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New data on *Cataglyphis nodus* (Brullé, 1833) (Hymenoptera, Formicidae) from Iran

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ABSTRACT. *Cataglyphis nodus* (Brullé) is one of the fairly known ants of Iran. Here, we present new data on a morphotype from Chaharmahal-o Bakhtiari province (Cheri - Mavarz) closely related to *C. nodus*. The examined specimens were morphologically had very similar characters with the original description of *C. nodus*. In order to explore the phylogenetic position of Iranian specimens, a fragment of the mitochondrial *Cytochrome c oxidase* subunit 1 gene (COI) was used. The Iranian specimens belong to a clade containing *C. holgerseni* Collingwood & Agosti; *C. niger* (André), *C. nodus* and *C. savignyi* (Dufour). Surprisingly, it was clustered with *C. holgerseni*, indicating existence of a possibly undescribed species. The final taxonomic conclusion and description of the possible new species postponed until examination of the type specimens of both *C. nodus* and *C. holgerseni*.

Key words: COI sequences, morphology, cryptic species

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INTRODUCTION

The ant genus *Cataglyphis* Foerster, 1850, is widely distributed in the semi-deserts and deserts of the Old world, from Central Asia to North Africa (Guénard et al., 2017). It was also recorded from other arid habitats, such as high altitude, mountain steppes, and forest steppes (Agosti, 1990; Brown, 2000). This genus currently contain 117 described species and subspecies (Bolton, 2022) and are among the commonest ants of the in various arid ecosystems, where the colonies construct crater nests on the ground. Workers forage mainly for dead insects and other invertebrates (Agosti, 1990; Collingwood & Agosti 1996; Brown, 2000; Lenoir et al., 2010). Some *Cataglyphis* species are also reported as flower pollinators and contributors to myrmecochorous seed dispersal (Wehner, 2020).

Until now, more than 270 species belonging to 37 genera of five subfamilies of the Formicidae (Formicinae, Myrmicinae, Ponerinae, Dolichoderinae and Dorylinae) are reported from Iran (Guénard et al., 2017; Bolton, 2022). As a result of historical events and predominance in open and arid habitats, Iran hosts one of the highest numbers of *Cataglyphis* species, so that, the genus with 36 valid species, represents more than about 14% of Iran's ant biodiversity (Khalili-Moghadam et al., 2021; Salata et al., 2021). Nevertheless, it seems that some of previous identifications require revision. The classification

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and nomenclature of ants in the genus *Cataglyphis* is notoriously challenging due mainly to a high intraspecific variability among local populations (Knaden et al., 2012). There has been disagreement with regard to the taxonomic position of some nominal taxa of the genus *Cataglyphis*, despite several studies based on morphology (Agosti, 1990), exocrine product chemistry (Dahbi et al., 2008), and molecular markers (Aron et al., 2016; Knaden et al., 2012).

Cataglyphis nodus (Brullé, 1833) originally described from Greece (Brullé, 1833:326), then reported from other parts of the world such as North Africa, Arabian Peninsula, central and southwest Asia, former U.S.S.R., Central and Southeast Europe (https://antmaps.org). This is a common ants species of Iran and was reported from various areas, including Alborz, Tehran, Bushehr (Paknia et al., 2008), Ardabil, Lorestan, North Khorasan, Qom (Pashaei Rad et al., 2018), Chaharmahal-o Bakhtiari (Khalili-Moghadam et al., 2019), Khorasan Razavi (Mortazavi et al., 2015) and Khuzestan (Shiran et al., 2013). However, there is no a comparative study on its different populations. Khalili-Moghadam et al. (2019) mentioned that Iranian samples are generally darker coloured and larger than typical populations from the type locality (Greece). The main question is whether these darker populations represent a separate morphotype or even a separate species. The comparison of male genitalia between different localities was not possible, because we haven't the male specimens. So, here, we used a fragment of *Cytochrome c oxidase* subunit I gene (COI) to investigate phylogenetic positon of Iranian sample of *C. nodus* among other related species.

MATERIAL AND METHODS

We collected specimens from six nests at altitudes above 1515 m in a mountainous area of Cheri (Mavarz - 32°08'51"N, 50°06'20"E - Fig. 1), Chaharmahal-o Bakhtiari province. The dominant sampling method was a direct collecting by hand. Individual specimens were collected on the ground or from nests under stones. All specimens (25 specimens) were preserved in 75% EtOH. The specimens were deposited in the Insect Collection of the Plant Protection Department (Shahrekord University). "*A priori*" identity of specimens were determined based on the relevant morphological characters (identification key by Radchenko, 1998) and by comparison with photographs of the type specimen (www.AntWeb.org - CASENT0903291) and also confirmed by Prof. Lech Borowiec (Department of Biodiversity and Evolutionary Taxonomy, University of Wrocław, Poland). Genomic DNA was extracted from the legs of two individual specimens (same locality/different nests) using the slightly modified standard salt-out method (Green & Sambrook, 2012). A 638 base pair fragment of the *Cytochrome c oxidase* subunit I gene (*COI*) was PCR-amplified using the modified primer pair LCO1490-JJ and HCO2198-JJ under standard conditions (Astrin & Stuben, 2008). The sequences produced in this study (ON381727, ON381728) were combined with previously published COI sequences of *Cataglyphis nodus* as well as other *Cataglyphis* species retrieved from NCBI, trimmed and aligned using BioEdit 7.1.3 (Hall, 1999). Based upon current phylogenetic studies on *Cataglyphis*, *Proformica nasuta* (KU749618, KU749624) was chosen as outgroup taxon (e. g. Kuhn et al. 2020). No evidence suggesting the existence of pseudo genes was found in the final alignment. Pairwise genetic distances with the Kimura-2-Parameter (K2P) correction was estimated by MEGA v11.0.10 (Tamura et al., 2021). The dataset used for the phylogenetic analyses consisted of 635 nucleotides, there were 493 invariable sites and 142 polymorphic sites, of which 104 sites were phylogenetically informative and 38 were singletons. Pairwise genetic distances between five species of *Cataglyphis* and Iranian specimens of *C. nodus* as well as outgroup taxon are shown in Table 1. For Bayesian inference (BI), the best-fit codon-partitioning schemes and the best-fit substitution models were selected using PartitionFinder2 (Lanfear et al., 2016) using the greedy algorithm and the Akaike information criterion (AIC). The BI analysis was performed using MrBayes 3.2.7 (Ronquist et al., 2012), with K81UF+G (1st), GTR+G (2nd) and TIM+I (3rd); as the best-fit model of evolution for each partition. The analyses were run for 10^{10} generations with a sample frequency of every 1000 generations. We conservatively discarded the first 25% of trees as burn-in.

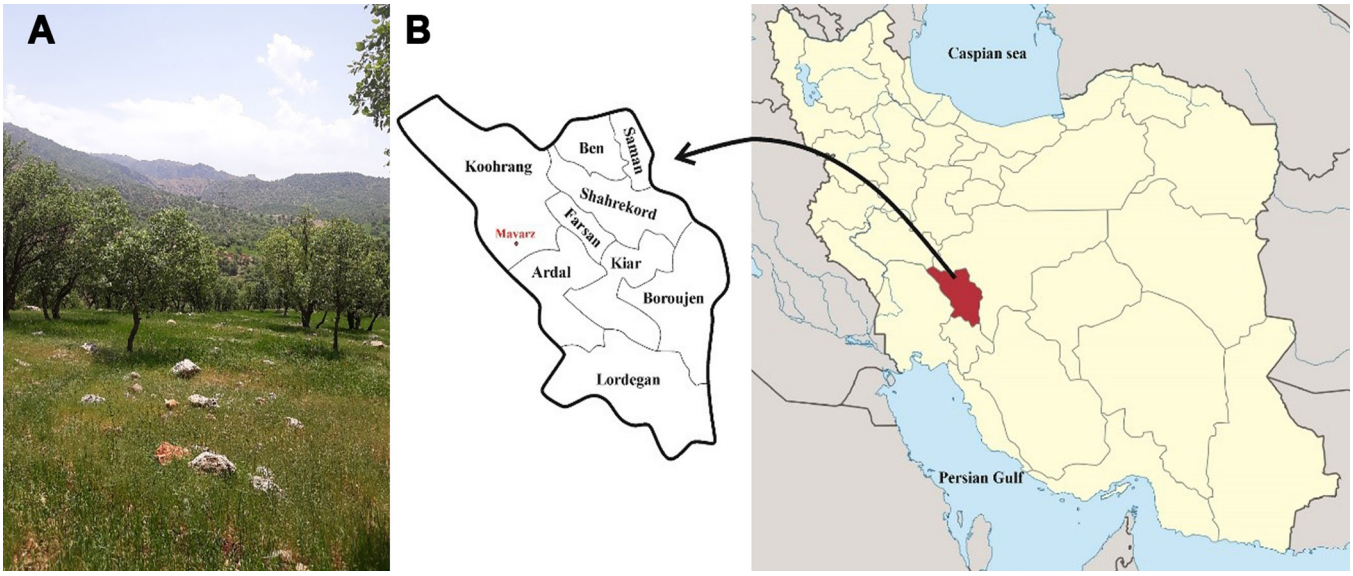

Figure 1. The locality, where the specimens of *Cataglyphis* c.f. *nodus* were collected. **A.** Habitat in Mavarz. **B.** Map of Chaharmahal-o Bakhtiari province (Iran).

RESULTS

Taxonomic Hierarchy

Class Insecta Linnaeus, 1758

Order Hymenoptera Linnaeus, 1758

Superfamily Vespoidea Latreille, 1802

Family Formicidae Latreille, 1802

Subfamily Formicinae Latreille, 1802

Genus *Cataglyphis* Foerster, 1850

Cataglyphis Foerster, 1850. Type species: *Cataglyphis fairmairei* Foerster, 1850:493 (junior synonym of *Formica bicolor* Fabricius, 1793:356; by monotypy).

Cataglyphis c.f. *nodus* (Brullé, 1833) (Fig. 2)

Diagnosis (Workers)**.** Morphologically, the examined specimens were most similar to the description of *Cataglyphis nodus* (Brullé, 1833) with the following characters: body bicoloured, head and thorax with different shades of red sometimes red colouration strongly reduced, abdomen black-brown to black, body with scarce erect setae, mesonotum not raised above pronotum, propodeum broadly rounded, petiole node-shaped, more or less rounded in dorsal view, first segment of flagellomere long, distinctly longer than second, third maxillary palpomere normal (not flattened), with shorter erect hairs more than 1.5 time as long as maximum palpomere width.

Molecular analysis. Our genetic analyses reveal that the minimum genetic distance between pairs of species referred to *C. niger* and Iranian specimens (*C. nodus*) was 1.61%, while the maximum divergence between *C. holgerseni* and *C. isis* was 8.53% (Table 1 and Fig. 3). The tree inferred from the Bayesian analysis showing position of Iranian specimens is in Figure 3. The Iranian specimens belong to a clade containing *C. holgerseni, C. niger, C. nodus* and *C. savignyi*. The Iranian specimens clustered with *C. holgerseni,* although lower posterior probability support (pp=0.65) were detected in the relationship between *C. holgerseni* and Iranian specimens (Fig. 3).

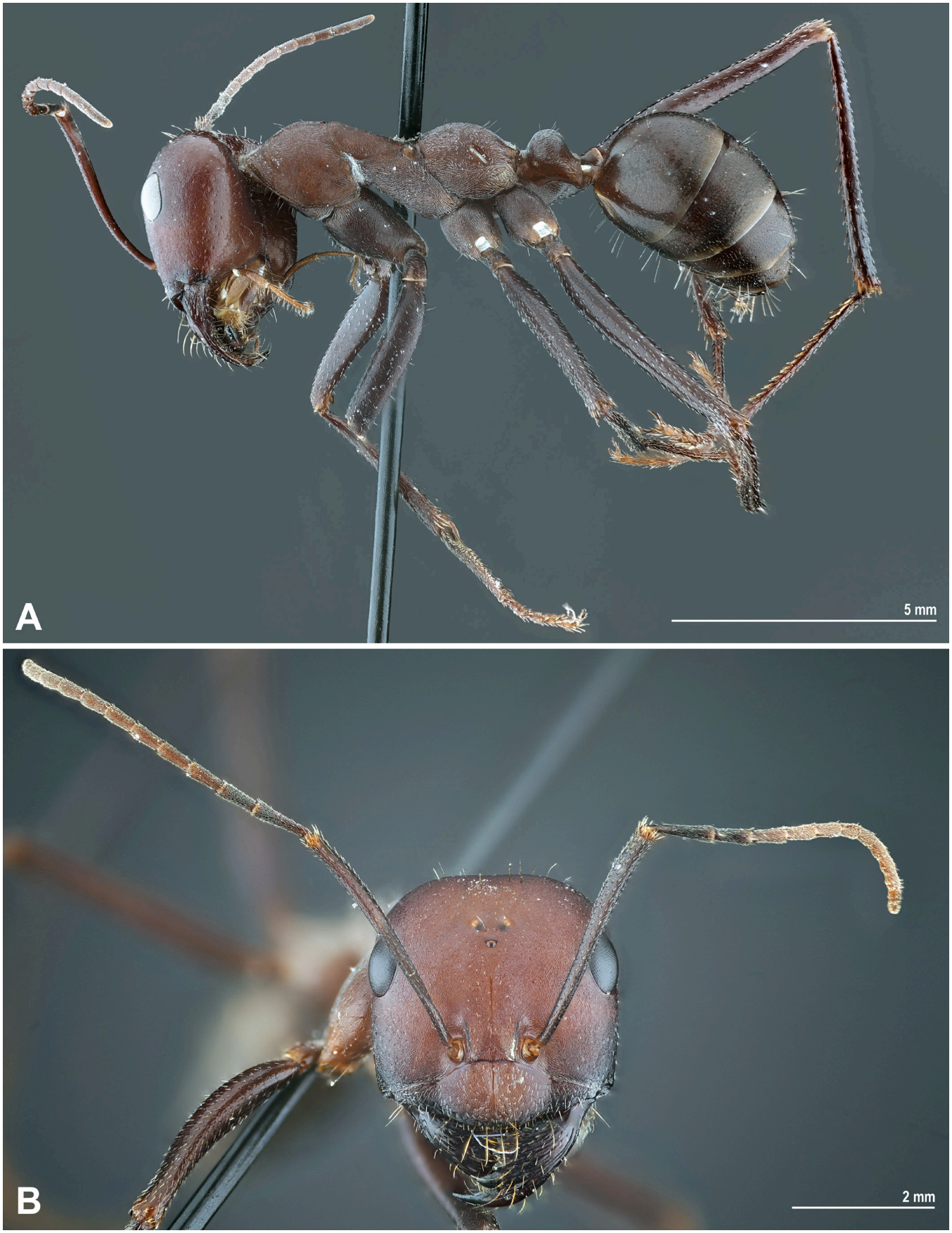

Figure 2. *Cataglyphis* c.f. *nodus* (Brullé, 1833) from Iran, worker. **A.** General habitus, lateral view; **B.** head.

Table 1. Corrected genetic distances (Kimura 2 parameters) among the species of *Cataglyphis* and *Proformica nasuta* as out-group taxon.

Taxa	*Proformica nasuta*	*C. nodus* (Iran)	*C. holgerseni*	*C. isis*	*C. niger*	*C. nodus*
Proformica nasuta	-	-	-	-	-	-
***C. nodus* (Iran)**	0.1528	-	-	-	-	-
C. holgerseni	0.1578	0.0423	-	-	-	-
C. isis	0.1583	0.0833	0.0853	-	-	-
C. niger	0.1499	0.0381	0.0272	0.0707	-	-
C. nodus	0.1575	0.0409	0.0297	0.0817	0.0161	-
C. savignyi	0.1544	0.0430	0.0247	0.0824	0.0205	0.0193

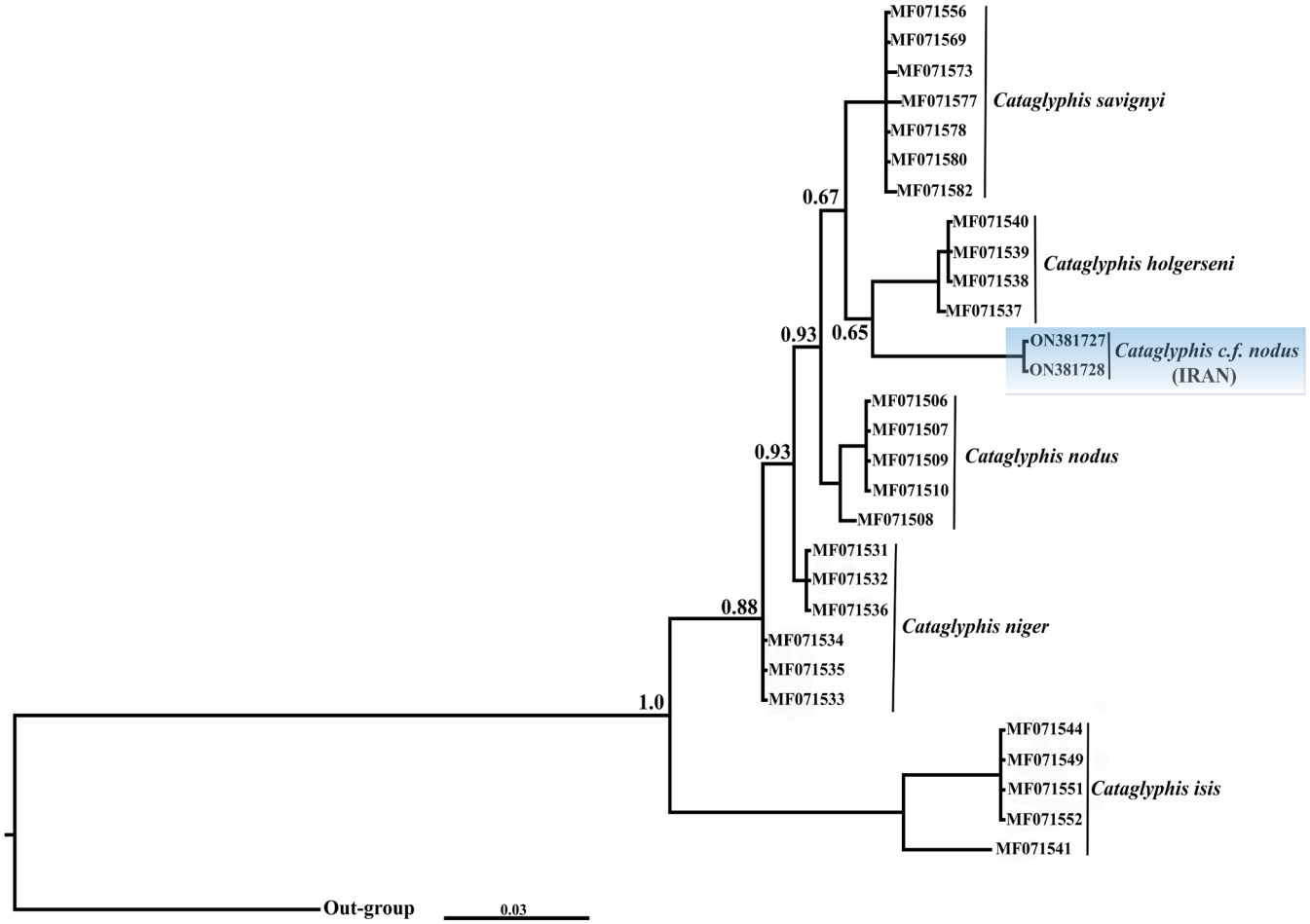

Figure 3. Bayesian Inference (BI) gene tree of *Cataglyphis* inferred from 638 base pairs of the COI sequence. The blue box represents the individuals from Iran. Other samples were extracted from NCBI.

DISCUSSION

The specimens of *Cataglyphis* c.f. *nodus* from Iran are generally darker in color and larger than specimens collected from type locality "Greece" (Lech Borowiec – per. comm.) (Table 2). The comparison of male genitalia between different localities was not possible, because we haven't the male specimens.

Table 2. Comparison of the morphological characters between *C. nodus*; *C. holgerseni* and Iranian specimens.

Characters	*Cataglyphis nodus*	*Cataglyphis holgerseni*	Iranian specimens
General body coloration	Bicoloured (head and thorax red, abdomen black-brown to black)	uniformly black	Bicoloured (head and thorax brown, abdomen black)
Body length	12 mm	6.8–13.2 mm*	13 mm
Propodeum	moderately convex	high-raised and massive	moderately convex
Setation on propodeum	with erect setae	without erect setae	with erect setae
Dorsal and descending part of propodeum	broadly rounded	rounded right angle	broadly rounded
Mesonotum	not raised above pronotum	raised above pronotum	not raised above pronotum

* Specimens from the type locality (Ionescu & Eyer, 2016)

Genetic markers represent a powerful tool for studies on intraspecific variability and made it possible to unravel cryptic species (genetically distinct but morphologically very similar species) in many taxonomic groups, including ants (e.g. Schär et al., 2022). The genetic differentiation (KP2) between Iranian specimens of *C. nodus* and other related congers (*C. holgerseni, C. niger, C. nodus, C. savignyi*) is more than 3.8% (Table 1), a value lying in above of the range of cut-off values (2–3%) used to consider clades of ants as distinct species (Ng'endo et al., 2013). The conflict between molecular and morphological data mostly rooted from the morphological characters which show homoplastic evolution. In this manner, morphological data may mislead phylogenetic and taxonomic interpretations (Wiens et al., 2003). Cryptic species are a major challenge for taxonomy in ants. Their reliable identification requires the application of elaborate methods such as genetic analyses. Complications caused by intraspecific polymorphism and interspecific hybridization necessitate integrating some methods in multi-source approaches. Cryptic biodiversity is not evenly distributed within the evaluated ant genera (Seifert, 2009). Concerning the ongoing discussion on the future roles of morphology and molecular in systematics we call for a combination of both whenever possible. Considering current genetic distance gap as well as phylogenetic potion (Fig. 3), the Iranian specimens of *C. nodus* studied here could be a putative new species, if have enough divergence from type specimens of *C. nodus*. But, the final taxonomic conclusion and description of the possible new species postponed until examination of the type specimens of both *C. nodus* (Greece) and *C. holgerseni* Collingwood & Agosti (Israel). So, we consider Iranian specimens as *C. nodus* untill clarification of its identity through examination of specimens from the other parts of country.

In conclusion, further fieldwork is needed to include more Iranian specimens of *Cataglyphis nodus* from their entire distribution range as well as specimens from Greece (as type specimens) and neighboring countries. An integrative approach using morphological, molecular and ecological data could shed light on taxonomic and evolutionary history of this species.

AUTHOR'S CONTRIBUTION

The authors confirm contribution in the paper as follows: A. Khalili-Moghadam: Conceiving the idea, collection of specimens, identification, preparing and revising the manuscript; H. Oraie: Conceiving the idea, preparing the manuscript, analysis and interpretation of the molecular data; both authors approved the final version of the manuscript.

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AVAILABILITY OF DATA AND MATERIAL

The specimens listed in this study are deposted in deposited in the Insect Collection of the Plant Protection Department, Shahrekord University, available from the curator, upon request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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یافته‌های جدید از *Cataglyphis nodus* (Brullé, 1833) (Hymenoptera, Formicidae) در ایران

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چکیـده: مورچه *Cataglyphis nodus* (Brullé, 1833) یکی از گونه‌های نسبتا شناخته شده ایران است. در این مقاله داده‌های جدیدی از یک مورفوتایپ از استان چهارمحال و بختیاری (چری- مورز) که نزدیک به *C. nodus* است، ارایه شد. نمونه‌های بررسی شده از نظر ریخت‌شناسی دارای خصوصیات بسیار مشابه با توصیف اصلی *C. nodus* بودند. به منظور بررسی موقعیت فیلوژنتیکی نمونه‌های ایرانی، از قطعه‌ای از زیرواحد ۱ ژن سیتوکروم اکسیداز C میتوکندریایی (COI) استفاده شد. نمونه‌های ایرانی متعلق در شاخه‌ای از تبارنمای فیلوژنتیک، شامل گونه‌های *C. holgerseni* Collingwood & Agosti؛ *C. niger* (André)؛ *C. nodus* و *C. savignyi* (Dufour) قرار گرفتند. نکته قابل توجه در تبارنمای تولید شده، هم‌شاخه شدن جمعیت ایرانی با گونه *C. holgerseni* بود. این موضوع، نشان‌دهنده وجود یک گونهٔ احتمالا توصیف نشده است. نتیجه‌گیری طبقه‌بندی نهایی و توصیف گونه جدید احتمالی تا بررسی نمونه‌های مرجع (Type) گونه‌های *C. nodus* و *C. holgerseni* به تعویق افتاد.

واژگـان کلیدی: توالی ژن سیتوکروم اکسیداز ۱، ریخت‌شناسی، گونه مخفی