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Molecular phylogeny of the desert ant genus *Cataglyphis* (Hymenoptera: Formicidae)

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Abstract

Since the middle of the 20th century ants of the genus *Cataglyphis* – inhabiting the southern part of the Palearctic region – have become model organisms for insect navigation and various other fields of biological research. Currently ca. 100 *Cataglyphis* species are described. However, although molecular-based phylogenetic analyses are common practice in ant systematics, to date phylogenetic analyses of *Cataglyphis* have been strictly morphology-based. Here we present the first molecular phylogeny based on mitochondrial DNA (754 bp) of 78 *Cataglyphis* specimens collected over a large part of the distributional range of the genus. By examining the same specimens based on morphological characters, we can conclude that major features of the morphology-based species-group phylogenies are supported by our molecular approach.

Key words: *Cataglyphis*, phylogeny, mtDNA, morphology, systematics.

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Introduction

Starting in the late 1960s (WEHNER 1968) desert ants of the genus *Cataglyphis* have become a neuroethological model system for the study of animal navigation, especially of visually guided behaviour such as the use of celestial and terrestrial orientation cues, odometry, and path integration (for reviews, see WEHNER 1982, 2003, 2008; for path integration, see WEHNER & SRINIVASAN 2003; for odometry, see WITTLINGER & al. 2006). In addition, and partly in connection with the studies mentioned above, several species of the genus have attracted the interest of biologists covering as widely a spectrum of research areas as optics (e.g., ZOLLIKOFER & al. 1995), brain research (e.g., WEHNER & al. 2007, SEID & WEHNER 2008, STIEB & al. 2010), locomotor physiology (e.g., ZOLLIKOFER 1994, SEIDL & WEHNER 2008), respiratory physiology (e.g., LIGHTON & WEHNER 1993), thermobiology (e.g., WEHNER & al. 1992, GEHRING & WEHNER 1995, CERDÁ & RETANA 1997, 2000, CLÉMENCET & al. 2010), ecology (e.g., HARKNESS 1977, WEHNER & al. 1983, HARKNESS & MAROUDAS 1985, LENOIR & al. 1990, CERDÁ & RETANA 1998, CERDÁ & al. 2002, DIETRICH & WEHNER 2003, KNADEN & WEHNER 2006), social behaviour and division of labour (RETANA & CERDÁ 1990, 1991) and various sociobiological aspects such as life history traits, genetic structure, colony reproduction, and sex-ratio determination (e.g., CAGNIANT 1982, 1988, LENOIR & CAGNIANT 1984, LENOIR & al. 1988, BERTON & al. 1991, PEARCY & al. 2004, KNADEN & WEHNER 2006, FOURNIER & al. 2008, LENOIR & al. 2009, TIMMERMANS & al. 2010).

What has lacked behind this upsurge of interest in *Cataglyphis* biology is an inquiry into the phylogenetic relationships of the genus. On a smaller taxonomic scale there have been various studies comparing limited numbers of species (e.g., HEFETZ & LLOYD 1985, TINAUT 1991, GÖKCEN & al. 2002, KNADEN & al. 2005, DAHBI & al. 2008). Furthermore, several new species have been described since SANTSCHI's "Etudes sur les *Cataglyphis*" (1929) – e.g., *C. sabulosa* (KUGLER 1981), *C. humeya* (TINAUT 1991), *C. floricola* (TINAUT 1993), *C. hannae* (AGOSTI 1994) and *C. zakharovi* (RADCHENKO 1997) or have been elevated to species rank (e.g., *C. fortis*: WEHNER 1983). However, a full modern treatment of the genus is still lacking and much in need. This is all the more clear if you consider that since FOERSTER (1850) established the genus, an enormous amount of renaming and reclassification has occurred at the species-group, species, and subspecies level. Even the entire genus was once combined with the North American genus *Myrmecocystus* (see FOREL 1878, corrected by WHEELER 1908), i.e., with ants that had convergently evolved many morphological traits adapting them to desert life in a similar way as in *Cataglyphis*. At the species level a major confusion has been caused by Forel's misidentifi-

cation of the type specimen of *C. viatica*, which FOREL (1890) placed (erroneously) in the *C. altisquamis* species group – the subgenus *Monocombus* of SANTSCHI (1929) – rather than (correctly) in the *C. bicolor* species group – the subgenus *Cataglyphis* of SANTSCHI (1929). It was only after AGOSTI (1990) and TINAUT (1991) had pointed out Forel's error that this major confusion about the *C. viatica-bicolor* assignment has been cleared.

To date the taxonomy of the genus *Cataglyphis* is based on morphological investigations mainly performed by EMERY (1906), FOREL (1908a) and SANTSCHI (1929) between the end of the 19th and the beginning of the 20th century. As all three investigators were elaborate "splitters", their nomenclatures are fraught with trinominal and tetranominal taxa such as *Cataglyphis viaticus* r. *adenensis* var. *bugnioni* (FOREL 1908b) or *Cataglyphis adenensis* st. *livida* var. *bugnioni* (SANTSCHI 1929). In fact some 100 subspecies, subtypes, varieties, etc., have been described, most of them representing only four species: *C. albicans* with 27, *C. bicolor* with 19, *C. cursor* with 13 and *C. viatica* with ten subclassifications. This confusion reflects the high intraspecific variability and, hence, the difficulty to identify valid morphological characters that are species- and not just population-specific. Therefore, in recent years the investigations have focused more and more on non-morphological characters. A restricted number of species has been investigated also biochemically (by comparing glandular secretions and cuticular hydrocarbons: e.g., HEFETZ & ORION 1982, KEEGANS & al. 1992, DAHBI & al. 1996, GÖKCEN & al. 2002, DAHBI & al. 2008) or molecular phylogenetics (by employing nuclear and mitochondrial DNA: KNADEN & al. 2005).

Here we present a preliminary molecular phylogeny inferred with mitochondrial DNA from 78 *Cataglyphis* worker specimens that were collected over a large part of the distributional range of the genus. The same specimens were also identified on the basis of morphological characters down to at least the species group level. We then compare the cladogram derived from the molecular analysis with the published phylogenetic schemes based on morphological data, which have been based on males and workers by AGOSTI (1990) and RADCHENKO (2001), respectively. The "trees" provided by the latter authors do not represent cladograms, even though named as such by AGOSTI (1990), but some kind of similarity diagrams based on assemblages of morphological characters.

Materials and Methods

Sample collection: Between 1995 and 2005, we collected 78 samples of *Cataglyphis* across a transect from western Morocco to eastern Mongolia and from northern Greece to southern Burkina Faso, i.e., a large part of the geographical distribution of this genus (Fig. 1). We have deposited the samples in the R. and S. Wehner *Cataglyphis* collection, Senckenberg Museum, Frankfurt, Germany. For collection codes of all samples see Table 1.

Molecular phylogeny: We extracted DNA from the mesosoma of single ants using the CTAB method with minor modifications (SAMBROOK & RUSSELL 2001). Proteinase K (20 mg / ml) was used instead of mercaptoethanol. It should be mentioned that we did not succeed in amplifying the universal primers used for DNA barcoding (FOLMER & al. 1994). For the analysis of mitochondrial DNA (mtDNA) the 3'end within the cytochrome oxidase one gene (CO1) was amplified using the primer COI-RLR (ROEHRDANZ 1993). For the 5'end we used the primers COIIR1 (KNADEN & WEHNER 2006) or COIIR1bic (KNADEN & al. 2005) amplifying with COI-RLR a portion of the CO1 gene (of which 754 bp could be sequenced), an intergenic spacer with extensive length polymorphism (e.g., within *Cataglyphis bicolor* 76 - 106 bp) and a leucine tRNA gene (for primer sequences see Tab. 2). Due to the length polymorphism we could not find any satisfying alignment for the intergenic spacer. Furthermore, the position of the reverse primer very close to the leucine tRNA gene resulted in less precise sequencing of this gene. Hence we excluded the non-coding intergenic spacer and the neighboring leucine tRNA from the analysis.

PCR amplifications were carried out in 50 µl reaction volumes containing 1 × Buffer A, 0.5 µl DMSO, 0.2 mM each dNTP, 10 pM each primer, about 50 ng DNA, and one unit Taq (Promega) with a PTC 100 (MJ Research) for 40 cycles (94°C, 75 s, 43°C, 75 s, 72°C, 135 s) after an initial 180 s denaturation step at 95°C and with final extension at 72°C for 300 s. PCR reactions were purified with the QIAquick PCR Purification Kit (Qiagen) under conditions specified by the manufacturer. PCR products were sequenced using the ABI-PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (ABI-Perkin Elmer) in 10 µl reaction volumes following the manufacturer's instructions and run on an ABI 3100 DNA sequencer.

Chromatograms were first checked by eye for base call accuracy and then aligned with the reverse strand from the same individual using the program Sequencher™, and sequences were examined for sequence agreement. Finally all sequences were checked for internal stop codons to exclude possible pseudogenes from analysis. For accession numbers of all samples see Table 1.

Sequence trace files of all sequences were edited with Sequencher v.4.7 (GeneCodes, Ann Arbor, Michigan, U.S.A.). Sequences were aligned by the ClustalW algorithm (THOMPSON & al. 1994) as implemented in MEGA 4 (TAMURA & al. 2007) and default parameters. JModeltest 0.1.1 (POSADA 2008) was used to choose the appropriate nucleotide substitution model for each marker according to the corrected Akaike Information Criterion (AICc) (AKAIKE 1974). Bayesian inference (BI) was implemented with Mr. Bayes 3.1.2 (HUELSENBECK & RONQUIST 2001). JModeltest suggested GTR + I + G (nst = 6 and rates = invgamma) as the best fitting model for the dataset. The following parameters for the BI analysis were set: 2,500,000 generations, trees sampled every 100th generation, TBR, four chains, and branch lengths saved. The burn-in was set to 25% of the samples (= 6,250). A 50% majority rule consensus tree was calculated from the obtained trees.

Maximum parsimony (MP) and neighbor joining (NJ) trees were calculated with MEGA 4 (TAMURA & al. 2007) with default parameters. We used the close-neighbor-interchange search method for the MP analysis. For both analyses 1000 bootstrap replicates were calculated.

The same specimens as were used in the phylogenetic analysis were also identified based on their worker morphology using not only the classical keys of EMERY (1906) and SANTSCHI (1929) and the revision by AGOSTI (1990), but also with regional keys such as COLLINGWOOD (1978) for the Iberian Peninsula, COLLINGWOOD (1985) and COL-

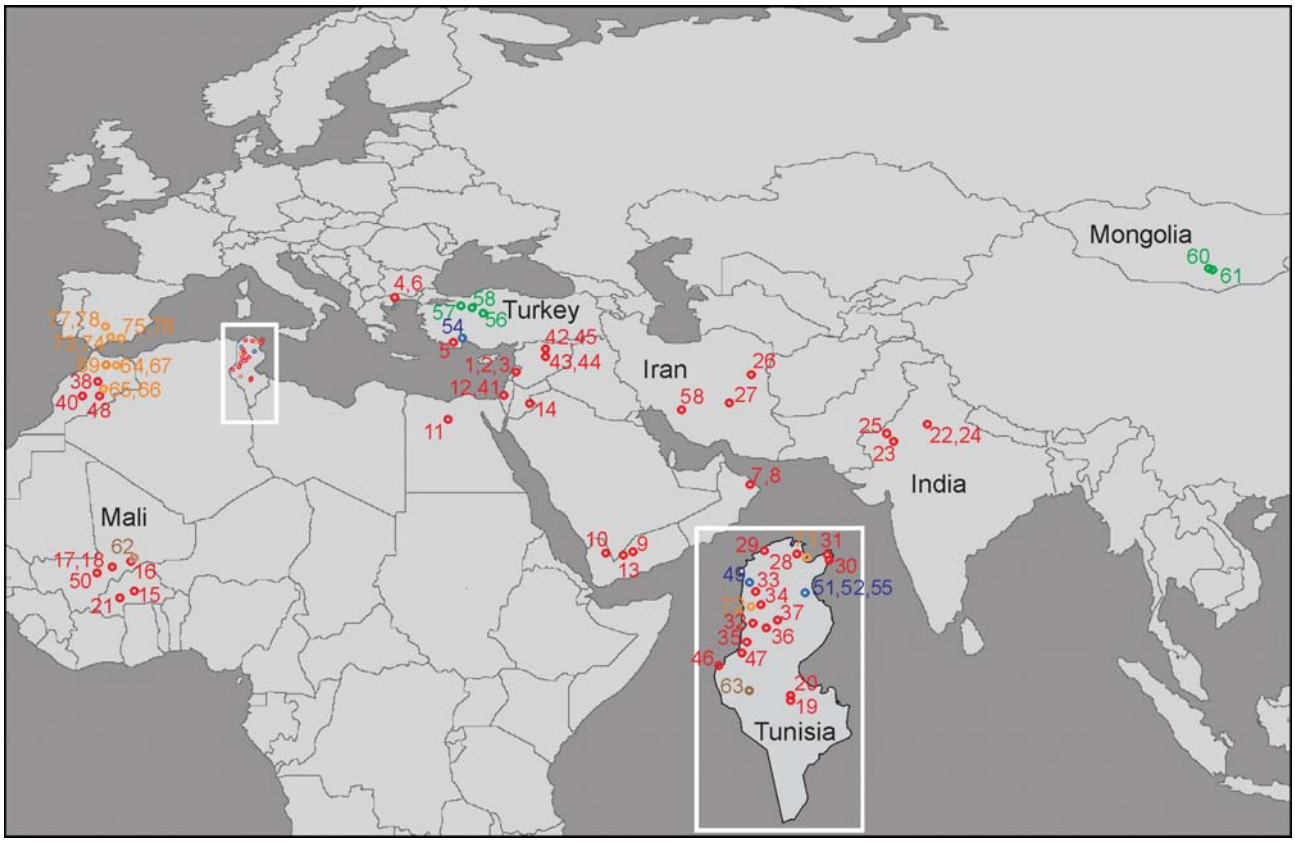

Fig. 1: Locations of the *Cataglyphis* samples used in this study. Numbers depict appearance within the phylogenetic tree (Fig. 2). Colors depict morphologically identified species groups. Red, *C. bicolor* group; blue, *C. albicans* group; green, *C. cursor* group; pink, *C. pallida* group; yellow, *C. altisquamis* group; brown, *C. bombycina* group. Inset figure depicts Tunisia, the country with the highest sample density in this study.

LINGWOOD & AGOSTI (1996) for Saudi Arabia, AGOSTI & COLLINGWOOD (1987) for the Balkan region and CAGNIANT (2009) for Morocco. For a list of the species names used see appendix (provided as digital supplementary material to this article, at the journal's web pages). We should mention here, that in some cases different authors use different name variants for the same species (e.g., *Cataglyphis nodus* vs. *C. noda*, *C. viaticus* vs. *C. viatica* (AGOSTI 1990, KNADEN & al. 2005, LENOIR & al. 2009). As the gender of the species name needs to conform to the gender of the genus, which is feminine in case of *Cataglyphis*, we always used the feminized version of the species name. Finally we compared the specimens we included with *Cataglyphis* type specimens in the collections of the museums of Basel, Genève, Paris, and Warsaw.

From the nine species groups described by AGOSTI (1990) we identified six (*Cataglyphis albicans* group, *C. altisquamis* group, *C. bicolor* group, *C. bombycina* group, *C. cursor* group, and *C. pallida* group, with the latter being represented by only one specimen). We then compared whether those specimens that were morphologically determined to belong to the same species group also clustered in the molecular-based phylogenies.

Results

Of the analyzed 754 bp 311 sites were variable, and 275 were parsimony informative. The mtDNA analysis resulted in a tree with two well supported clades. Clade one further splits into a *Cataglyphis bicolor* and a *C. albicans* clade, while clade two contains the *C. cursor*, the *C. bombycina*, and the *C. altisquamis* clades, whose separation was supported by BI, but not by NJ or MP. Of the 78 specimens we included, 77 clustered at the level of species groups as predicted by the morphological determination (Fig. 2), while the only representative of the *C. pallida* group appeared within the *C. cursor* clade. However, especially within the *C. bicolor* clade the assemblage of species was quite mixed with, e.g., *C. viatica*, *C. bicolor*, and *C. savignyi* appearing at several statistically well supported positions within the clade. We conclude that for an in-depth analysis across the *Cataglyphis* species groups, the CO1 sequence used in our approach would need to be complemented by additional mtDNA genes and / or nuclear genes.

We next checked whether the 48 specimens from the *Cataglyphis bicolor* group clustered according to their geographical origin. However, this was not the case (Figs. 1, 2). For example, specimens from Greece appeared in the same clade as specimens collected in Yemen, and specimens from Tunisia clustered with those from Burkina Faso.

Discussion

Until recently the most thorough classification of ants of the genus *Cataglyphis* was based on the morphology of male genitalia (for a review, see AGOSTI 1990). The majority of ant individuals belongs to the worker caste, while males are rare and can be collected only during the restricted time frame of nuptial flights. We therefore revisited the morphology-based classification of this genus with a mo-

Tab. 1: List of samples used for the molecular analysis. 1st column, position in the phylogenetic tree (Fig. 2); 2nd column, morphology-based assigned species names; 3rd column, collection numbers under which these samples are stored in the Rüdiger and Sibylle Wehner *Cataglyphis* Collection at the Senckenberg Research Institution (Leibniz Institute) in Frankfurt/Main, Germany; 4th column, country of collection; 5th column, sampling site; 6th column, GenBank accession numbers.

	Name	Coll. code	Origin	Geographical coordinates	Acc. number		Name	Coll. code	Origin	Geographical coordinates	Acc. number
1	*C. viatica*	1692	Lebanon	N33°48' E35°51'	JN630724	40	*C. bicolor*	1938	Morocco	N32°26' W6°20'	JN630763
2	*C. bicolor*	1689	Lebanon	N34°02' E36°12'	JN630725	41	*C. isis*	1643	Israel	N31°23' E34°36'	JN630764
3	*C. bicolor*	1691	Lebanon	N33°48' E35°51'	JN630726	42	*C. diehlii*	1625	Syria	N34°58' E38°52'	JN630765
4	*C. bicolor*	1998	Greece	N40°40' E23°14'	JN630727	43	*C. isis*	1613	Syria	N34°51' E38°54'	JN630766
5	*C. bicolor*	1843	Turkey	N36°21' E30°22'	JN630728	44	*C. diehlii*	1602	Syria	N34°52' E38°55'	JN630767
6	*C. bicolor*	1997	Greece	N40°40' E23°15'	JN630729	45	*C. diehlii*	1626	Syria	N34°59' E38°55'	JN630768
7	*C. abyssinica*	1965	Oman	N22°45' E59°15'	JN630730	46	*C. isis*	1828	Tunisia	N34°25' E7°59'	JN630769
8	*C. abyssinica*	1964	Oman	N22°45' E59°15'	JN630731	47	*C. diehlii*	1679	Tunisia	N34°22' E8°26'	JN630770
9	*C. abyssinica*	1716	Yemen	N15°22' 47°02'	JN630732	48	*C. savignyi*	1935	Morocco	N32°15' W4°31'	JN630771
10	*C. abyssinica*	1715	Yemen	N15°24' E45°19'	JN630733	49	*C. albicans*	2240	Tunisia	N35°56' E8°50'	JN630772
11	*C. abyssinica*	2290	Egypt	N28°26' E28°49'	JN630734	50	*C. albicans*	1983	Mali	N14°08' W5°05'	JN630773
12	*C. niger*	1645	Israel	N31°22' E34°34'	JN630735	51	*C. fortis*	1675	Tunisia	N35°45' E10°31'	JN630774
13	*C. niger*	1718	Yemen	N15°23' E46°08'	JN630736	52	*C. fortis*	1674	Tunisia	N35°45' E10°30'	JN630775
14	*C. niger*	2299	Jordan	N30°16' E36°12'	JN630737	53	*C. albicans*	1637	Morocco	no information	JN630776
15	*C. spec*	2279	Burkina Faso	N11°45' W2°51'	JN630738	54	*C. viaticoides*	1833	Turkey	N36°54' E30°49'	JN630777
16	*C. savignyi*	1975	Mali	N15°53' W1°15'	JN630739	55	*C. albicans*	1677	Tunisia	N35°45' E10°32'	JN630778
17	*C. savignyi*	1972	Mali	N14°32' W3°13'	JN630740	56	*C. cursor*	1801	Turkey	N39°06' E33°24'	JN630779
18	*C. savignyi*	1971	Mali	N14°32' W3°13'	JN630741	57	*C. cursor*	1784	Turkey	N39°54' E30°12'	JN630780
19	*C. savignyi*	1493	Tunisia	N33°37' E9°55'	JN630742	58	*C. cursor*	1795	Turkey	N39°30' E31°36'	JN630781
20	*C. savignyi*	1492	Tunisia	N33°37' E9°54'	JN630743	59	*C. pallida*		Kazakstan	no information	JN630782
21	*C. spec*	2281	Burkina Faso	N11°02' W4°19'	JN630744	60	*C. aenescens*	1650	Mongolia	N43°32'E104°25'	JN630783
22	*C. longipedem*	1880	India	N28°05' E76°34'	JN630745	61	*C. aenescens*	1651	Mongolia	N43°34'E104°26'	JN630784
23	*C. longipedem*	1867	India	N26°34' E72°20'	JN630746	62	*C. bombycina*	1975	Tunisia	N33°46 E9°15'	JN630785
24	*C. longipedem*	1878	India	N28°05' E76°35'	JN630747	63	*C. bombycina*	1499	Mali	N15°53' W1°15'	JN630786
25	*C. longipedem*	1872	India	N26°55' E71°52'	JN630748	64	*C. gaetula*	2271	Morocco	N34°39' W2°56'	JN630787
26	*C. oasium*	1988	Iran	N26°09' E48°02'	JN630749	65	*C. mauritanica*	1932	Morocco	N32°31' W4°29'	JN630788
27	*C. bellicosa*	1893	Iran	N30°03' E57°17'	JN630750	66	*C. mauritanica*	1929	Morocco	N32°41' W4°44'	JN630789
28	*C. viatica*	1538	Tunisia	N37°02' E10°06'	JN630751	67	*C. gaetula*	2272	Morocco	N34°41' W2°58'	JN630790
29	*C. viatica*	1532	Tunisia	N37°14' E9°12'	JN630752	68	*C. mauritanica*	1918	Morocco	no information	JN630791
30	*C. viatica*	1488	Tunisia	N36°50' E11°05'	JN630753	69	*C. mauritanica*	1915	Morocco	N33°49' W4°50'	JN630792
31	*C. viatica*	1483	Tunisia	N36°56' E10°52'	JN630754	70	*C. mauritanica*	1921	Morocco	no information	JN630793
32	*C. bicolor*	1513	Tunisia	N35°13' E8°43'	JN630755	71	*C. mauritanica*	2245	Tunisia	N36°52' E10°20'	JN630794
33	*C. viatica*	1521	Tunisia	N35°57' E8°50'	JN630756	72	*C. mauritanica*	2236	Tunisia	N35°09' E8°46'	JN630795
34	*C. viatica*	1514	Tunisia	N35°33' E9°05'	JN630757	73	*C. velox*	1670	Spain	N36°50' E3°36'	JN630796
35	*C. viatica*	1529	Tunisia	N34°53' E8°30'	JN630758	74	*C. velox*	1666	Spain	N37°10' W3°35'	JN630797
36	*C. bicolor*	1559	Tunisia	N35°13' E9°08'	JN630759	75	*C. humeya*	1663	Spain	N36°50' W2°27'	JN630798
37	*C. bicolor*	1554	Tunisia	N35°26' E9°35'	JN630760	76	*C. humeya*	1662	Spain	N36°50' W2°26'	JN630799
38	*C. bicolor*	1905	Morocco	N33°54' W5°33	JN630761	77	*C. hispanica*	1659	Spain	N37°46' W3°49'	JN630800
39	*C. viatica*	1902	Morocco	no information	JN630762	78	*C. hispanica*	1658	Spain	N37°46' W3°48'	JN630801

Tab. 2: Primers used for the amplification of CO1 mtDNA.

Name	Sequence, 5'-3'	Length	Position honeybee genome	Reference
COI-RLR	ttgatttttggtcatccagaagt	24 bp	2492	CROZIER & CROZIER (1993)
COIIR1	taggagaatttgartttgtagag	24 bp	3376	KNADEN & WEHNER (2006)
COIIR1bic	tgggagaatttgaattttgaagtg	24 bp	3376	KNADEN & al. (2005)

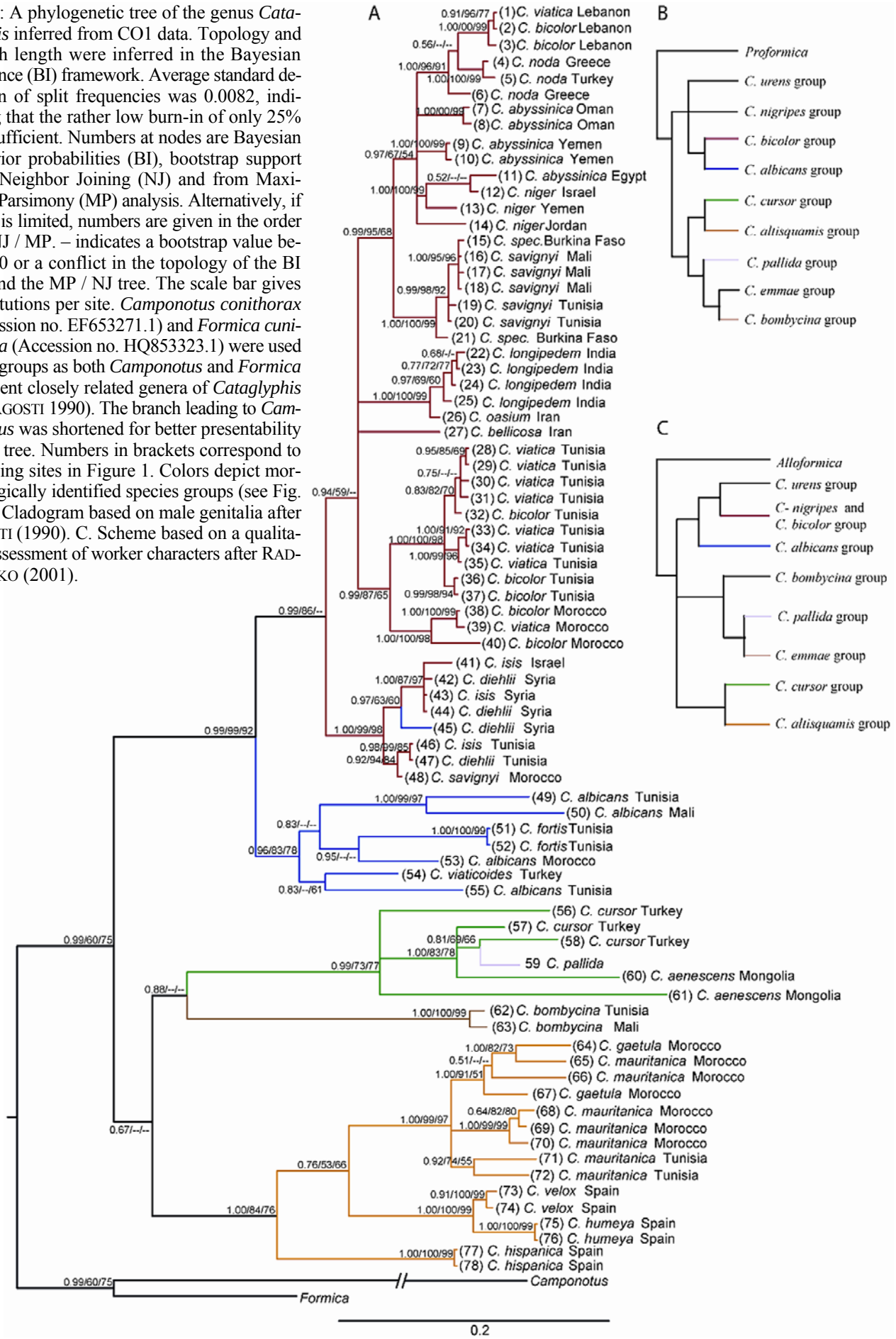

Fig. 2: A phylogenetic tree of the genus *Cataglyphis* inferred from CO1 data. Topology and branch length were inferred in the Bayesian Inference (BI) framework. Average standard deviation of split frequencies was 0.0082, indicating that the rather low burn-in of only 25% was sufficient. Numbers at nodes are Bayesian posterior probabilities (BI), bootstrap support from Neighbor Joining (NJ) and from Maximum Parsimony (MP) analysis. Alternatively, if space is limited, numbers are given in the order BI / NJ / MP. – indicates a bootstrap value below 50 or a conflict in the topology of the BI tree and the MP / NJ tree. The scale bar gives substitutions per site. *Camponotus conithorax* (Accession no. EF653271.1) and *Formica cunicularia* (Accession no. HQ853323.1) were used as outgroups as both *Camponotus* and *Formica* represent closely related genera of *Cataglyphis* (see AGOSTI 1990). The branch leading to *Camponotus* was shortened for better presentability of the tree. Numbers in brackets correspond to sampling sites in Figure 1. Colors depict morphologically identified species groups (see Fig. 1). B. Cladogram based on male genitalia after AGOSTI (1990). C. Scheme based on a qualitative assessment of worker characters after RADCHENKO (2001).

lecular-based approach. HEBERT & al. (2003) showed that the CO1's sequence variability allows assigning taxa not only to animal phyla or insect orders, but often to specific species. Since then the CO1 sequence has been widely used as a so-called DNA barcode for the identification of genera, subgenera (ants: MARUYAMA & al. 2008) and species (e.g., ants: species-group phylogeny for *Pheidole*: MOREAU 2008; moths: BEHERE & al. 2007; snails: KANE & al. 2008; birds: TAVARES & BAKER 2008). As our study was initiated before the barcode era, and as we were not able to amplify the universal primers used for barcoding (FOLMER & al. 1994) we did not analyze the region of CO1 that is now commonly used as a standard for barcoding. However, the region of CO1 that we used has also been used for phylogenetics in ants (BACCI & al. 2009, SEPPÄ & al. 2011). Furthermore the ratio of parsimonious informative bp of 36.4% lies well within the ratios described for the barcoding region of other ant genera (*Lasius*: 8%, MARUYAMA & al. 2008; *Pheidole*: 48%, MOREAU 2008). Therefore this mitochondrial gene seems to be well suited for comparing morphological and molecular phylogenies in *Cataglyphis*. We analyzed 78 specimens that were collected from across the geographical distributional range of this genus. Unfortunately the CO1 sequences were not sufficient to resolve the phylogeny in full detail, which suggests the need to include additional mitochondrial and / or nuclear genes in future studies. However, despite the unresolved base of the tree the analysis of the CO1 sequences supports the hitherto existing morphology-based phylogeny (Fig. 2) with most of the 78 taxa clustering in species groups as predicted by morphology.

First, at the species-group level the *Cataglyphis bicolor* group clusters with the *C. albicans* group (Fig. 2), which is in accord with the morphology-based species-group diagrams provided by AGOSTI (1990) (Fig. 2b) and RADCHENKO (2001) (Fig. 2c). The resolution of our phylogeny does not allow us to confirm the monophyletic origin of the *C. altisquamis* and the *C. cursor* group. The only disagreement of our analysis with the previously published ones (AGOSTI 1990, RADCHENKO 2001) consists in placing the single *C. pallida* specimen we were able to include in our study within the *C. cursor* group. Based on worker morphology and the anatomy of the male genitalia it should have clustered with *C. bombycina* (see AGOSTI 1990; RADCHENKO 2001). The specimen included here was collected in Kazakhstan, i.e., within the distributional range of *C. pallida* (see KARAVAIEV 1910), together with males and females from the same nest. We were able to confirm our identification based on the male genitalia. The latter shared characters with those of the *C. cursor* group (stipes and squamula), the *C. bombycina* group (subgenital plate) and even the *C. altisquamis* group (subgenital plate, volsella, and lacinia). On the basis of *C. emeryi* male genitialia morphology AGOSTI (1990) placed the *C. pallida* group close to the *C. emmae* group. However, the genitalia of *C. emmae* differ significantly from those of *C. pallida*. Future analyses will be necessary to determine whether the morphology-based description of the *C. pallida* group is valid.

Second, at the species level roughly two scenarios can be inferred. In scenario one most specimens used in the present study are grouped in the same clade and belong to the same geographical area. This is the case for *Cataglyphis longipedem* (India), *C. noda* (Turkey, Greece), *C. fortis* (Tunisia), *C. cursor* (Turkey) as well as *C. hispanica*, *C. velox* and *C. humeya* from Spain. In scenario two "species" such as *C. bicolor* (Morocco, Tunisia, Lebanon) and *C. savignyi* (Morocco, Tunisia, Mali) appear to have very wide geographic ranges. The latter case could be due to (I) intrinsic characteristics of the species such as particular dispersal abilities, (II) lack of resolution from the molecular data, (III) or incorrect identification of the specimens. That species-specific differences can occur with respect to argument (I) has been shown by KNADEN & WEHNER (2006), but dispersal performances are unlikely to explain the occurrence of, e.g., *C. bicolor* in Lebanon and Morocco. Due to the difficulty to access some of the distributional areas of *Cataglyphis* the transect includes some sampling gaps. However, these gaps cannot account for the phylogenetic clustering of geographically distant samples.

Argument (II) is at least partially supported by the study of KNADEN & al. (2005), in which *Cataglyphis bicolor* and *C. viatica* could not be separated by the mitochondrial CO1 gene, but could be separated clearly by nuclear microsatellite genes. Another case in point is *C. gaetula*, which on the basis of morphological characters is clearly separated from *C. mauritanica*, but appears intermixed with the latter species in the molecular phylogeny. In many cases CO1 data have discrimination potential at the species level (see below), but this is not true in all cases. Argument (III) seems to be an inescapable one as worker morphology does indeed provide only limited identification potential. To be really certain in this respect we again checked all specimens of the scenario two with the criteria of the published keys available, and reconfirmed our species level identifications. Given the amount of variability in morphological characters of the worker caste, especially in the species rich *C. bicolor* and *C. albicans* species groups, any identifications and phylogenies based on worker characters alone will suffer from imperfections. Taken together, the appearance of individual "species" at different positions within the phylogeny seems to be due to species identification problems based on worker morphology rather than on a lack of resolution from our molecular data. Note that what we have labeled, e.g., "*C. bicolor*" represents what according to current taxonomy based on morphological characters of the worker caste has to be identified as "*C. bicolor*". This is not to say that all taxa labeled this way as "*C. bicolor*" belong to one species.

The *Cataglyphis bicolor* species group appears as the most variable based on morphology, but there is at least one important conclusion that can be derived from our molecular phylogenetic analysis. Based on a comparison of glandular secretions of *C. viatica* and *C. bicolor* from Morocco and some *Cataglyphis* group specimens collected in Burkina Faso, DAHBI & al. (2008) suggested that the *C. bicolor*-group species have evolved separately north and south of the Sahara. However, in our mtDNA analysis the specimens collected in Burkina Faso (Fig. 2A, Nos. 15 and 21) and in Mali (Fig. 2A, Nos. 16, 17, and 18) clustered within the specimens collected north of the Sahara. Therefore, our data do not support the hypothesis raised by DAHBI & al. (2008). We suggest that the evolution of the *C. bicolor* group, and probably of the *C. albicans* group as well, followed a Secular Migration model (see LOMOLINO & al. 2006) in so far as populations might have expanded gradually through the Arabian peninsula and African conti-

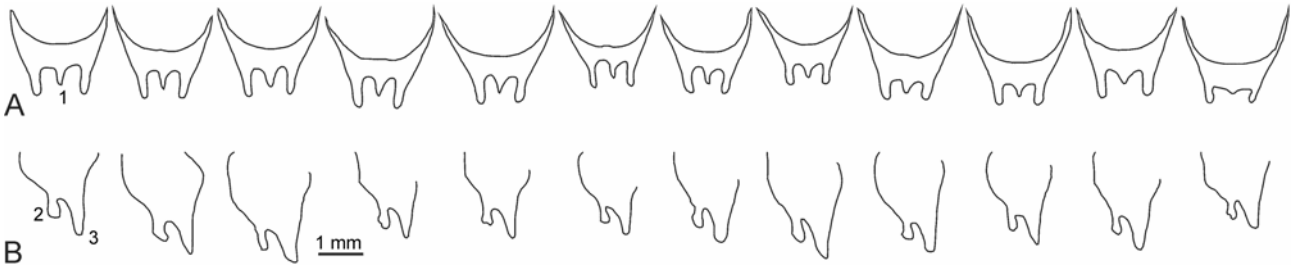

Fig. 3: Genitalia of *Cataglyphis noda* males collected from a single nest in Greece. A. Subgenital plates sorted for the relative length of the medial processes (1). B. Corresponding squamulas. Please note the variability of the median appendix (2) and the shape of the stipes (3). Both genitalia parts usually are used for the identification of species (AGOSTI 1990). Compare high intraspecific variability of *C. noda* with the relatively low interspecific variability given by AGOSTI (1990, figs. 13 - 42).

nent and by this produced diversification during range expansion.

In conclusion, the molecular approach applied in the present account provides a valuable tool to assist species determination based on worker morphology. For the morphological determination of *Cataglyphis* ants to the species level, one usually needs access to the male sexual organs (AGOSTI 1990). As most samples of *Cataglyphis* belong to the worker caste (sexuals can be collected only during the restricted time frame of nuptial flights), the specimens used in our study could often be determined only down to the species-group level. As mentioned above nuclear microsatellite genes might be necessary to separate species if mtDNA analyses do not suffice (KNADEN & al. 2005).

When collecting *Cataglyphis noda* males during a transect through Greece we found unexpectedly high variability in the males' genitalia (data not shown). In order to check, whether this variability was due to population-specific differences, we also collected 100 males from a single nest. Again we found striking differences in the shapes of the different parts of the genitalia that resembled the variability of the total Greek population (Fig. 3). The morphological classification of AGOSTI (1990) did not include any intraspecific variability but was based on interspecific variability only. We conclude that although both the anatomy-based and the molecular-based phylogenies yielded comparable results, due to potentially high intraspecific variability identifications based on morphology should be considered with care.

As the main result of the present study we find that the major features of the morphology-based species-group phylogenies as provided by AGOSTI (1990) and RADCHENKO (2001) are supported by our molecular approach, and that the sequence data add phylogenetic resolution to worker-based morphological identification. Furthermore, our study emphasizes the strong need for geographically fine-scale studies on the species and population level in *Cataglyphis*.

Finally, in studying the evolution of various modes of navigation in different *Cataglyphis* species we will heavily rely on knowledge of the phylogenetic relationships between the species in question. This comparative approach focuses especially on the salt-pan species *C. fortis*, which inhabits flat, unstructured environments nearly completely avoid of visual landmarks, and various representatives of the *C. bicolor* species group, which occur in a variety of habitats cluttered with grass tussocks, shrubs, and / or loosely scattered trees and rocks, and hence of a variety of visual cues. This leads to the question of whether all *Cataglyphis* species are equipped with the same navigational toolkit (consisting of one or several compasses, odometers, path integrators and landmark-guidance routines), but that the weight that any particular species puts on these particular modes of navigation is adapted to the particular ecological niche occupied by the species in question (see, e.g., the comparative study including *C. albicans*, *C. bicolor*, *C. fortis*, *C. noda*, and *C. rubra* on the peripheral olfactory pathway; STIEB & al. 2011). As in all such studies phylogeny-based and experience-based traits must be disentangled (see, e.g., the intergeneric study of the relative importance of different mechanisms of navigation in *C. fortis* in North Africa and *Melophorus bagoti* in central Australia; BÜHLMANN & al. 2011), the phylogenetic status of the animals participating in the various experimental programmes must be known in the first place.

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