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First DNA-barcode for the genus *Aegyptobia* (Trombidiformes: Tenuipalpidae) and molecular barcodes of spider mites (Trombidiformes: Tetranychidae) from Iran

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Species delimitation of phytophagous mite is a key step in pest control because members of this group are responsible for significant crop losses worldwide (Jeppson *et al*. 1975; Helle and Sabelis 1985; Migeon and Dorkeld 2019). Usually, researchers use morphological characters to identify species within a tetranychoid genus, including aedeagus morphology, dorsal striations, and pretarsus characters (Ehara 1956; Ehara and Gotoh 1990; Arabuli *et al*. 2019), but the small size of these mites (less than 0.5 mm) and the limited number of potential diagnostic characters make morphological identification difficult in some cases (Wauthy *et al*. 1998; Gotoh *et al*. 2009; Matsuda *et al*. 2013). Therefore, DNA barcoding techniques are used as a complimentary tool to facilitate species identification (Bennur *et al*. 2015). DNA barcoding supports the conventional descriptions of new mite species (the morphological approach) and helps detect cryptic species (Skoracka *et al*. 2012) or validate the species status within populations of mites from different hosts or from various niches (Glowska *et al*. 2014; Dabert *et al*. 2015). The mitochondrial COI gene is the most extensively sequenced gene region, and is an established DNA barcoding marker for species delimitation (Pentinsaari *et al*. 2016).

In this study, we determined the COI sequences of two species of the family Tetranychidae (*Oligonychus afrasiaticus* and *O. ununguis*) and a species of the family Tenuipalpidae (*Aegyptobia beglarovi*) collected from Iran. These are the first barcodes of tetranychoid mites from the country.

Oligonychus afrasiaticus specimens were collected from the date fruit in Bam County (Kerman province), while *O. ununguis* and *A. beglarovi* were collected from the leaves of *Cupressus sempervirens* (Cupressaceae) in Kerman province in 2020. Microscope slides were prepared for morphological identification. DNA was extracted from individual female specimens of each species using method described by Anderson and Fuchs (1998) with some modifications as follows: each mite was removed from ethanol and after removing the alcohol was transferred to a microtube containing 15 µl of 2x lysis buffer (120 µg/ml proteinase K, 0.1 M KCl, 0.02 M TRIS -HCl pH 8.3,

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5 mM MgCl2, 0.9% Tween 20, 0.9% NP40 and 0.02% gelatin). Then the mite was crushed using a sterile plastic pestle in a 1.5 mL microcentrifuge tube bottom. To facilitate cell disruption after vortexing and centrifuging, the samples were incubated at 65 ℃ for three hours, then at 95 ℃ for 15 min, and 5 min at 4 ℃. Finally, the samples were centrifuged at 14000 rpm for 5 min, then DNA solution was collected in a 0.5 mL tube and was stored at −20 ℃ for subsequent PCR assays.

A region of the COI gene was amplified by PCR using universal COI primers LCO1490: 5'-ggtcaacaaatcataaagatattgg-3' and HC02198: 5'-taaacttcagggtgaccaaaaaatca-3' described by Folmer *et al*. (1994). All PCR products were purified using MG Gel extraction SV kit (Macrogen, Korea) and direct Sanger sequencing for both directions by Macrogen Inc. (South Korea). Obtained sequences were compared to sequences in the GenBank using the BLAST function. In this study, the nucleotide sequence of COI of *O. afrasiaticus* was most similar to orthologous sequences from *Tetranychus neocaledonicus* (JX075251) and *Oligonychus* sp. (MT472382), with identity of 87.78% and 87.03%, respectively. The COI sequence of *O. ununguis* showed 92.18% identity with *Oligonychus* sp. (KM826727) followed by 91.95% identity with Tetranychidae sp. (JX834351). Our COI fragment is at the moment the first record in GenBank for species *A. beglarovi*. The COI sequences of *A. beglarovi* was most similar to orthologous sequences from *Raoiella* sp. (JF928424) and *Brevipalpus obovatus* (MG458800), with identities of 85.89% and 83.23%, respectively.

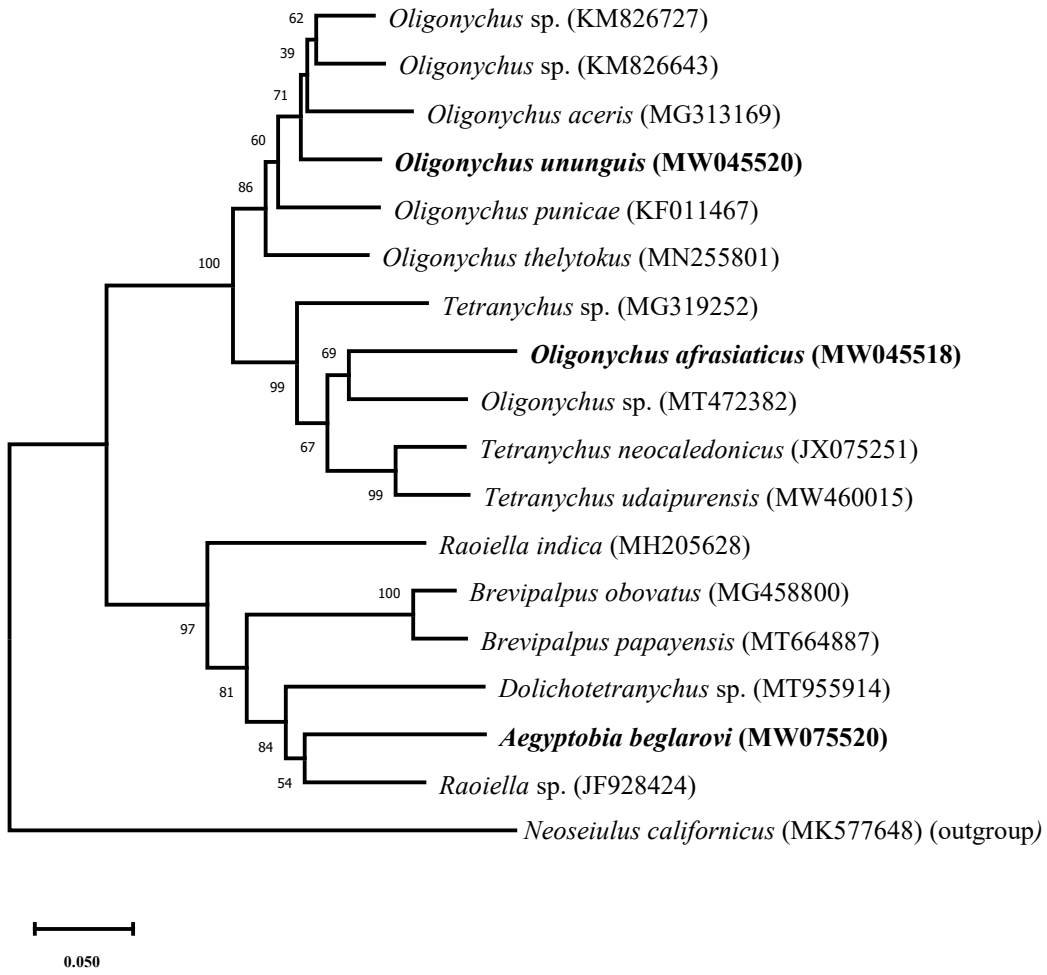

Figure 1. Neighbor-Joining tree of the COI sequences using Tamura-Nei model. Scale bar represents number of nucleotide substitutions per site. Bootstrap was 1000 replicates. Numbers on nodes represent bootstrap values.

After aligning and trimming, the final length of the COI alignment was 657 bp. The phylogenetic tree was generated by the neighbor-joining method based on Tamura-Nei model using

Software MEGA X (Kumar *et al*. 2018). *Neoseiulus californicus* (MK577648) was used as an outgroup in the phylogenetic analysis (Fig. 1).

Our sequences have been deposited in GenBank with accession numbers MW045520 (*O. ununguis*), MW045518 (*O. afrasiaticus*), and MW075520 (*A. beglarovi*).

The phylogenetic tree constructed on the basis of COI gene sequence agrees with morphological taxonomy and can accurately describe the phylogenetic relationships among Tetranychidae and Tenuipalpidae (Fig. 1). In pest management strategies, early detection and identification of pests are really important steps to take necessary control action. Though morphologically adult male can be identified by male genitalia, it is hard to identify the juveniles and females correctly on the basis of morphological features at species level. In this regards, molecular identification can be a great supporting tool for identifying any stages and sexes of spider mites (Jahan *et al*. 2013).

There was no sequence information for *Aegyptobia* in the GenBank; therefore, the sequence information reported in this study will be useful for molecular taxonomy of this species. The genus *Aegyptobia* is the third largest genus of the Tenuipalpidae. Some species of this genus have previously been reported from Iran among which *A. beglarovi* is one of the dominant species in the country (Farzan *et al*. 2012; Farzan and Asadi 2013, 2015; Khanjani *et al*. 2013). We hope that our work, together with similar efforts, could provide the platform for a uniform, accurate, practical and easy-to-use method for tetranychoid species identification.

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