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Article

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

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ABSTRACT
Otobius megnini is a tick species which it's 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 datawas 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.

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INTRODUCTION

Otobius megnini (Dugès, 1883) (Acari: Argasidae), the spinose ear tick, is a tick species which it's 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 burneti*, 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 Saeej *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).

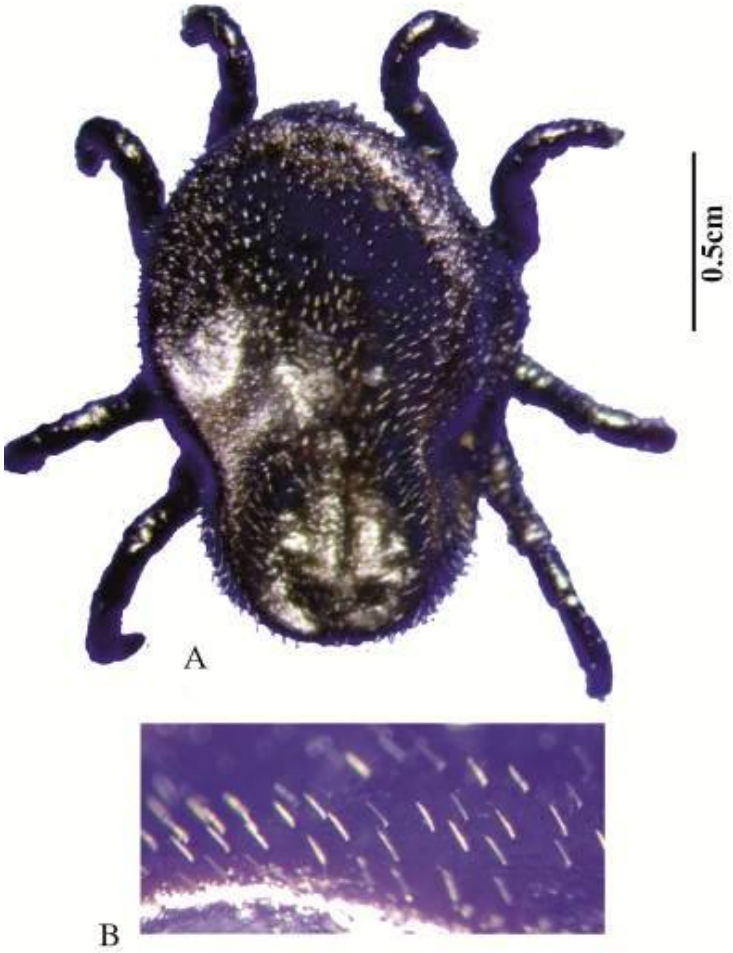

Figure 1. *Otobius megnini* collected from Iran (Famenin County): A. Nymph dorsal aspect; B. a section of it's spiny cuticle.

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*.

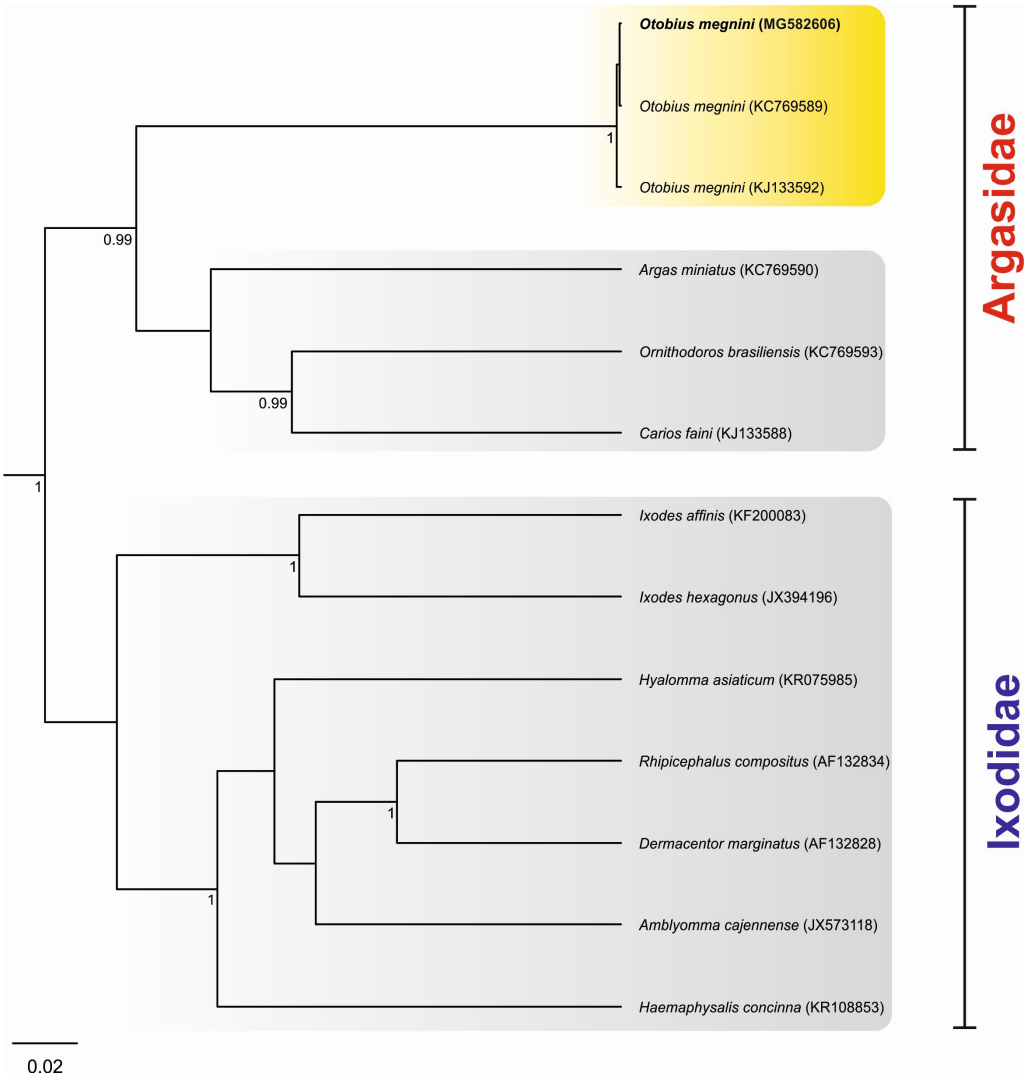

Figure 2. Phylogenetic tree of ixodid and argasid ticks including *Otobius megnini* taxa inferred from *COI* sequence data constructed using Bayesian Inference (BI) method; main clades in right side of tree were separated with grayish rectangular shapes and yellow colored box for *O. megnini*. Taxa are 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 it's 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* toIran 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.

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REFERENCES

Bates, P.G. (2012) *External parasites of small ruminants, a practical guide to their prevention and control*. CABI, Oxford, UK. 256 pp.

Bowman, D.D., Hendrix, C.M., Lindsay, D.S. & Barr, S.C. (2002) *Feline clinical parasitology*. Iowa State University Press, Iowa USA, 359 pp.

Bursali, A., Keskin, A. & Tekin, S. (2012) A review of the ticks (Acari: Ixodida) of Turkey: species diversity, hosts and geographical distribution. *Experimental and Applied Acarology*, 57 (1): 91–104.

Colbenson, H. (1962) Spinose ear tick, *Ornithodoros* (*Otobius*) *megnini*, from pronghorn antelope. *The Journal of Parasitology*, 48 (4): 630–630.

Diyes, G. & Rajakaruna, R. (2016) Seasonal dynamics of spinose ear tick *Otobius megnini* associated with horse otoacariasis in Sri Lanka. *Acta tropica*, 159 170–175.

Doyle, J.J. & Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19: 11–15.

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, 29(8): 1969–1973.

Estrada-Peña, A. & Jongejan, F. (1999) Ticks feeding on humans: a review of records on human-biting ixodoidea with special reference to pathogen transmission. *Experimental and Applied Acarology*, 23(9): 685–715.

Estrada-Peña, A., Nava, S., Horak, I.G. & Guglielmone, A.A. (2010) Using ground-derived data to assess the environmental niche of the spinose ear tick, *Otobius megnini*. *Entomologia Experimentalis et Applicata*, 137(2): 132–142.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3(5): 294–299.

Guglielmone, A., Mangold, A. & Aufranc, C. (1992) *Haemaphysalis juxtakochi*, *Ixodes pararicinus* (Ixodidae) and *Otobius megnini* (Argasidae) in relation to the phytogeography of Argentina. *Annales de Parasitologie Humaine et Comparee,* 67(3): 91–93 (In French).

Gouy, M., Guindon, S. & Gascuel, O. (2010) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution*, 27 (2): 221–224.

Hoogstraal, H. (1972) The influence of human activity on tick distribution, density, and diseases. *Wiadomosci Parazytologiczne,* 18 (4–6): 501–511.

Jellison, W., Bell, E., Huebner, R., Parker, R. & Welsh, H. (1948) Q fever studies in southern California: IV. occurrence of *Coxiella burneti* in the spinose ear tick, *Otobius megnini*. *Public Health Reports (1896-1970)*, 63(46): 1483–1489.

Jensen, L.A., Snow, R.L. & Clifford, C.M. (1982) Spinöse ear tick, *Otobius megnini* attached to the conjunctiva of a child's eye. *The Journal of Parasitology*, 68(4): 528.

Kaufmann, J. (1996) *Parasitic infections of domestic animals: a diagnostic manual*. Birkhäuser Verlag, Basel, Switzerland, 423 pp.

Keirans, J.E. (1992) Systematics of the Ixodida (Argasidae, Ixodidae, Nuttalliellidae): an overview and some problems. *In*: Fivaz, B., Petney, T. and Horak, I.G. (Eds.), *Tick vector biology: medical and veterinary aspects.* Springer-Verlag, Berlin Germany, pp. 1–21.

Keirans, J.E. & Pound, J.M. (2003) An annotated bibliography of the spinose ear tick, *Otobius megnini* (Dugès, 1883) (Acari: Ixodida: Argasidae) 1883–2000. *Systematic and Applied Acarology Special Publications,* 13 (1): 1-68.

Kingston, J. (1936) Spinose ear tick in India. *Journal of the Royal Army Veterinary Corps*, 7: 142–143.

Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33 (7): 1870–1874.

Munaó Diniz, L., Belluomini, H., Travassos Filho, L. & da Rocha, M. (1987) Presence of the ear mite *Otobius megnini* in the external ear canal of lions (*Panthera leo*). *The Journal of Zoo Animal Medicine*, 18(4): 154–155.

Nava, S. & Guglielmone, A.A. (2009) Difficulties to control natural infestation with *Otobius megnini* (Acari: Argasidae) nymphs in cattle with systemic biocides. *Research in Veterinary Science*, 87 (2): 258–259.

Nava, S., Mangold, A.J. & Guglielmone, A.A. (2009) Field and laboratory studies in a neotropical population of the spinose ear tick, *Otobius megnini*. *Medical and Veterinary Entomology*, 23(1): 1–5.

Nuttall, G.H.F., Warburton, C., Cooper, W.F. & Robinson, L.E. (1908). Part 1. The Argasidae. *In*: Nuttall, G.H.F., Warburton, C., Cooper, W.F. & Robinson, L.E. (Eds.), *Ticks: A monograph of the Ixodidae.* Cambridge University Press, Cambridge, UK, pp. 1–80.

Paktinat Saeej, S., Sadeghi Namaghi, H., Monfaredi, F. & Panahi Laeen, H. (2012) Fauna of Ixodidae and Argasidae (Acari: Metastigmata) associated with domestic animals in Razavi Khorasan province, Iran. Proocedings of the 20th Iranian Plant Protection Congress, Shiraz, Iran, p. 463.

Rahbari, S., Taghipour-Bazargani, T. & Seifollahy, M. (1990) The first report of occurrence of *Otobius megnini* in Iran. *Journal of Veterinary*, 45: 81–93.

Rich, G. & Gregson, J. (1968) The first discovery of free-living larvae of the ear tick, *Otobius megnini* (Duges), in British Columbia. *Journal of the Entomological Society of British Columbia*, 65: 22–23.

Schad, G. (1958) First report of *Ornithodorus* (*Otobius*) *megnini* from the pronghorn antelope, antilocapra americana. *The Journal of Parasitology*, 44(5): 514–514.

Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Peña, A., Horak, I.G., Latif, A.A., Pegram, R.G. & Preston, P.M. (2007) *Ticks of domestic animals in Africa: a guide to identification of species*. Bioscience Reports, Edinburgh, UK., 221 pp.

White, S.D., Scott, K.V. & Cheney, J.M. (1995) *Otobius megnini* infestation in three dogs. *Veterinary Dermatology*, 6(1): 33–35.

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

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چکیده

گونه‌ای کنهٔ نرم به نام *Otobius megnini* (Dugès, 1883) (Acari: Argasidae)، معروف به کنهٔ خاردار گوش، کنه‌ای تک میزبانه است که لاروها و پوره‌ها در عمق مجرای خارجی گوش طیف گسترده‌ای از تک‌سُمی‌ها خون‌خواری می‌کنند. تعداد ۱۲ نمونه کنهٔ 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 ارایه 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؛ کنهٔ نرم؛ کنهٔ خاردار گوش.

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