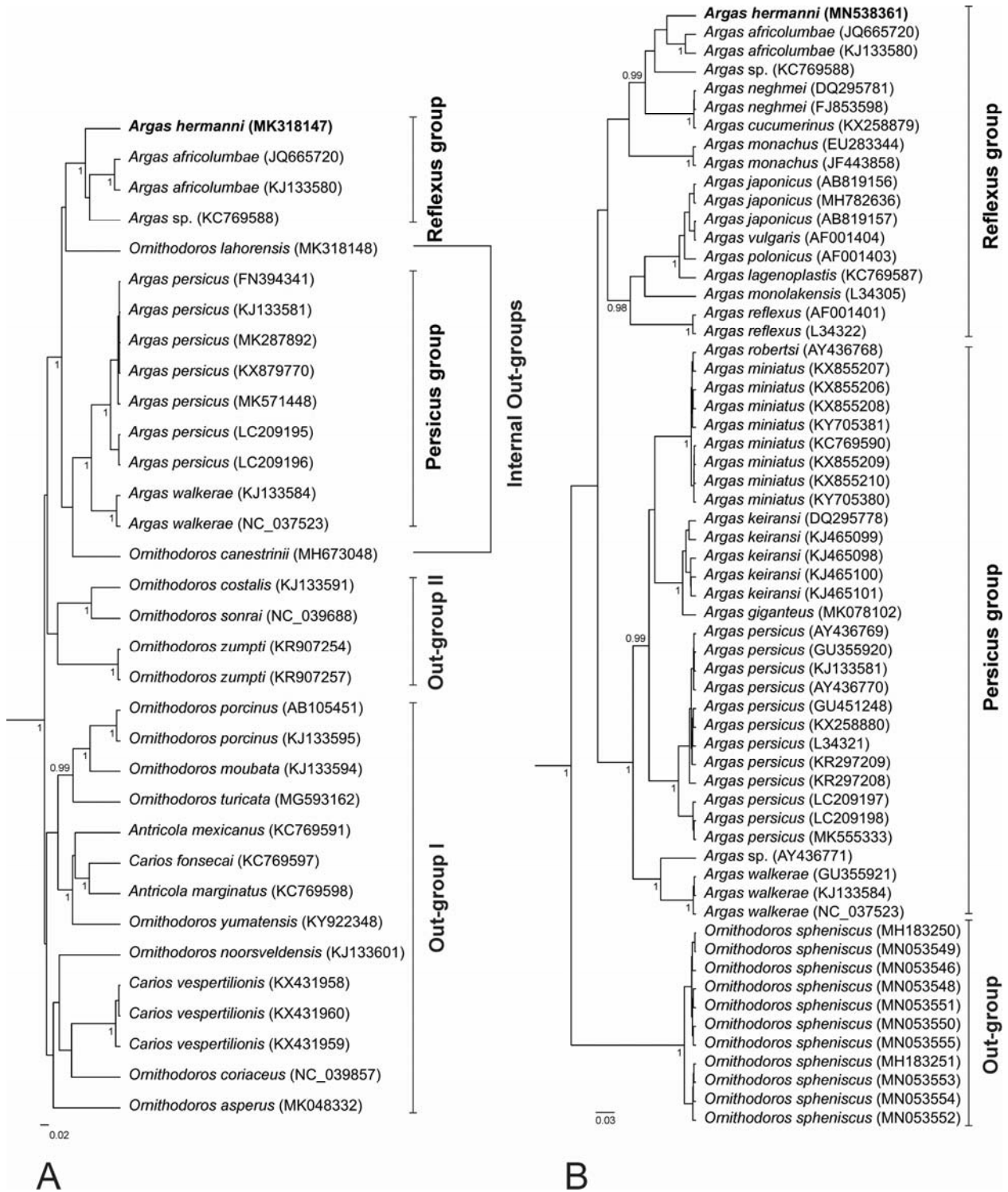


Figure 2. Phylogenetic relationships among argasid tick taxa and *Argas* species derived from the Bayesian inference (BI) generated based on analysis of partial *COI* (A) and 16S rRNA (B); numbers below each node show posterior probability value (10 million reiterations). Taxon labels give the species name followed by GenBank accession numbers in parentheses; the taxon sequenced in the present study is highlighted in bold. Branch lengths are proportional to the evolutionary distances.

Our phylogenetic analysis supported the paraphyly and monophyly of the genus *Argas* according to *COI* and 16S rRNA phylogenetic trees, respectively. Two closely related taxa of the genus *Alveonatus canestrinii* Grebenyuk, 1951 and *Al. lahorensis* Schulze, 1941) appear inside the clade

Argas (COI phylogenetic tree). Historically, the classification of the Argasidae has been the subject of considerable disagreement (Klompen and Black 1996). In our analysis, the genus *Argas* was isolated from *Ornithodoros* (16S rRNA phylogenetic tree). This study is the first record of the occurrence of *A. hermanni* from Iran supported by mitochondrial DNA evidences.

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گزارش گونه *Argas hermanni* Audouin (Acari: Argasidae) عضو جدید فون کنه‌های دامی ایران

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چکیده

گروه گونه‌ای *Argas reflexus* کنه‌های نرم انگل پرندگان را شامل می‌شوند. نمونه‌های بالغ گونه‌ای کنه *Argas* از درون اماکن مسکونی انسانی نزدیک لانه‌های کبوتر اهلی در استان لرستان واقع در غرب ایران پیدا شد. در ادامه، نمونه‌ها بر اساس ویژگی‌های ریخت‌شناسی به عنوان *A. hermanni* Audouin, 1827 تشخیص داده شد. آرایه‌شناسی این گونه با تحلیل BLAST توالی‌های نوکلئوتید میتوکندریایی مورد تأیید قرار گرفت. رویکرد بارکد DNA قادر است تشخیص ریخت‌شناسی گونه‌های کنه را مورد تأیید قرار دهد. این مطالعه نخستین گزارش *A. hermanni* از ایران است که با استفاده از شواهد مولکولی توالی‌های DNA حمایت می‌شود.

واژگان کلیدی: بارکدینگ DNA؛ استان لرستان؛ کنه‌های انگل کبوتر؛ درخت تبارشناسی؛ گروه رفلکسوس.

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