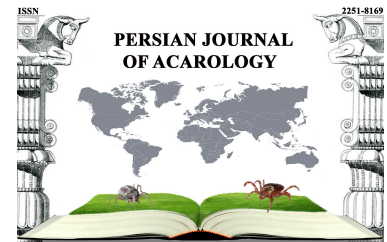




*Persian J. Acarol.*, 2018, Vol. 7, No. 2, pp. 209–216.  
<http://dx.doi.org/10.22073/pja.v7i2.34812>  
Journal homepage: <http://www.biotaxa.org/pja>



## Article

### *Otobius megnini* (Acari: Argasidae) in Iran: exotic or established?

Asadollah Hosseini-Chegeni<sup>1, 2</sup>, Javad Khedri<sup>3</sup>, Zakkyeh Telmadarraiy<sup>4</sup> and Faezeh Faghihi<sup>5\*</sup>

1. Department of Plant Protection, Faculty of Agriculture, University of Lorestan, Lorestan, Iran; E-mail: Hosseinichegeni@gmail.com
2. Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Lorestan, Iran
3. Department of Pathobiology, Shahid Bahonar University of Kerman, Kerman, Iran; E-mail: Javadkhedri1362@yahoo.com
4. Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; E-mail: Telmadarraiy@tums.ac.ir
5. Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran; E-mail: Faezefaghihi@yahoo.com & Faghihi.f@iums.ac.ir

\* Corresponding author

#### ABSTRACT

*Otobius megnini* is a tick species which its larvae and nymphs feed deep in the external ear canal of a variety of ungulates. In this study, twelve adult and four nymph specimens were collected from cattle hosts in Sistan and Baluchestan, and Hamedan Provinces. The specimens were identified using morphological key and the data was confirmed by molecular assays. In the present study, we could find *O. megnini* in tick fauna of Iran with new hosts for this species.

**KEY WORDS:** Cattle; molecular assay; *COI*; soft tick; spinose ear tick.

**PAPER INFO.:** Received: 11 December 2017, Accepted: 1 March 2018, Published: 15 April 2018

#### INTRODUCTION

*Otobius megnini* (Dugès, 1883) (Acari: Argasidae), the spinose ear tick, is a tick species which its larvae and nymphs feed deep in the external ear canal of a variety of species [primarily ungulates] (Kaufmann 1996). The non-parasitic adults live on the ground and have no functional mouthparts. There are many reports from many different parts of the world that *O. megnini* bite humans (Estrada-Peña and Jongejan 1999). Although no pathogens were known to be transmitted by *O. megnini*, considerable damages to the ears, eardrums and auricular nerves of hosts have been reported, due to feeding (Bates 2012). However, it has been reported that *Coxiella burnetii*, the agent of Q fever, was found on *O. megnini* collected in southern California (Jellison *et al.* 1948). The genus *Otobius* contains two species, *O. megnini*, a parasite of large mammals and *O. lagophilus*, which feeds on the genera *Lepus* and *Sylvilagus* (Keirans 1992). *Otobius megnini* was first described from Mexico by Dugès in 1883 as *Argas megnini* (Nuttall *et al.* 1908). The arid lands of southwestern North America are thought to be the original center of *O. megnini* (Keirans and Pound 2003). This American tick species has been widely distributed to far-reaching areas of the world via transportation by their animal hosts (Hoogstraal 1972; Bowman *et al.* 2002). *Otobius megnini* has

been reported from Asian countries including Turkey (Bursali *et al.* 2012), India (Kingston 1936), and some other countries with low decisiveness (Keirans 1992; Keirans and Pound 2003). In Iran, *O. megnini* was reported by Rahbari *et al.* (1990) from three dairy cattle for the first time, in December 1987. Since then, *O. megnini* was not detected in tick samples collected from Iran for many years. In 2012, Paktinat Saej *et al.* (2012) reported *O. megnini* from an unknown host in northeastern border of Iran, Khorasan-e Razavi Province. In the present study, we provide some distributional data and new hosts for this species from Iran.

## MATERIALS AND METHODS

### Tick sampling

Tick specimens were collected from Iranian Sistani cattle in abattoir of Mohammad Abad, Hamoun County, Sistan and Baluchestan Province located in southeastern Iran (30° 52' 47.58" N 61° 27' 40.15" E) during the year 2016. Other specimens were also collected from Famenin County, Hamedan Province (35° 6' 47.91" N 48° 58' 30.13" E) in western Iran during the year 2017. They were deposited in Razi Herbal Medicine Research Center, Lorestan University of Medical Sciences, Lorestan, Iran. The voucher specimens used in this study were retained in Razi Herbal Medicine Research Center, Lorestan University of Medical Sciences, Lorestan, Iran.

### Morphological identification and molecular confirmation

The specimens were identified based on Walker's taxonomical key (2007). The most important characters for identification of *O. megnini* were their spiny integuments (Fig. 1) and well developed hypostomes; adults with granular integuments and vestigial hypostomes. For molecular confirmation, DNA of a single nymph sample was extracted by cTAB methods (Doyle and Doyle 1987), then a 693bp fragment of cytochrome oxidase subunit 1 (*COI*), was amplified by polymerase chain reaction (PCR) using primers submitted by Folmer *et al.* (1994) with minor modifications. The primer sequences were as follows; forward primer (Ffol: 5'- GGT CAA CAA ATC ATA AAG ATA TTG G -3') and reverse primer (Rfol: 5'- CAG GGT GMC CAA AAA ATC -3'). PCR reactions for *COI* gene were carried out in Corbett® thermocycler (Australia) by initial denaturation (5 min. at 95 °C), 5 cycles of denaturation (60 sec. at 95 °C), annealing (60 sec. at 55 °C), extension (50 sec. at 72 °C), and final extension step (5 min. at 72 °C). For each 25µl of final volume reaction, 12.5 µl RedMaster PCR® 2X, 1 µl of each forward and reverse primers (10 pM), and 4 µl gDNA template (100ng/µl), as well as 6.5 µl deionized water were used. Amplifications were confirmed by 1% agarose gel electrophoresis. The desired band was purified using GeneJET Gel Extraction Kit®. Then, the purified PCR product was submitted for sequencing to FAZA biotech company (Iran). The sequences were manually checked using FinchTV® software to correct any sources of error or ambiguities. Homologies with the available sequence data in GenBank were checked using BLAST analysis. Finally, the sequence was submitted in GenBank and the accession number (MG582606) was assigned.

### COI phylogenetic tree

All *COI* sequences were included a single sequence obtained from this study and the sequences of GenBank (AF132828: *Dermacentor marginatus*, AF132834: *Rhipicephalus compositus*, JX394196: *Ixodes hexagonus*, JX573118: *Amblyomma cajennense*, KC769589: *Otobius megnini*, KC769590: *Argas miniatus*, KC769593: *Ornithodoros brasiliensis*, KJ133588: *Carios faini*, KJ133592: *Otobius megnini*, KR075985: *Hyalomma asiaticum*, KR108853: *Haemaphysalis concinna*) aligned using SeaView4 software (Gouy *et al.* 2010). The genetic distances among the sequences were calculated using Maximum Composite Likelihood (MCL) model in MEGA7 software (Kumar *et al.* 2016). Afterwards, in order to construct the phylogenetic tree of the ticks, the aligned sequences were analyzed using BEAST® software (version 1.8.4) (Drummond *et al.*

2012) based on the Bayesian Inference (BI) method that uses Markov chain Monte Carlo (MCMC) algorithms for Bayesian phylogenetic inference. For this purpose, 13 taxa (including a sequence of the present study as well as the GenBank data sequences as in- and out-group submitted from different areas of the world) were used for constructing *COI* phylogenetic trees. The constructed clades of phylogenetic trees were reorganized based on support posterior probability of 0.9 values and the reasonable genetic distance differences within and between the clade members. An ixodid taxa were examined as out-group in the phylogenetic tree.

## RESULTS

### *Morphological identification and molecular confirmation*

Number of 12 adults of *Otobius megnini* was collected from Sistani cattles (*Bos indicus*) in Sistan and Baluchestan Province. Moreover, four nymphs of *O. megnini* were collected from cattle host in Hamedan Province. A *COI* target fragment was amplified and sequenced from a nymph specimen collected in Famenin County, Hamedan Province. The BLAST showed 99% similarity between a sequence of this study with two *COI* sequences of *Otobius megnini* submitted from South Africa (KJ133592) and Madagascar (KC769589).

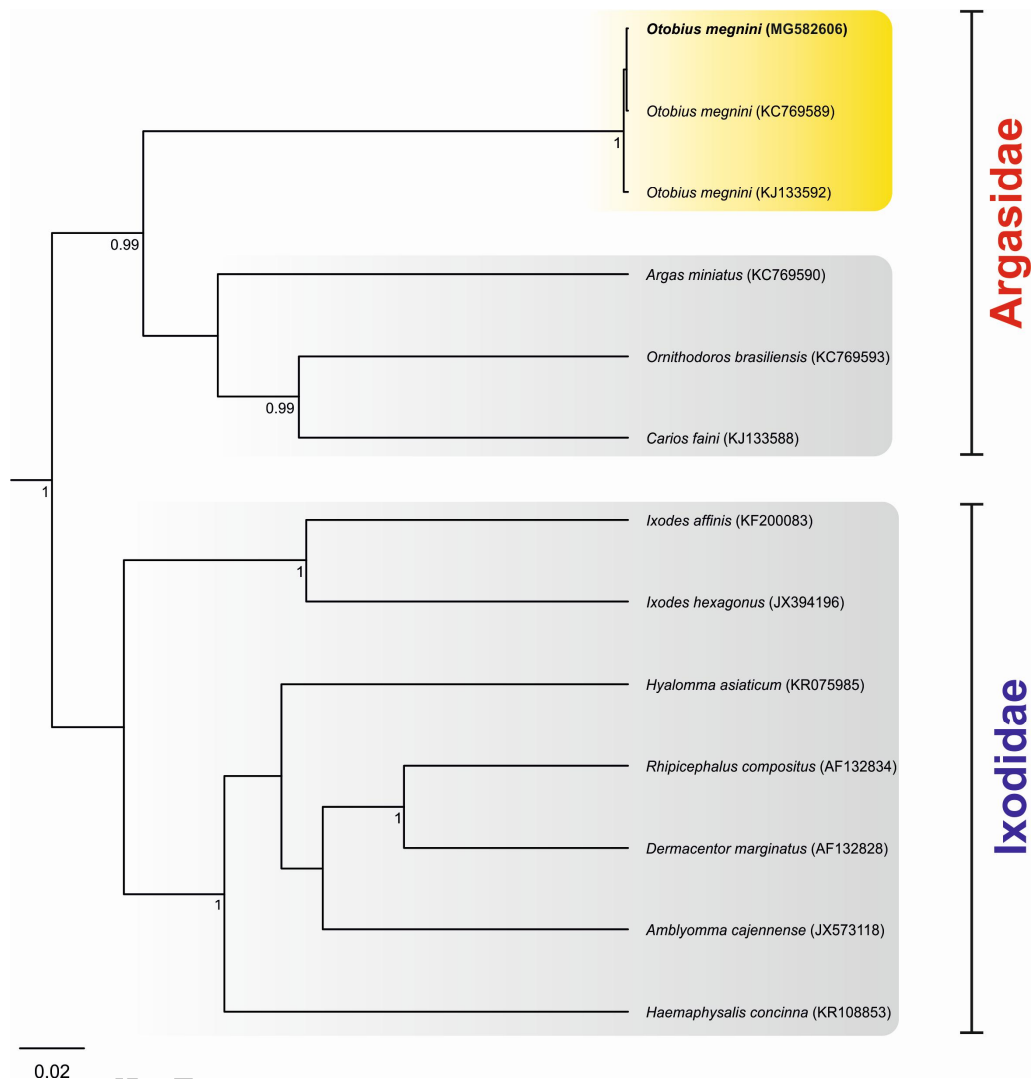


**Figure 1.** *Otobius megnini* collected from Iran (Famenin County): A. Nymph dorsal aspect; B. a section of it's spiny cuticle.

### *COI phylogenetic tree*

A phylogenetic tree constructed using BEAST software with data BI method; including in-

group and out-group tick taxa were shown in Figure 2. The presented phylogeny indicated that the Iranian *O. megnini* would be group with the GenBank *O. megnini* sequences data submitted from Madagascar and South Africa. No intraspecies variation in terms of genetic distance was found between *COI* sequences of clade *O. megnini*.



**Figure 2.** Phylogenetic tree of ixodid and argasid ticks including *Otobius megnini* taxa inferred from *COI* sequence data constructed using Bayesian Inference (BI) method; main clades in right side of tree were separated with grayish rectangular shapes and yellow colored box for *O. megnini*. Taxa are as species name following GenBank accession number, taxon of the present study indicated as bold. Nodes indicated with bootstrap value. Branch lengths are proportional to evolutionary changes. The members of Ixodidae clade are indicated as out-group.

## DISCUSSION

*Otobius megnini* is one of the most important argasid tick species which has been distributed in various parts of the world far from its origin in North America through livestock movement (Keirans 1992; Keirans and Pound 2003). The transportation of livestock to Iran was the main cause for the entrance of *O. megnini* to Iran in 27 years ago (Rahbari *et al.* 1990). In the present study, we detected two native sites of infestation of domestic animals with *O. megnini* in southeastern and western Iran on cattle and sheep hosts. Both adults and immature stages (nymphs) of *O. megnini* were collected. In a previous report by Rahbari *et al.* (1990), only nymphal stages were reported.

The controversy between our data and Rahbari's might be due to establishment and inbreeding of *O. megnini* in the collection sites of the present study. The ear tick is more able to establish in outside of origin in North America as well as, in new areas; including Europe, Asia, and Africa. *Otobius megnini* has been previously collected from animals other than sheep and cattle, including dogs (White *et al.* 1995), lions (Munaó Diniz *et al.* 1987), antelope (Schad 1958; Colbenson 1962), horses (Diyes and Rajakaruna 2016), and even from a human child (Jensen *et al.* 1982). Guglielmone *et al.* (1992) detected *O. megnini* in arid and semiarid lands of Argentina on cattle and sheep hosts. In the present study, immature stages of *O. megnini* were detected parasitizing animal hosts; however, free living larvae have been seen in some natural occasions (Rich and Gregson 1968). The control of *O. megnini* through sanitary measures and applications of biocides into the ears is recommended as the most reasonable approach on cattle (Nava and Guglielmone 2009). The difference in biological properties among populations of this tick species representing different forms due to environmental niches of different climatic conditions in which each population uses particular bio-geographical adaptations (Nava *et al.* 2009; Estrada-Peña *et al.* 2010). In this regard, we suggest the evaluation of the tick different populations to prove the species status and biological specifications of the tick species. Following the findings of Diyes and Rajakaruna (2016) on different pure bred and the mixed bred horses, studying of the parasitic effects of ticks on bred and nun-bred races of cattles in terms of their economic importance is suggested. The present study provides some distributional data, with new hosts found for *O. megnini* in Iran.

#### ACKNOWLEDGMENTS

The authors would like to thank the bounteous villagers and ranchers living within Hamedan province, as well as the workers of the abattoir of Mohammad Abad in Sistan and Baluchestan province for their help with tick sampling.

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## کنه *Otobius megnini* (Acari: Argasidae) در ایران: وارد شده یا مستقر؟

اسداله حسینی چگنی<sup>۱</sup>،<sup>۲</sup>، جواد خدري<sup>۳</sup>، زکيه تلمادره‌ای<sup>۴</sup> و فائزه فقیهی<sup>۵\*</sup>

۱. گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه لرستان، لرستان، ایران؛ رایانامه: [Hosseinichegeni@gmail.com](mailto:Hosseinichegeni@gmail.com)

۲. مرکز تحقیقات داروهای گیاهی، آزمایشگاه مولکولی، لرستان، ایران

۳. گروه پاتوبیولوژی، دانشگاه شهید باهنر کرمان، کرمان، ایران؛ رایانامه: [Javadkhedri1362@yahoo.com](mailto:Javadkhedri1362@yahoo.com)

۴. گروه حشره شناسی و کنترل ناقلین، دانشکده بهداشت، دانشگاه علوم پزشکی تهران، تهران، ایران؛ رایانامه: [Telmadarraiy@tums.ac.ir](mailto:Telmadarraiy@tums.ac.ir)

۵. مرکز تحقیقات سلولی و مولکولی، دانشگاه علوم پزشکی ایران، تهران، ایران؛ رایانامه: [Faezefaghihi@yahoo.com](mailto:Faezefaghihi@yahoo.com)

\* نویسنده مسئول

### چکیده

گونه‌ای کنه نرم به نام *Otobius megnini* (Dugès, 1883) (Acari: Argasidae)، معروف به کنه خاردار گوش، کنه‌ای تک میزبان است که لاروها و پوره‌ها در عمق مجرای خارجی گوش طیف گسترده‌ای از تک‌سُمی‌ها خون‌خواری می‌کنند. تعداد ۱۲ نمونه کنه کامل و چهار پوره از گاو در استان‌های سیستان و بلوچستان، و همدان جمع‌آوری شد. نمونه‌ها بر اساس کلید ریخت‌شناسی به عنوان *O. megnini* شناسایی شدند، سپس نتیجه بر اساس روش‌های مولکولی مورد تأیید قرار گرفت. در مطالعه حاضر، داده‌های مربوط به پراکندگی و میزبان‌های جدید برای این گونه ارائه شد و نویسندگان به این نتیجه رسیدند که *O. megnini* به احتمال گونه‌ای استقرار یافته در فون کنه‌های ایران است.

**واژگان کلیدی:** دام؛ بررسی مولکولی؛ ریخت‌شناسی؛ کنه نرم؛ کنه خاردار گوش.

**اطلاعات مقاله:** تاریخ دریافت: ۱۳۹۶/۹/۲۰، تاریخ پذیرش: ۱۳۹۶/۱۲/۱۰، تاریخ چاپ: ۱۳۹۷/۱/۲۶