



Phylogenetic affinities of *Cataglyphis bazoftensis* (Hymenoptera: Formicidae) from Iran

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Abstract. The ant *Cataglyphis bazoftensis* Khalili-Moghadam Salata & Borowiec, 2021 has been recently described in Iran. Here, new materials of *C. bazoftensis* were collected from five new localities in Chaharmahal and Bakhtiari province, Iran. All the specimens examined were morphologically consistent with the description of *C. bazoftensis*. At the molecular level, based on phylogenetic trees obtained from the mitochondrial Cytochrome C oxidase subunit I gene (COI), *C. bazoftensis* appears as a sister taxon of *Cataglyphis kurdistanica* Pisarski, 1965, separated from each other by a genetic distance of 9.79 %.

Keywords: ant, *Cataglyphis*, Iran, COI sequences, phylogeny

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Introduction

Ants are one of the most prosperous living beings on the planet (Hölldobler & Wilson, 1990). The genus *Cataglyphis* Förster, 1850 shows the highest diversity in arid ecosystems. It is a moderately large ant genus comprising 99 species and 18 valid subspecies divided into several species groups (Agosti, 1990; Collingwood & Agosti, 1996; Brown, 2000; Bolton, 2022). Many *Cataglyphis* species are distinctly polymorphic, which causes challenges in their determination and makes it necessary to prepare new species descriptions based on nest samples (Khalili-Moghadam & Oraie, 2023). Furthermore, genetic investigations have indicated the existence of cryptic species and a high level of hybridization (Ionescu & Eyer, 2016; Eyer *et al.*, 2017). Thus, the genus *Cataglyphis* with several species groups, has some confusing taxonomic problems.

One such problem concerns the *Cataglyphis altisquamis* species group, distributed through a wide geographic range extending from Portugal and Morocco to Central Asia. Workers from this species group are characterized by the following combination of characteristics: relatively large (Weber's length up to 5 mm); body dull, uniformly yellow-black to black or bicolored with black gaster, petiole cuneiform or pseudo-nodiform, head finely reticulate with punctulate frons (Agosti, 1990; Radchenko, 1998; Khalili-Moghadam *et al.*, 2021; Guénard *et al.*, 2017).

A recently described species within the *Cataglyphis altisquamis* species group is *Cataglyphis bazoftensis* from Iran (Khalili-Moghadam *et al.*, 2021). According to morphological data, *C. bazoftensis* bears a striking resemblance to *Cataglyphis kurdistanica* Pisarski, 1965.



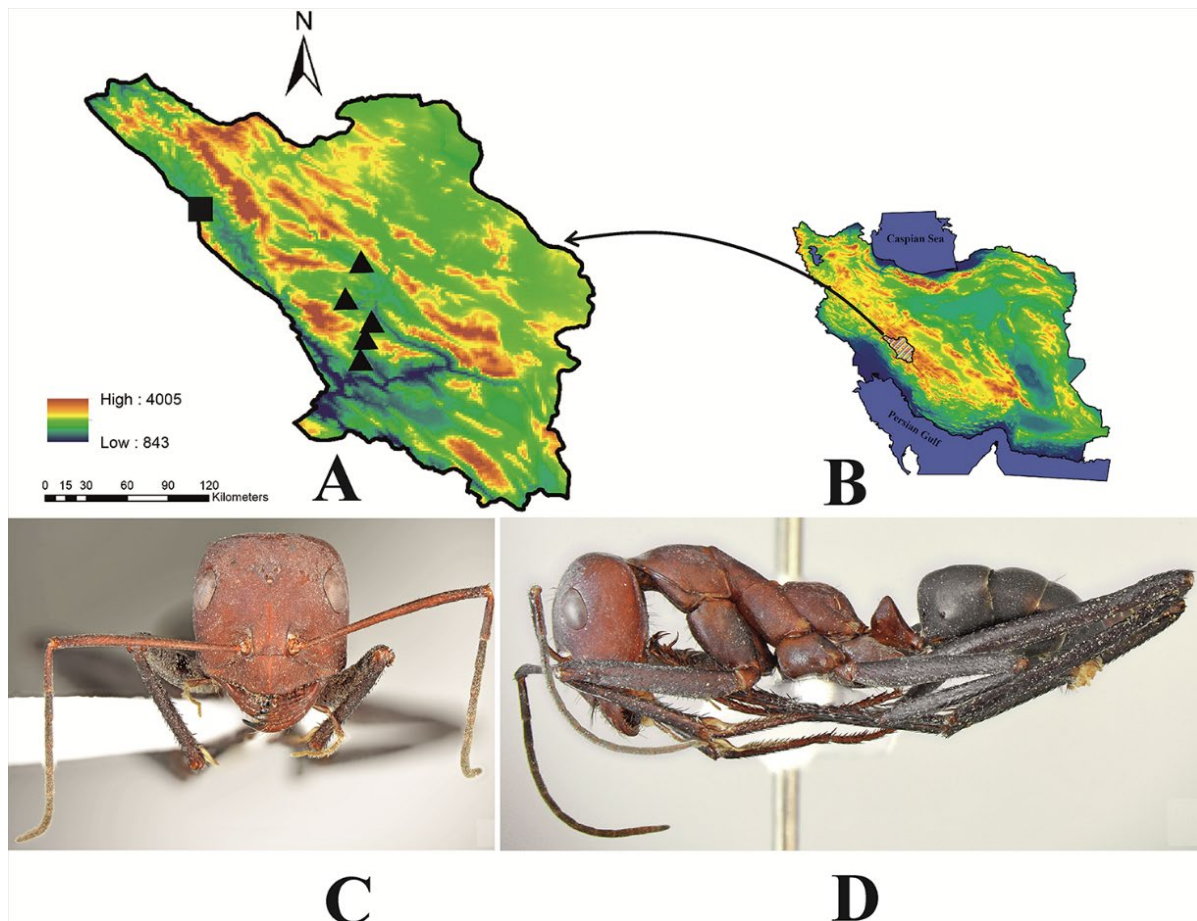


Fig. 1. Distribution of *Cataglyphis bazoftensis* Khalili-Moghadam, Salata & Borowiec, 2021 in Iran. Square shows the type locality and the triangles refer to new records. A. map of Chaharmahal va Bakhtiari Province, B. map of Iran, C. Head in full-face view; D. lateral view of the body (major worker).

Nevertheless, a significant differentiation between the two species is the presence of a soldier caste in *C. kurdistanica*, which is absent in *C. bazoftensis*. Additionally, *C. kurdistanica* exhibits long, black, and erect setae along the posterior margin of the head and propodeum, which is not observed in *C. bazoftensis* (Khalili-Moghadam *et al.*, 2021).

Here, we explored the phylogenetic position of *C. bazoftensis* within *C. alitisquamis* species group using a fragment of the Cytochrome C oxidase subunit I gene (*COI*). A 642 base pair fragment of *COI* was used which corresponds to positions 2354-2995 of the *Camponotus atrox* mtDNA sequence (accession numbers: KT159775). In addition, we provide new evidences for the occurrence of *C. bazoftensis* outside of its type locality (Koohrang, Bazoft region, 32.2969 / 49.9358) in Iran.

Materials and methods

During a field study on ant's biodiversity in the Chaharmahal and Bakhtiari Province of Iran in 2021, ten new specimens of *Cataglyphis bazoftensis* were collected from its type locality (Koohrang, Bazoft region, 32.2969 / 49.9358); fifteen from Kiar county near Firoz-Abad (26 IIX 2021, 31.86861/ 50.58888, 2051 m); Eslam-Abad (17 IIX 2021, 32.09777/ 50.54944, 1898 m), and Ardal county near Lirabi (14 IX 2021, 31.95666/ 50.48777, 1948 m) and Gandomkar (09 X 2021, 31.80472/ 50.56416, 1887-1941 m) around Karoon 4 Dam (09 X 2021, 31.72444/ 50.54527, 1789 m) (Fig. 1 & 2).

The specimens were preserved in 75 % ethanol and deposited in the Insect collection, Department of Plant Protection, Agricultural College, Shahrekord University, Shahrekord, Iran. Identification of the specimens followed relevant morphological keys such as Radchenko (1998), Khalili-Moghadam *et al.* (2021) and compared with materials (Paratypes specimens) preserved in the Department of Plant Protection.

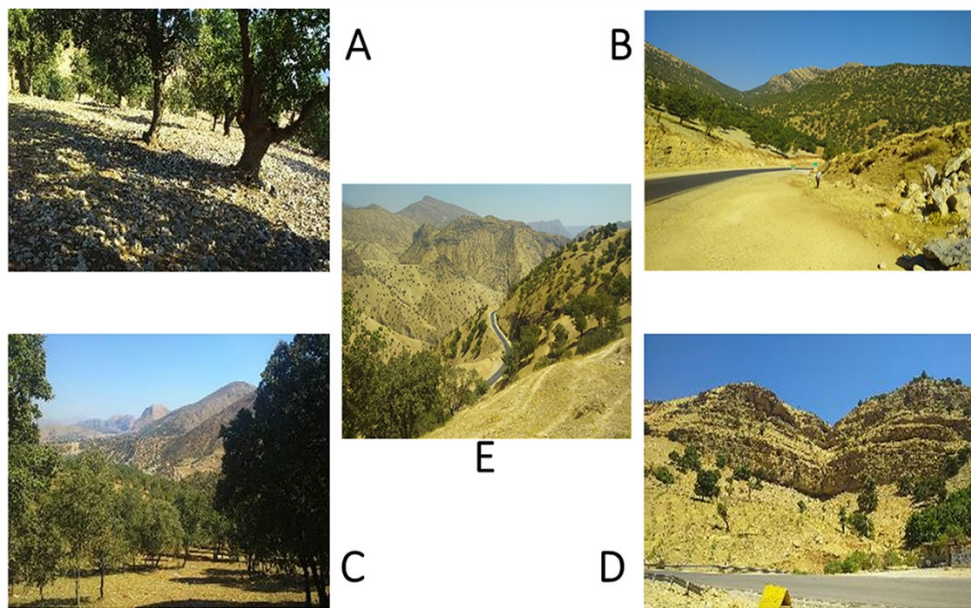


Fig. 2. Habitat of new records of *Cataglyphis bazoftensis* in Chaharmahal and Bakhtiari Province, Iran (Kiar county, A: Firoz-Abad, B: Eslam-Abad; Ardal county, C: Lirabi, D: Gandomkar, F: around Karoon 4 Dam)

To evaluate the phylogenetic relationships among *Cataglyphis bazoftensis* and other members of the *C. alitisquamis* species group, we conducted an analysis of the partial sequences of Cytochrome C oxidase subunit I gene (COI) gene. For this purpose, we used three specimens of *C. bazoftensis* from its type locality. We extracted DNA from the ants' legs using the salting-out method (Green & Sambrook, 2012). Amplification conditions for the partial mitochondrial COI gene with the primers ChR58 and ChF746 (Darras & Aron, 2015) consisted of 36 thermal cycles, as Kuhn *et al.* (2020) described.

The amplification products were sequenced on an automated sequencer ABI 3730XL (GeneAzma Genetic Group, Isfahan, Iran) according to standard protocols.

Alignments were first conducted using Clustal X 1.81 (Thompson *et al.*, 1997) in MEGA v11.0.10 (Tamura *et al.*, 2021) with default parameters, and the alignment was checked by eye and manually revised when necessary. The alignment was deposited in TreeBASE (www.treebase.org) with accession no. S30431. We calculated pairwise divergence between species using the uncorrected p-distance implemented in MEGA v11.0.10 (Tamura *et al.*, 2021). Phylogenetic relationships among species were assessed with Maximum Likelihood (ML) and Bayesian Inference (BI) criteria. For this, the data set partitioned by codon positions and 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 ML analysis was conducted in IQ-TREE (Nguyen *et al.*, 2015). We performed 1000 ultrafast bootstraps to assess heuristic support for inferred clades, and we considered support values (UFboot) ≥ 95 to be strong support for monophyly (Minh *et al.*, 2013). Bayesian Inference analyses were conducted using Mr.Bayes 3.2.7 (Ronquist *et al.*, 2012). The analyses were run for 20^{10} generations with a sampling frequency of every 1.000 generations. We conservatively discarded the first 25% of trees as burn-in. As outgroups, we used one species of *Bajcaridris sp.* (KX118481) and one distantly related species of *Camponotus japonicus* (NC_061037, OK509076) retrieved from NCBI (Agosti, 1990; Knaden *et al.*, 2012; Aron, *et al.*, 2016).

Results

Taxonomic Hierarchy

Class Insecta Linnaeus, 1758

Order Hymenoptera Linnaeus, 1758

Family Formicidae Latreille, 1809

Subfamily Formicinae Latreille, 1809

Genus *Cataglyphis* Foerster, 1850

Type species

Cataglyphis fairmairei Förster, 1850: 493 (junior synonym of *Formica bicolor* Fabricius, 1793: 356; by monotype)

Cataglyphis bazoftensis

Khalili-Moghadam, Salata & Borowiec, 2021

Diagnosis

Morphologically, the newly found specimens were consistent with the description of the type-specimen of *Cataglyphis bazoftensis* with the following combination of characters in the worker caste: body bicolored, head, mesosoma and gaster brown to black, if red-brown then posterior margin of the head without black, erect setae; mesonotum in major workers distinctly bicolored, head, mesosoma, and petiole reddish; petiole in both, major and minor workers conical; the surface of femora and tibiae covered with black, thick, and decumbent setae; soldier caste absent (Khalili-Moghadam *et al.*, 2021).

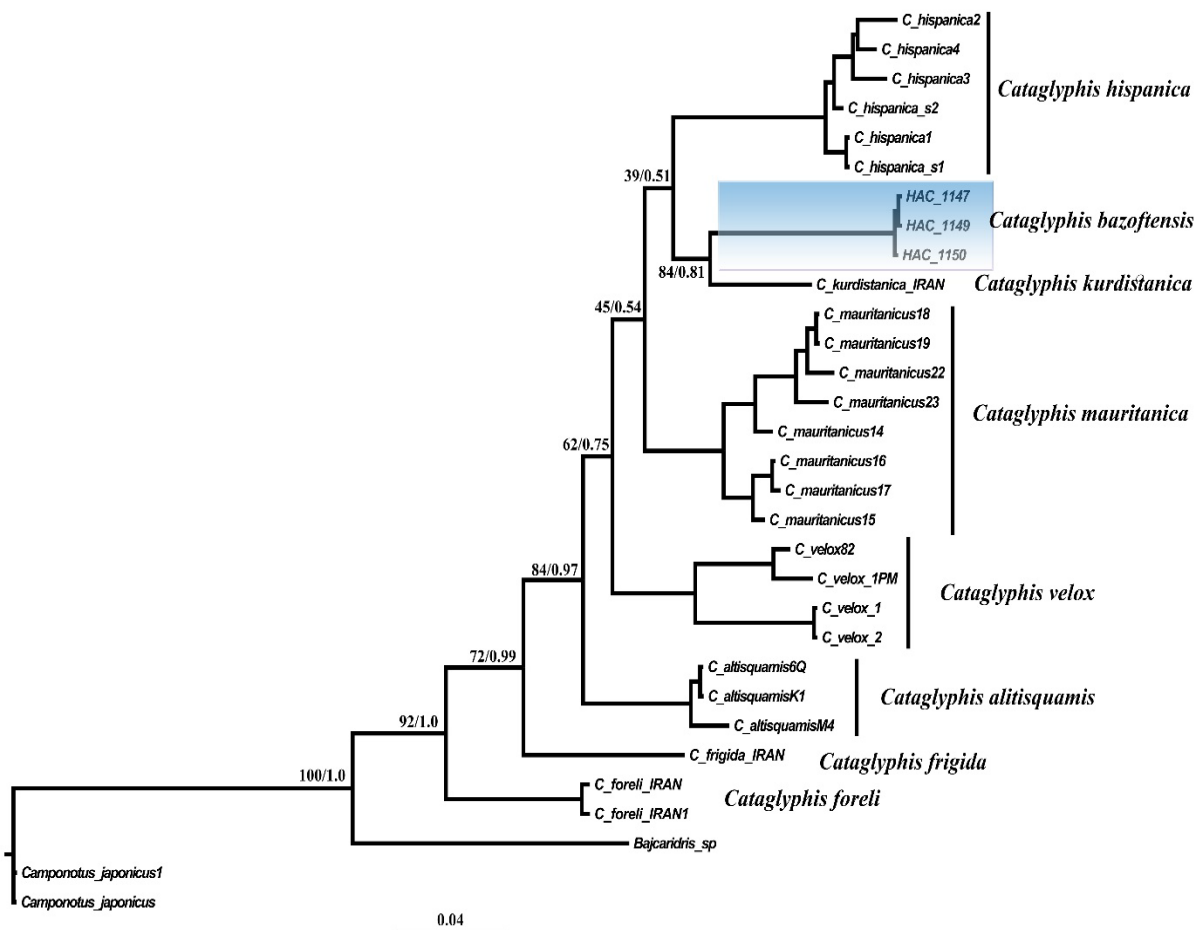


Fig. 3. Phylogeny estimate inferred from 520 base pairs of the COI sequences. The tree is a 50 % majority-rule consensus generated by Mr Bayes. Posterior probability values and ML bootstrap values are reported along branches. The blue box represents the individuals of *Cataglyphis bazoftensis*. Other samples were downloaded from NCBI (more information is presented in Table 2).

Distribution

Iran, Chaharmahal va Bakhtiari Province, Koohrang county, Bazoft region, 32.2969 / 49.9358), as type locality;

Kiar county, Firoz-Abad village (26 IIX 2021, 31.86861/ 50.58888, 2051 m), Eslam-Abad village (17 IIX 2021, 32.09777/ 50.54944, 1898 m); Ardal county, Lirabi village (14 IX 2021, 31.95666/ 50.48777, 1948 m), Gandomkar village (09 X 2021, 31.80472/ 50.56416, 1887-1941 m) and around Karoon 4 Dam (09 X 2021, 31.72444/ 50.54527, 1789 m) as new locality (Fig. 2). The new locality is about 80 km South East away from the type locality. Plant cover and climate conditions are similar (inside a deciduous oak forest surrounded by a grazing area). All collecting sites are warmer than other regions of Chaharmahal and Bakhtiari Province.

Table 1- List of published sequences of *Cataglyphis altisquamis* species group used in this study along with their localities and NCBI accession numbers.

Species	Abbreviated names on the tree	Locality	Gene bank Accession Number
<i>C.kurdistanica</i>	C_kurdistanica_IRAN	Iran	MK810365
<i>C.frigida</i>	C_frigida_IRAN	Iran	MK810369
<i>C.foreli</i>	C_foreli_IRAN1	Iran	MK810356
<i>C.foreli</i>	C_foreli_IRAN	Iran	MK810357
<i>C.mauritanica</i>	C_mauritanicus22	Tunisia	EF139795
<i>C.mauritanica</i>	C_mauritanicus19	Tunisia	EF139793
<i>C.mauritanica</i>	C_mauritanicus18	Tunisia	EF139792
<i>C.mauritanica</i>	C_mauritanicus14	Tunisia	EF139788
<i>C.mauritanica</i>	C_mauritanicus17	Tunisia	EF139791
<i>C.mauritanica</i>	C_mauritanicus23	Tunisia	EF139796
<i>C.mauritanica</i>	C_mauritanicus15	Tunisia	EF139789
<i>C.mauritanica</i>	C_mauritanicus16	Tunisia	EF139790
<i>C.velox</i>	C_velox82	Spain	MK810379
<i>C.velox</i>	C_velox 2	Spain	MK810371
<i>C.velox</i>	C_velox1	Spain	MK810362
<i>C.velox</i>	C_velox1PM	Spain	KX118483
<i>C.hispanica</i>	C_hispanica1	Spain	MK810360
<i>C.hispanica</i>	C_hispanica2	Spain	KP420173
<i>C.hispanica</i>	C_hispanica3	Spain	KP420174
<i>C.hispanica</i>	C_hispanica4	Spain	KP420175
<i>C.hispanica</i>	C_hispanica4	Spain	MK810360
<i>C.hispanica</i>	C_hispanica4	Spain	MK810361
<i>C.altisquamis</i>	C_altisquamis6Q	Israel	MK810350
<i>C.altisquamis</i>	C_altisquamisK1	Israel	MK810351
<i>C.altisquamis</i>	C_altisquamisM4	Israel	MK810377

Table 2. Uncorrected p-distance among members of *Cataglyphis alitisquamis* species group with special reference of *C. bazoftensis*

	<i>C.bazoftensis</i>	<i>C.kurdistanica</i>	<i>C.mauritanica</i>	<i>C.alitisquamis</i>	<i>C.velox</i>	<i>C.foreli</i>	<i>C.frigida</i>	<i>C.hispanica</i>
<i>C.kurdistanica</i>	0.0979							
<i>C.mauritanica</i>	0.0971	0.0917						
<i>C.alitisquamis</i>	0.1083	0.0938	0.0878					
<i>C.velox</i>	0.1130	0.1021	0.1079	0.1010				
<i>C.foreli</i>	0.1271	0.1188	0.1049	0.1181	0.1318			
<i>C.frigida</i>	0.1292	0.1083	0.1174	0.1097	0.1219	0.1167		
<i>C.hispanica</i>	0.1257	0.1003	0.1167	0.1053	0.1168	0.1299	0.1243	

Molecular analysis

A 642 base pair fragment of the Cytochrome C oxidase subunit I gene (*COI*) was produced in this study (OQ352245 (HAC_1149); OQ352246 (HAC_1147); and OQ352247 (HAC_1150)). They were combined with the *COI* sequences of the *Cataglyphis alitisquamis* species group proposed by Agosti, 1990 and available on the National Center for Biotechnology Information (NCBI) (Table 2).

We trimmed our *COI* sequences to the same length as those from GenBank, so the final data-set for phylogenetic reconstruction comprised sequences of 520 bp for 30 specimens. The final alignment of 520 bp contained 429 invariable (monomorphic) sites and 168 variable (polymorphic) sites, 157 of which were parsimoniously informative. Uncorrected genetic divergence (P-distance) among the species of the outgroup taxon is shown in Table 2. The average genetic divergence between species ranged from 9.71 to 12.92% (Table 1).

The topologies of the BI and ML trees were generally consistent, so only the BI tree is presented in Fig. 3. Our data identified a major clade, including *Cataglyphis alitisquamis* species group, with bootstrap value of 100%. The sister-group relationship of *C. bazoftensis* and *C. kurdistanica* is recovered by medium support (PP 0.81, ML 84%).

Discussion

This research provides preliminary evidence of the existence of *C. bazoftensis* in a significantly large area of the western Chaharmahal and Bakhtiari Province, located more than 80 km away from its type locality (Fig. 1). It is anticipated that the endemic taxon of *C. bazoftensis* will have a broader distribution in the habitats of the western foothills of the Zagros Mountains (Khalili-Moghadam *et al.*, 2021).

According to our observations, *C. bazoftensis* occupies relatively same habitats at elevations below 2000 m within the Chaharmahal and Bakhtiari Province. Nevertheless, additional field work is necessary to determine its precise distribution range and ecological requirements.

This study is the first attempt to resolve the phylogenetic position of one of the Iranian endemic ants (*C. bazoftensis*) among *Cataglyphis alitisquamis* species group using a *COI* sequence. Our phylogenetic reconstruction using two methods (ML and BI) generally produced congruent results, in which all representatives of *Cataglyphis alitisquamis* species group analyzed formed a well-supported group (Fig. 3). Our results are consistent with the classification based on morphological characteristics proposed by Agosti (1990). However, one sequence (MK810369) represented as *Cataglyphis frigida persica* (Emery, 1906) clustered with *Cataglyphis foreli* (Ruzsky, 1903) (Fig. 3 and Table 1). This is in line with Kuhn *et al.* (2020), except that *C. frigida persica* was thought to be a subspecies of the *cursor* group when first described (Agosti, 1990; Paknia *et al.*, 2008).

Our phylogenetic analysis revealed a clade including *Cataglyphis mauritanica* (Emery, 1906), *C. bazoftensis* and *C. kurdistanica* with strong support (ML 90%, pp = 0.91) (Fig. 3). They share similar morphological characteristics such as the shape of petiole, black, thick, and decumbent setae on the femora and tibiae, and sculpture on the abdomen. However, they differ in key features such as body color and posterior margin of the head with or without black, erect setae (Table 3). *Cataglyphis bazoftensis* formed a cluster with *C. kurdistanica* with strong support (ML 90%, pp = 0.91).

Based on morphological characters, *C. bazoftensis* is the most similar to *C. kurdistanica* (Khalili-Moghadam *et al.*, 2021). *Cataglyphis kurdistanica* could be easily distinguished from *C. bazoftensis* by presence of soldier caste, posterior margin of head and propodeum with long, black and erect setae (Khalili-Moghadam *et al.*, 2021). The genetic differentiation (p-distance) between *C. mauritanica*, *C. bazoftensis* and *C. kurdistanica* is more than 9 % (Table 1), a value lying very above the range of cut-off values (2-3%) used to consider clades of ants as distinct species (Hebert *et al.*, 2004; Ng'endo *et al.*, 2013). Table 3 presents a comparison of the morphological characters between different species belonging to the *C. alitisquamis* species group and *C. frigida persica*.

According to the literature, significant morphological distinctions exist between *C. foreli* and *C. frigida persica* (Agosti, 1990). These differences include the uniformly brownish-black body, conical petiole, and densely sculptured, dull abdomen with sparse erect hairs on the posterior margin of the head in *C. foreli*, while *C. frigida persica* exhibits a bicolored body with a uniformly light brown head and mesosoma, a dark brown abdomen, squamiform petiole, and a shining abdomen with smooth or finely sculptured features and no black, erect setae on the posterior margin of the head. These findings suggest further field surveys and a comprehensive morphological study. If conclusive evidence supports the close relationship between *C. frigida persica* and *C. foreli*, it may be necessary to revise Agosti's morphological key.

Table 3. The comparison of main morphological characters among different species of *Cataglyphis alitisquamis* species group.

species	body color	shape of petiole	sculpture on abdomen	black, thick, and decumbent setae on femora and tibiae	solider caste	black, erect setae on posterior margin of head
<i>C. bazoftensis</i>	bicolored, head and mesosoma uniformly red, abdomen dark brown to black	conical	dull and distinctly microreticulate	✓	×	×
<i>C. kurdistanica</i>	bicolored, head and mesosoma uniformly red, abdomen dark brown to black	conical	dull, densely sculptured	✓	✓	✓
<i>C. foreli</i> eyes large, 0.8–1× as long as genae	uniformly brownish black	wide-cuneiform	dull, densely sculptured	×		without or with sparse erect hairs
<i>C. frigida persica</i>	bicolored, head and mesosoma uniformly light brown, abdomen dark brown	squamiform	shining, with smooth or finely sculptured	×		×
<i>C. mauritanica</i>	uniformly blackbrown	conical	dull, densely sculptured	✓		×
<i>C. alitisquamis</i> eyes small, 0.6–0.5× length of genae	uniformly black or black-brown	conical	dull, densely sculptured	×		without or with sparse erect hairs
<i>C. velox</i>	head and mesosoma uniformly orangish-red, abdomen black	(Conical) with triangular petiolar scale	slightly shining	×		×
<i>C. hispanica</i>	uniformly black	conical	dull, densely sculptured	×		×

In summary, our study has demonstrated that *C. bazoftensis* is broadly distributed in the western foothills of Zagros Mountain, Iran. Our phylogenetic analyses have corroborated previous morphological investigations, indicating that this species is a *C. alitisquamis* species group. Furthermore, we have established the close relationship between *C. bazoftensis* and *C. kurdistanica*, despite the significant genetic differences between them (>9% p-distance). Additional molecular analyses are required to elucidate the intraspecific variability of *C. bazoftensis*. Additionally, extensive fieldwork is necessary to identify potentially undiscovered species in this region and shed light on the biodiversity of Iranian ants.

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پیوندهای فیلوژنتیکی (*Cataglyphis bazoftensis* (Hymenoptera: Formicidae) از ایران

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مکیده

گونه *Cataglyphis bazoftensis* Khalili-Moghadam Salata & Borowiec 2021 به تازگی از ایران توصیف شده است. در این مطالعه نمونه هایی از *C. bazoftensis* از پنج محل جدید در استان چهارمحال و بختیاری جمع آوری شد. تمام نمونه های مورد بررسی از نظر ریخت شناسی با توصیف *C. bazoftensis* مطابقت داشتند. آنالیزهای تبارشناسی مبتنی بر توالی های زیرواحد I ژن سیتوکروم اکسیداز C میتوکندریایی (COI) نشان می دهد که این گونه تاکنون خواهری *Cataglyphis kurdistanica* Pisarski, 1965 است، و با فاصله ژنتیکی ۹.۷۹ درصد از همدیگر جدا می شوند.

کلمات کلیدی: مورچه، *Cataglyphis*، ایران، COI sequences، فیلوژنی

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