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Combined hybridization and mitochondrial capture shape complex phylogeographic patterns in hybridogenetic *Cataglyphis* desert ants

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ABSTRACT

Some species of *Cataglyphis* desert ants have evolved a hybridogenetic mode of reproduction at the social scale. In hybridogenetic populations, two distinct genetic lineages coexist. Non-reproductive offspring (workers) are hybrids of the two lineages, whereas sexual offspring (males and new queens) are produced by parthenogenesis and belong to the mother queen lineage. How this unusual reproductive system affects phylogeographic patterns and speciation processes remains completely unknown to date. Using one mitochondrial and four nuclear genes, we examined the phylogenetic relationships between three species of *Cataglyphis* (*C. hispanica*, *C. humeya* and *C. velox*) where complex DNA inheritance through social hybridogenesis may challenge phylogenetic inference. Our results bring two important insights. First, our data confirm a hybridogenetic mode of reproduction across the whole distribution range of the species *C. hispanica*. In contrast, they do not provide support for hybridogenesis in the populations sampled of *C. humeya* and *C. velox*. This suggests that these populations are not hybridogenetic, or that hybridogenesis is too recent to result in reciprocally monophyletic lineages on nuclear genes. Second, due to mitochondrial introgression between lineages (Darras and Aron, 2015), the faster-evolving COI marker is not lineage specific, hence, unsuitable to further investigate the segregation of lineages in the species studied. Different mitochondrial haplotypes occur in each locality sampled, resulting in strongly structured populations. This micro-allopatric structure leads to over-splitting species delimitation on mitochondrial gene, as every locality could potentially be considered a putative species; haploweb analyses of nuclear markers, however, yield species delimitations that are consistent with morphology. Overall, this study highlights how social hybridogenesis varies across species and shapes complex phylogeographic patterns.

1. Introduction

Genetic tools are increasingly used to delimit species boundaries and to infer species phylogenies (Bickford et al., 2007; Flot, 2015; Fontaneto et al., 2015; Fujita et al., 2012). Species delimitation hypotheses are strengthened by means of multilocus datasets evaluating genealogical concordance of unlinked markers, that most likely mirror the speciation processes at the genome scale (Hudson and Coyne, 2002; Dettman et al., 2003; Dupuis et al., 2012). Within species, the mixing effects of recombination cause loci to have distinct genealogical histories, but genetic drift and long-term divergence lead to genealogical concordance at loci across the genome (Ellegren et al., 2012; Payseur, 2010). However, in some cases, different loci may uncover discordant information regarding the species tree (Dávalos et al., 2012). Such incongruences may stem from different evolutionary processes, such as incomplete lineage sorting, gene selection or gene duplication (Castoe et al., 2009; Degnan and Rosenberg, 2009; Pamilo and Nei, 1988; Rokas et al., 2003). They may also reflect divergences from classic mating systems, like parthenogenesis, hybridization, introgression, hybridogenesis, gynogenesis or androgenesis (Doucet-Beaupré et al., 2012; Ghiselli et al., 2007; Hedtke and Hillis, 2011; Passamonti, 2007; Pigneur et al., 2011; Mallet, 2005; Muñoz et al., 2010; Saitoh et al., 2004). Although phylogenetic incongruences often challenge phylogenetic inferences, resolving their causes may be informative to unravel complex speciation and diversification events, providing a more comprehensive picture of the evolutionary processes underlying speciation (Dávalos et al., 2012; Rokas et al., 2003; Degnan and Rosenberg, 2009; Toews and Brelsford, 2012).

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The desert ant genus *Cataglyphis* comprises approximately one hundred species that are sparsely distributed across arid lands and deserts of the Old World, from Spain to Mongolia (Lenoir et al., 2009). Phylogenies of this genus have long been based on phenotypic traits, including morphology (Agosti, 1990; Radchenko, 2001; Tinaut, 1990a, 1990b) and chemical compounds (Dahbi et al., 1996, 2008; Keegans et al., 1992; Oldham et al., 1999). Subsequent molecular phylogenies based on nuclear (nuDNA) and mitochondrial DNA (mtDNA) supported morphology-based species-group phylogenies (Aron et al., 2016; Knaden et al., 2012).

Although the phylogenetic positions of many *Cataglyphis* species have been resolved, relationships within some groups remain ambiguous (Aron et al., 2016; Knaden et al., 2012). This is notably the case in the *altisquamis* group (Dahbi et al., 1996; Tinaut, 1990a). Recent population genetic studies have shown that at least three species of this group have evolved a hybridogenetic mode of reproduction at the social scale that affects DNA inheritance and potentially leads to erroneous inferences regarding their evolutionary history and species delimitation: *Cataglyphis hispanica* (Leniaud et al., 2012; Darras et al., 2014a, 2014b), *C. velox* and *C. mauritanica* (Eyer et al., 2013). Two distinct genetic lineages coexist within each population (Fig. 1). Colonies are headed by queen(s) mated with male(s) from the other lineage. Queens use sexual reproduction to produce inter-lineage hybrid workers that perform colony-maintenance tasks (foraging, defending the nest, caring for the brood), but they use asexual reproduction to produce reproductive offspring, *i.e.* new queens and males. New queens are produced by thelytokous parthenogenesis and are diploid clones of their mother. Males are produced by arrhenotokous parthenogenesis and are haploid, as is usually the case in Hymenoptera. As a consequence, the two lineages are maintained genetically distinct over generations.

The three species *Cataglyphis hispanica*, *C. velox* and *C. humeya* are endemic to the Iberian Peninsula. Previous studies, based on microsatellite marker loci, showed that hybridogenesis occurs in 14 populations of *C. hispanica* (Darras et al., 2014a) and two populations of *C. velox* (Eyer et al., 2013). Whether a similar reproductive system occurs in other populations of *C. velox* and in the species *C. humeya* remains unknown. Furthermore, extensive studies in *C. hispanica* demonstrated a strong incongruence between data based on mitochondrial and microsatellite sequences: while microsatellite markers indicate the presence of two distinct hybridogenetic lineages across the species range, mtDNA does not recover the two lineages as reciprocally monophyletic (Darras and Aron, 2015). Instead, mtDNA variation presents a strong geographic structure incongruent with the nuclear lineages, showing mitochondrial introgression between lineages. How this unusual reproductive system affects phylogeographic patterns and speciation processes remains completely unknown to date.

In this paper, we investigated how complex DNA inheritance through social hybridogenesis challenges phylogenetic inferences in *Cataglyphis* ants of the *altisquamis* group. We used one mitochondrial and four nuclear genes to infer divergence events in *C. hispanica*, *C. velox* and *C. humeya*. First, using nuDNA, we examined whether reproduction by hybridogenesis occurs across the whole distribution range of *C. velox* and *C. humeya*, as is the case in *C. hispanica*. Second, using phylogeographic analyses based on mtDNA, we studied how social hybridogenesis shapes patterns of genetic variation in natural populations. Finally, we deciphered the phylogenetic relationships among the three species by applying several species delimitation approaches to our nuDNA and mtDNA data set. Our results show that, contrasting with *C. hispanica* and previous results at population level, *C. velox* and *C. humeya* are not hybridogenetic across their entire distribution range or that hybridogenesis is too recent to result in reciprocally monophyletic lineages on nuclear genes. In addition, in the three species studied the mitochondrial COI marker is not lineage specific, consistent with mitochondrial introgression between lineages. COI is hypervariable and yields absurd species delimitation over-splitting every locality as a putative species. Overall, this study shows how combined interbreeding between genetic lineages for workers production, asexual production of breeders within each lineage, and mitochondrial capture between lineages may challenge phylogenetic inferences.

2. Materials and methods

2.1. Sampling and molecular techniques

The three morphologically described species *Cataglyphis hispanica*, *C. humeya* and *C. velox* occupy reduced, non-overlapping areas of the Iberian Peninsula (Tinaut, 1990a). We examined 35 popula-

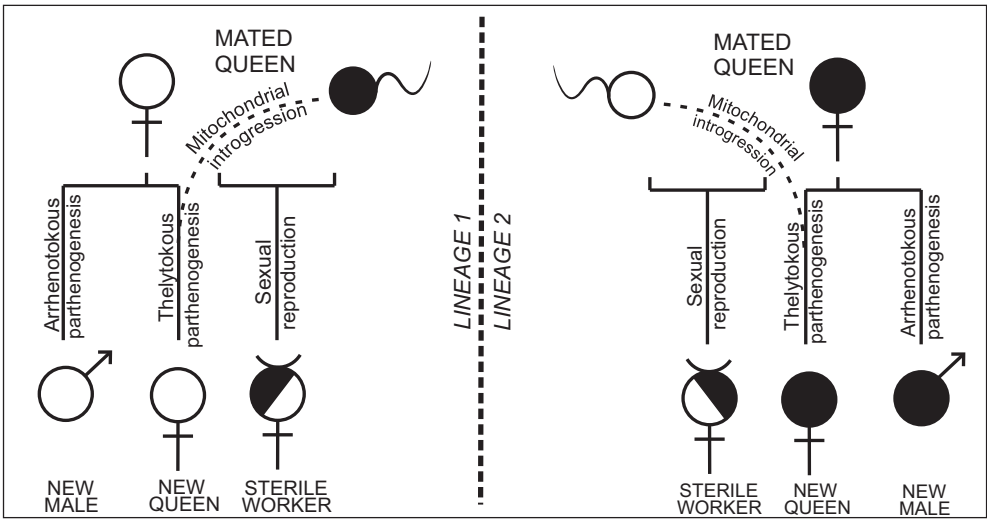

Fig. 1. Reproductive system in hybridogenetic species of *Cataglyphis* desert ants. Queens carry sperm from another lineage than their own. Sterile workers are produced sexually, whereas queens are produced by thelytokous parthenogenesis. As is usually the case in Hymenoptera, males arise from unfertilized eggs and are haploid (arrhenotokous parthenogenesis). As a result, sterile workers carry a hybrid nuclear genome, while new males and new queens inherit the pure maternal nuclear genome. Black and white colors represent nuclear genomes from two distinct lineages.

tions of *C. hispanica* (including 11 populations for which social hybridogenesis was previously reported; Darras et al., 2014a). Male and female sexuals were successfully assigned to each of the two genetic lineages on the basis of microsatellite analyses (Darras et al., 2014a; Leniaud et al., 2012). For *C. velox*, we analyzed 27 populations including two that were previously reported as hybridogenetic based on microsatellite analyses of queens and the content of their spermatheca (Eyer et al., 2013; Eyer, unpublished data). We also examined 7 populations of the species *C. humeya* for which the reproductive system has not yet been studied. Overall, our sampling comprised 89 individuals (58 workers and 31 queens) span across 71 localities, covering the entire range of these 3 species (Fig. 2 and Table S1, Supplementary Material). As outgroups, we used 4 individuals of *C. mauritanica* (another species of the *altisquamis* group) from one location in Morocco.

Total genomic DNA was extracted from 4 legs of each individual using a standard phenol-chloroform extraction protocol (Sambrook et al., 1989). Eight markers were sequenced: a fragment of the mitochondrial cytochrome *c* oxidase subunit I gene (COI, 626 bp) and seven nuclear markers: AbdA (from the *Abdominal A* gene, 606 bp), 28S (28S rRNA gene, 1152 bp), 18S (18S rRNA gene, 713 bp), Wg (*wingless* gene, 416 bp), Lr (long-wavelength rhodopsin, 414 bp), EF1 (elongation factor 1, 344 bp) and EF2 (elongation factor 2, 389 bp). These markers are usually informative for phylogenetic inferences in ants (Brady et al., 2006; Moreau et al., 2006; Ward and Downie, 2005; Ward et al., 2010), and primers are widely available. Primer sequences, annealing temperatures and bibliographic sources are provided in Table S2 (Supplementary Material). Low polymorphism was found for the markers AbdA, 28S and 18S; they were therefore discarded from our analyses.

PCR amplifications were carried out in 25 μL mixes containing 2.0 μL of DNA template, 2.0 mM buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs (MBI Fermentas), 0.4 mM of each primer and 2.0 units of *Taq* polymerase (MBI Fermentas), and performed on a TProfessional thermocycler (Biometra). The thermal cycling program for the amplification included a denaturation step (3 min at 94 °C) followed by 35 cycles of denaturation (30 s at 92 °C), annealing (40 s at 48–60 °C, depending on the primers; Table S2), elongation (45 s at 72 °C), and a final elongation step (10 min at 72 °C). PCR products were purified with the Nucleofast PCR purification kit

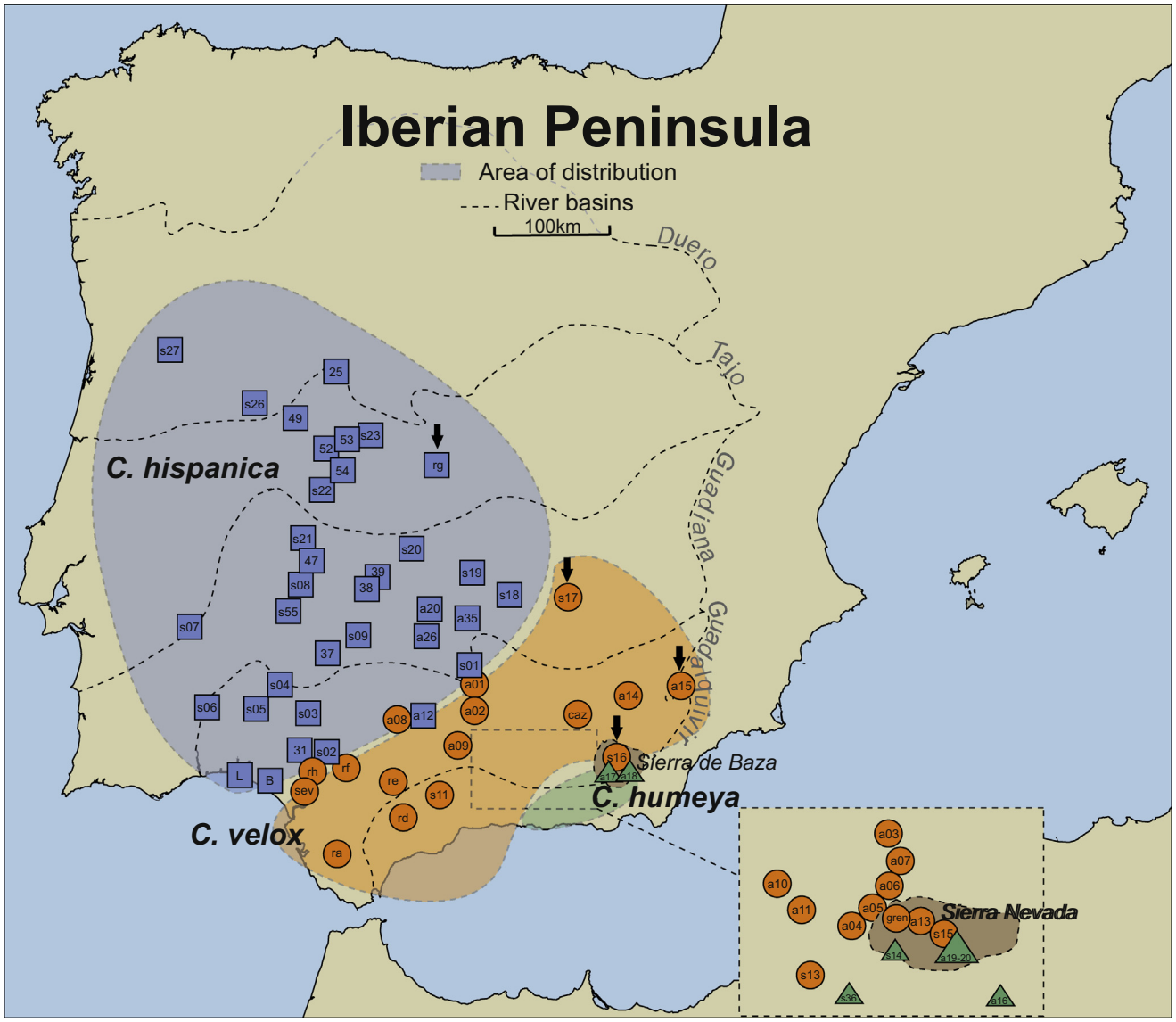

Fig. 2. Sampling sites and distribution areas (dotted grey areas) of the three *Cataglyphis* species studied, in the Iberian Peninsula. Rivers basins are delimited by dotted lines. Squares: *C. hispanica*; circles: *C. velox*; triangles: *C. humeya*. Arrows indicate the localities Vs16, Vs17, Va15 and Hrg.

(Macherey-Nagel, Düren, Germany) and sequenced with the ABI BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, U.S.A.). Sequencing was performed on an ABI 3730 Genetic Analyzer (Applied Biosystems). Base calling and contig assemblies were performed using CodonCode Aligner (CodonCode Corporation, Dedham, MA, USA). Sequences were aligned using the MUSCLE algorithm (Edgar, 2004) implemented in CodonCode Aligner. All DNA sequences were deposited in the GenBank database (Benson et al., 2015); accession numbers are given in Table S1 (Supplementary Material).

2.2. Network and haploweb analyses

Phylogenetic relationships between haplotypes were represented on network analyses for the mitochondrial marker COI and the four nuclear markers Wg, Lr, EF1 and EF2, on both workers and queens. Networks were produced by the median-joining method (MJM; Bandelt et al., 1999) implemented in the program NETWORK v.4.6.1.1 (available at http://www.fluxus-engineering.com/). In hybridogenetic populations, queens are produced asexually and harbor only lineage-specific genes, while workers are all hybrids. In the absence of prior information on the reproductive system of each population, we chose to treat all workers as potential hybrids and to base our nuclear analyses on the inferred parental haplotypes. Parental haplotypes were inferred using PHASE with 0.90 thresholds and using known haplotypes (identified from homozygous individuals) as references (Stephens et al., 2001); the PHASE input files were constructed using SeqPHASE (Flot, 2010). All samples were sequenced for their mitochondrial gene, except 8 individuals from four populations (*s10*, *caz*, *a14* and *a15*) due to amplification failure. For the nuclear markers, curves connecting the haplotypes found co-occurring in heterozygous individuals were drawn atop each haplotype network (haplonet), thereby turning it into a haplotype web (haploweb; Flot et al., 2010). This makes it possible to detect groups of individuals that share a common pool of alleles for a given marker and may therefore by considered as species.

2.3. Phylogenetic analyses

Due to the low polymorphism of the nuclear genes (see Results), the construction of phylogenetic trees was performed using only the mitochondrial marker COI (for information, the phylogenetic trees for each nuclear gene are given in the Supplementary Material, see Fig. S1). The simplest models of nucleotide substitution fitting our data were selected on the basis of the Akaike information criterion corrected for small samples (AICc; Akaike 1974; Hurvich and Tsai 1989). We calculated the best partitioning scheme and its substitution models using PartitionFinder v1.0.1 (Lanfear et al., 2012). We investigated three different partitions for the mitochondrial marker COI (1st, 2nd and 3rd codon positions). PartitionFinder recommended two partitions. The Hasegawa-Kishino-Yano (HKY; Hasegawa et al., 1985) model with a proportion of invariant sites (+I) and gamma distribution of variation (+G) was identified as the most appropriate model for the first partition containing the 1st and 2nd codon positions. The General Time-Reversible (GTR; Lanave et al., 1984; Tavaré 1986) model +I +G was selected as the best evolutionary model for the second partition (comprising the 3rd codon positions of the COI mitochondrial marker).

The phylogenetic relationships among COI haplotypes were investigated using Maximum Likelihood (ML) and Bayesian Inference (BI). The ML analysis was carried out with the PhyML online web server (Guindon and Gascuel, 2003). Nodal support was assessed by bootstrap resampling (1000 pseudoreplicates). Bayesian inference (BI) analysis was carried out with MrBayes version 3.2 (Ronquist et al., 2012). For Bayesian inference, four Markov chains Monte Carlo (MCMC; 3 hot and 1 cold) were run over 4 million generations and a tree was sampled every 100 generations. To ensure that the MCMC chains were not trapped in a local optimum, Bayesian analyses were run twice independently. Trees congruence was confirmed in Tracer v.1.7.4 (Drummond et al., 2012) if the Effectives Samples Sizes (ESSs) for the parameters and the analysis generated similar results across the runs, with minimum ESSs of 200 for each parameter. The first 25% sampled trees (obtained before parameter stabilization occurred) were discarded as burn-in. The remaining trees were used to assess tree topology, branch lengths and nodal support using posterior probabilities.

2.4. Phylogeographic analyses

Because the polymorphism of the nuclear genes was low, phylogeographic analyses were performed using the mitochondrial marker COI. Comparisons within and between species were performed based on nucleotide diversity and genetic divergence using MEGA v. 5.0 (Tamura et al., 2011).

Genetic variations at different hierarchical levels (among species, among localities within species and within localities) were quantified by performing an Analysis of Molecular Variance (AMOVA) using Arlequin v. 3.5.1.2 (Excoffier and Lischer 2010). The variance components were tested statistically using non-parametric randomization tests on 10,000 permutations.

Patterns of genetic divergence among sampling localities were explored using SAMOVA (Spatial Analysis of Molecular Variance; Dupanloup et al., 2002) as implemented in SPADS v.1.0 (Dellicour and Mardulyn, 2014). The SAMOVA algorithm identifies groups of populations that are phylogeographically homogeneous and maximally differentiated from each other, taking into account the geographic distances. The presence of 2–71 (number of localities) genetic groups (K) was tested. The inter-group differentiation (Φ_{CT}), which represents the part of the molecular variation explained by a K-group structure, was estimated for each value of K. The maximal value of Φ_{CT} is expected to yield the most likely number of genetic groups. Since our study species have non-overlapping areas of distribution, we performed three independent analyses. To study geographical structuring within species, analyses were first performed for *C. hispanica* and *C. velox* separately (data were not sufficient for analyses of *C. humeya*). Then, to examine inter-specific differentiation, an analysis was performed on the combined data set of the three species.

The significance of the correlation between genetic differentiation and geographical distance was assessed using a Mantel test (Mantel, 1967), as implemented in Arlequin v.3.5.1.2 (Excoffier and Lischer, 2010).

2.5. COI-based species delimitation

We further examined the phylogenetic status of *C. hispanica*, *C. humeya* and *C. velox* on the basis of three tree-based species delimitation methods: the Automatic Barcode Gap discovery (ABGD; Puillandre et al., 2012), the Generalized Mixed Yule-Coalescent model (GMYC; Pons et al., 2006) and the Poisson Tree Processes approach (PTP; Zhang et al., 2013). These methods are frequently used to delimit independently evolving species based on single-locus data (e.g., Lahaye et al., 2008; reviewed in Fontaneto et al., 2015; Fujita et al., 2012). ABGD detects significant gaps between intraspecific and interspecific diversity (barcode gap) and uses it to partition the data (*i.e.*, initial partitioning). The procedure is recursively applied to newly obtained groups of sequences to conduct a second round of splitting (*i.e.*, recursive partitioning). ABGD analyses were conducted on the web interface (http://wwwabi.snv.jussieu.fr/public/abgd/). Prior maximum divergence of intraspecific

diversity P was investigated over a range of 0.001 to 0.010 under a Kimura's 2-parameter model (K2P, Kimura, 1980). In contrast, both PTP and GMYC models detect shifts in branching rates between intra- and interspecies branching events (Fujisawa and Barraclough, 2013; Zhang et al., 2013). Branching patterns within genetic clusters reflect the neutral coalescent processes supposed to occur within species, whereas branching patterns among genetic clusters reflect speciation events (modeled as a Yule process). These methods exploit the predicted difference in branching rate under the two modes of lineage evolution, assessing the point of highest likelihood of the transition (*i.e.*, threshold; Pons et al., 2006; Zhang et al., 2013). One drawback of these approaches, however, is that they can only delineate species that are monophyletic in the gene tree on which the analysis is run (Fontaneto et al., 2015); besides, GMYC-based approach, and possibly PTP approaches as well, do not perform well on species-poor datasets (Dellicour and Flot, 2015).

As recommended by Tang et al. (2014), we used a model-based gene tree (obtained using PhyML; Guindon and Gascuel, 2003) for PTP analyses and ultrametric trees generated with BEAST (Drummond et al., 2012) for the GMYC analysis. For the GMYC model, we used a Bayesian implementation of this method (bGMYC; Reid and Carstens, 2012). This procedure allows multiple-threshold algorithms and improves delimitation by performing more exhaustive searches based on Bayesian Markov Chains Monte Carlo. The bGMYC R package (R Development Core Team, 2010; Reid and Carstens, 2012) was run on 100 ultrametric gene trees obtained using BEAST v.1.7.4 (Drummond et al., 2012). The BEAST input files were generated using BEAUti v.1.7.4 (Drummond et al., 2012) following a relaxed molecular clock model (uncorrelated lognormal) and the two partition models suggested by PartitionFinder v1.0.1 (Lanfear et al., 2012). MCMC chains were run over 20 million generations and a tree was sampled every 1000 generations. Tree congruence across runs and Effectives Sample Sizes (ESSs) were confirmed visually using Tracer v.1.7.4. The first 25% trees were discarded as burn-in and 100 trees were randomly selected among the remaining trees to run bGMYC package. In contrast with bGMYC, PTP does not require an ultrametric tree as input; instead this model uses branch lengths to estimate the mean expected number of substitutions per site between two branching events. PTP species delimitations were conducted on the Web server of the Exelixis Lab (http://species.h-its.org/ptp/), using rooted phylogenetic tree obtained using PhyML. The analysis was run with the following parameters: 500,000 MCMC generations, 100 thinning trees and 10% burn-in.

3. Results

3.1. Haplotype variation

Among the 147 nuclear sequences analyzed (excluding the outgroup *C. mauritanica*), we found 5 variable nucleotide positions (SNPs) for Wg resulting in 5 unique haplotypes, 8 SNPs for Lr (8 haplotypes), 9 SNPs for EF1 (7 haplotypes) and 4 SNPs for EF2 (5 haplotypes). For the mitochondrial marker COI, a total of 222 nucleotide positions were variable (176 parsimony informative). For 8 individuals from four populations (*s10*, *caz*, *a14* and *a15*), reliable COI sequences could not be obtained because of amplification failure. A total of 72 unique haplotypes were found among the 81 COI sequences analyzed.

3.2. Haploweb analyses of the nuclear markers

For all nuclear genes, the haplotypes of *C. hispanica* clearly differed from those of *C. velox* and *C. humeya* (Fig. 3a). Except for the weakly polymorphic marker Wg, the haplotypes of *C. hispanica* segregated into two distinct genetic clusters that were only connected by dashed lines on the haplowebs (*i.e.*, by sexually produced workers). This clustering was consistent with the assignment of individuals to two different lineages based on the microsatellite loci (Darras et al., 2014a); in other words, all the individuals previously assigned to one microsatellite lineage by Darras et al., (2014a) belonged to one cluster, while all the individuals assigned to the other microsatellite lineage belong to the other cluster. In line with a hybridogenetic mode of reproduction, 100% of *C. hispanica* queens were homozygous, while 100% of workers were heterozygous and harbored one allele from each genetic lineage. All the haplotypes of each lineage grouped together, regardless of their geographical origin. Almost all individuals of each lineage shared the same haplotype, indicative of a low intra-lineage polymorphism in this species.

The situation of *C. velox* and *C. humeya* is more puzzling. These two morphologically defined species shared many haplotypes and were therefore not distinguishable molecularly; in addition, haplotypes did not segregate into hybridogenetic lineages. Moreover, a variable proportion of workers were homozygous (10–60% for *C. velox* and 61–86% for *C. humeya*). Such a pattern occurred in the 7 populations of *C. humeya* studied and the 25 newly sampled populations of *C. velox*; quite surprisingly, it was also observed in the two populations of *C. velox* where a hybridogenetic mode of reproduction had been previously documented based on microsatellite loci. Two localities of *C. velox* are particularly interesting because workers were strictly homozygous for all markers (*i.e.*, complete absence of detectable hybridogenesis): *a15* in the most eastern part of the distribution, and *s16* in the Sierra de Baza (Fig. 3a).

3.3. Phylogenetic analyses of the COI marker

ML and BI phylogenetic analyses of COI led to similar topologies, hence only the BI tree and the MJM network are presented here (Figs. 3b and 4). Both ML bootstrap values and BI posterior probabilities are indicated on the BI tree to estimate nodal support. For each species, mitochondrial haplotypes were structured following a geographical pattern: closely related haplotypes always came from adjacent localities. Mitochondrial haplotypes did not segregate into hybridogenetic lineage-specific clusters. This pattern occurred for *C. hispanica* despite the fact that hybridogenetic lineages are well defined on both nuclear sequence markers (see above) and microsatellite markers (Darras et al., 2014a). It is also observed in the two populations of *C. velox* where two lineages were previously detected based on microsatellites loci (Eyer et al., 2013). This may be explained by introgression or capture of mtDNA among nuclear lineages (Darras and Aron, 2015) or, alternatively, by incomplete lineage sorting. Moreover, polymorphism of mitochondrial DNA was extremely high; each locality was characterized by one or several private haplotypes (except the adjacent localities *52/53* and *38/39* in *C. hispanica*, *a03*/*a08* in *C. velox* and *17/18* in *C. humeya*), resulting in strongly differentiated populations. Both the phylogenetic tree and the median-joining network highlighted this highly structured pattern, with the occurrence of several mitochondrial subgroups in each species (Figs. 3b and 4).

C. hispanica formed a well supported monophyletic group. However, this species was divided into two major phylogroups, which correspond to the southern and northwest regions of its distribution (Phylogroups I and II, respectively; Fig. 3b). One haplotype (*Hrgw*) appeared quite isolated, which may result from a lack of sampling in this region.

C. velox and *C. humeya* together formed a clade, but the two species were not reciprocally monophyletic. Although the two species appeared less structured than *C. hispanica*, some geographical sub-

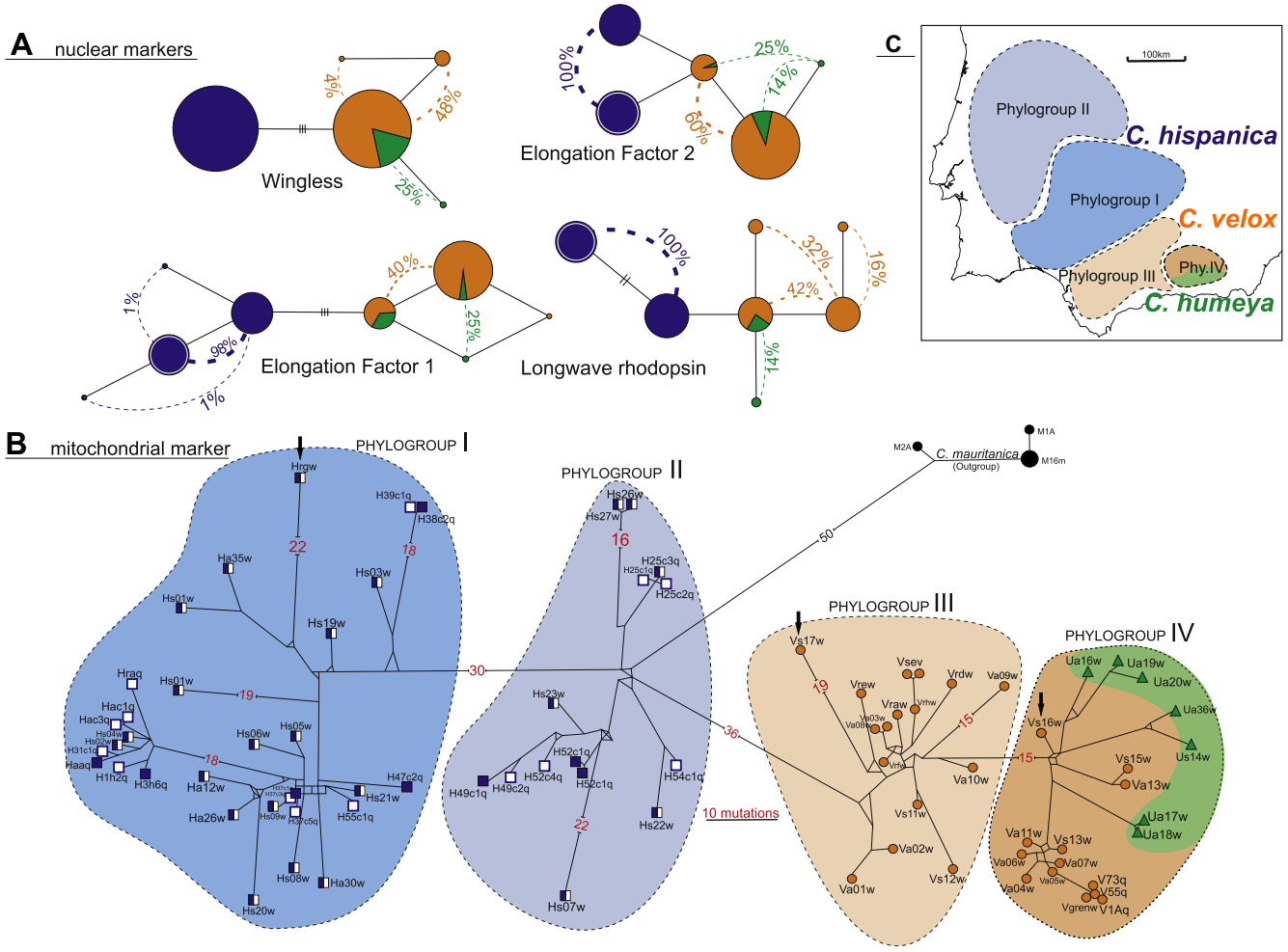

Fig. 3. Haplotype webs (haplowebs) for the four nuclear genes studied (A) and haplotype network (haplonet) for the mitochondrial marker COI (B). Each shape and color (*i.e.*, blue squares: *C. hispanica*; orange circles: *C. velox*; green triangles: *C. humeya*) represents a species. Circle sizes are proportional to the number of haplotypes observed in the dataset and the branch lengths indicate the number of mutations between haplotypes. In the nuclear haplowebs (A), dashed lines between two haplotypes represent the proportion of heterozygous workers carrying these two haplotypes (for hybridogenetic lineages, one expects 100% of the workers to be heterozygous); the sampled queens were all homozygous. The thickness of these lines is proportional to the number of individuals. For mitochondrial network (B), the number of mutations (>15) is indicated on the branches. Dashed lines delimit phylogroups as defined in the phylogenetic analyses. For *C. hispanica*, each of the two nuclear lineages is represented by a filled condition (*i.e.*, full or empty for pure-lineage males and queens; and mixed for hybrid workers). Arrows point to the haplotypes Vs16w, Vs17w and Hrgw. The geographic distribution of the phylogroups is indicated on a map (C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

grouping was observed. Phylogroup III comprised the populations of *C. velox* inhabiting the Western lowland in the Guadalquivir's valley. Phylogroup IV was composed of both *C. velox* and *C. humeya* individuals from the high-altitude populations of the Sierra Nevada. The haplotype *Vs17w* was the sole representative of *C. velox* from the eastern distribution; samples of the 4 *C. velox* populations from this region (*s10*, *caz*, *a14* and *a15*) could not be used due to amplification failures.

3.4. AMOVA analyses

For the COI gene, the mean percentage of sequence divergence between species under Kimura's 2-parameter model (K2P) ranged from 6.3% (between *C. humeya* and *C. velox*) to 14.1% (between *C. hispanica* and *C. velox*) and 14.8% (between *C. hispanica* and *C. humeya*).

The mean diversity within species (5.9%) was higher than the mean diversity between species (4.9%). Across the entire mitochondrial data set (excluding the outgroup *C. mauritanica*), the AMOVA showed that 41.8% of the genetic variation was due to differences among the three species studied, whereas 55.1% of the genetic variation was found among localities within species. Almost no genetic variation occurred within localities (3.1%). In line with these results, the SAMOVA indicated a strong spatial pattern across populations. The variation of inter-group differentiation (Φ_{CT}) increased with the number of genetic groups (K) both within species and for all species taken together (Fig. S2). For each analysis, the maximum value of Φ_{CT} (*i.e.*, the most probable K) was found for the maximum value of K, consistent with localities being genetically differentiated from each other. Furthermore, interspecific SAMOVA analysis failed to distinguish between the different species (*i.e.*, SAMOVA does not give the highest Φ_{CT} for K = 3 groups).

Overall, a significant relationship between geographical and genetic distances was detected within species, as well as for all species taken together (Mantel test, $P > 0.001$ for both data sets).

3.5. COI-based species delimitation

Because applying the ABGD approach with the default value for relative gap width (X = 1.5) did not produce a result for our data set, we used the highest value that could be applied (X = 1.0). ABGD

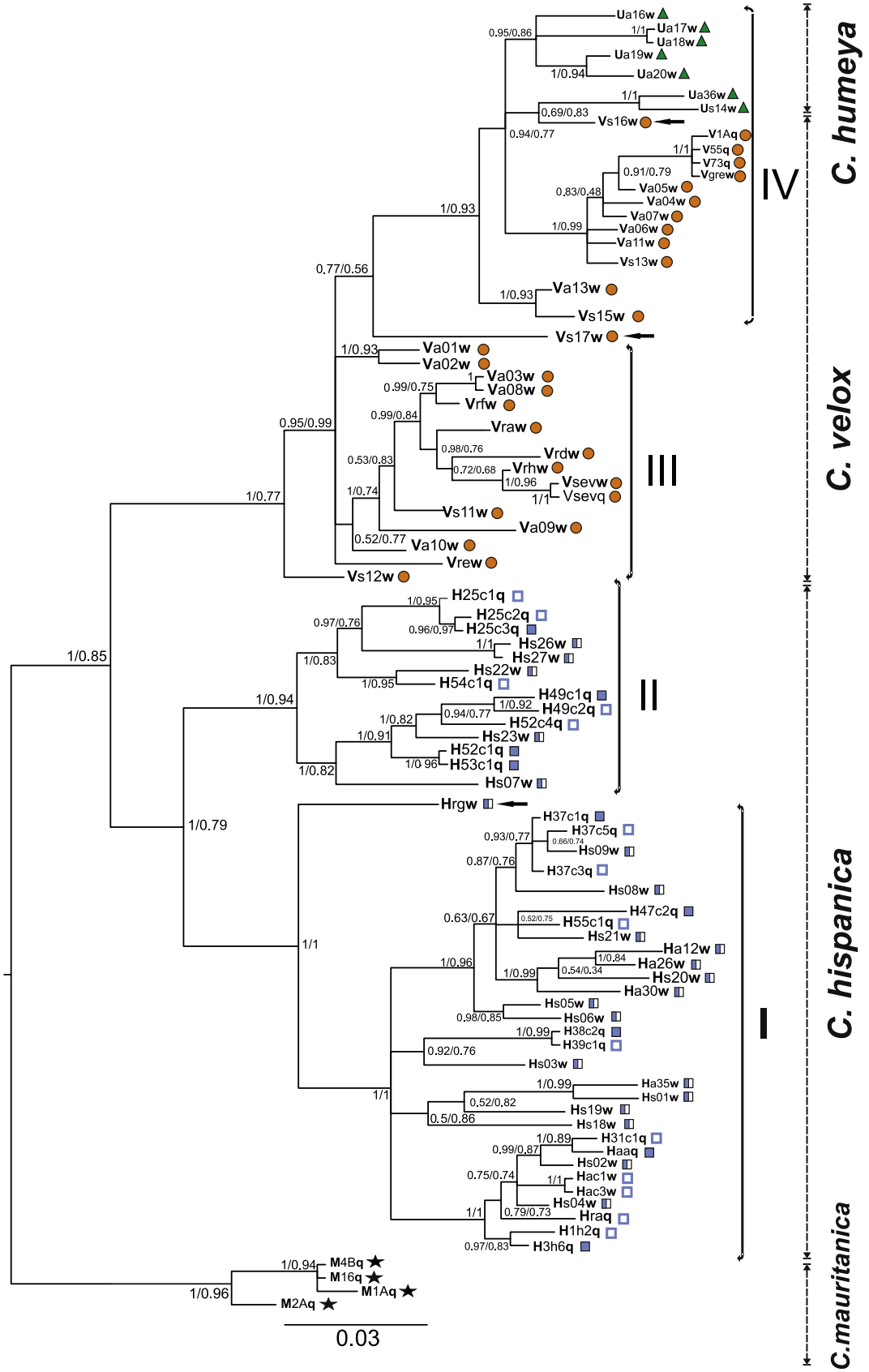

Fig. 4. Bayesian inference tree of 85 COI haplotypes of *Cataglyphis* ants. Bayesian probabilities/bootstrap values (from ML tree) are given to estimate branches support. The tree is rooted using the COI haplotypes of *C. mauritanica* as an outgroup. Each shape (*i.e.*, squares: *C. hispanica*; circles: *C. velox*; triangles: *C. humeya*; star: *C. mauritanica*) represents a species. For *C. hispanica*, each of the two nuclear lineages is represented by a filled condition (*i.e.*, full or empty for pure-lineage males and queens; and mixed for hybrid workers). Arrows point to the haplotypes Vs16w, Vs17w and Hrgw.

analysis failed to distinguish between the four 'morphological' species (*C. hispanica*, *C. humeya*, *C. velox* and the outgroup *C. mauritanica*) and it did not recover either the COI phylogroups defined above. Rather, both initial and recursive partitioning analyses clustered the mitochondrial sequences into 67 candidate species (Fig. 5) with a prior of intraspecific divergence up to 0.0046 (Fig. S3).

Similarly, the bGMYC analysis did not confidently discriminate the four species or the phylogroups using the COI marker (Fig. 5). For *C. hispanica*, two species were suggested, corresponding to the two phylogroups defined above (Fig. 3b). However, the posterior probability of this assignment was lower than 0.5 (X ± SE = 0.380 ± 0.004 and 0.415 ± 0.012 for Phylogroups I and II, respectively). Also, a single species comprising *C. humeya* and *C. velox* was proposed, but with a probability less than 0.5 (X ± SE = 0.334 ± 0.004). Rather, bGMYC analysis confidently clustered the mitochondrial data set into 71 putative species ($P > 0.95$ level), each corresponding to a single locality.

Equally, the Poisson Tree Processes method (PTP) failed to differentiate the four 'morphological' species based on our mitochondrial COI marker, and strongly overestimated the number of species (Fig. 5). This model considered every locality as a species and did not support any other cluster with a posterior probability higher than 0.50 (X ± SE = 0.167 ± 0.157; Fig. S4).

4. Discussion

Our results bring two insights into the phylogeography of three desert ants belonging to the *Cataglyphis* group *altisquamis* in which reproduction by social hybridogenesis and mitochondrial capture has been documented (Leniaud et al., 2012; Eyer et al., 2013; Darras and Aron, 2015). First, they do not provide support for hybridogenesis in the populations sampled of *C. velox* and *C. humeya*. Nuclear haplotypes do not clearly segregate into hybridogenetic lineages and a variable proportion of workers are homozygous (10–60% for *C. velox* and 61–86% for *C. humeya*). The results obtained for *C. velox* diverge from those of a previous study based on microsatellite analyses (Eyer et al., 2013) indicating hybridogenetic lineages in two populations of this species. They also contrast with the genetic pattern found in *C. hispanica* where 100% of workers are heterozygous, and harbor one allele from each genetic lineage across its whole distribution range (Darras et al., 2014a). This suggests that hybridogenetic lineages are either absent in some populations of *C. velox* and *C. humeya* and/or too recent to allow

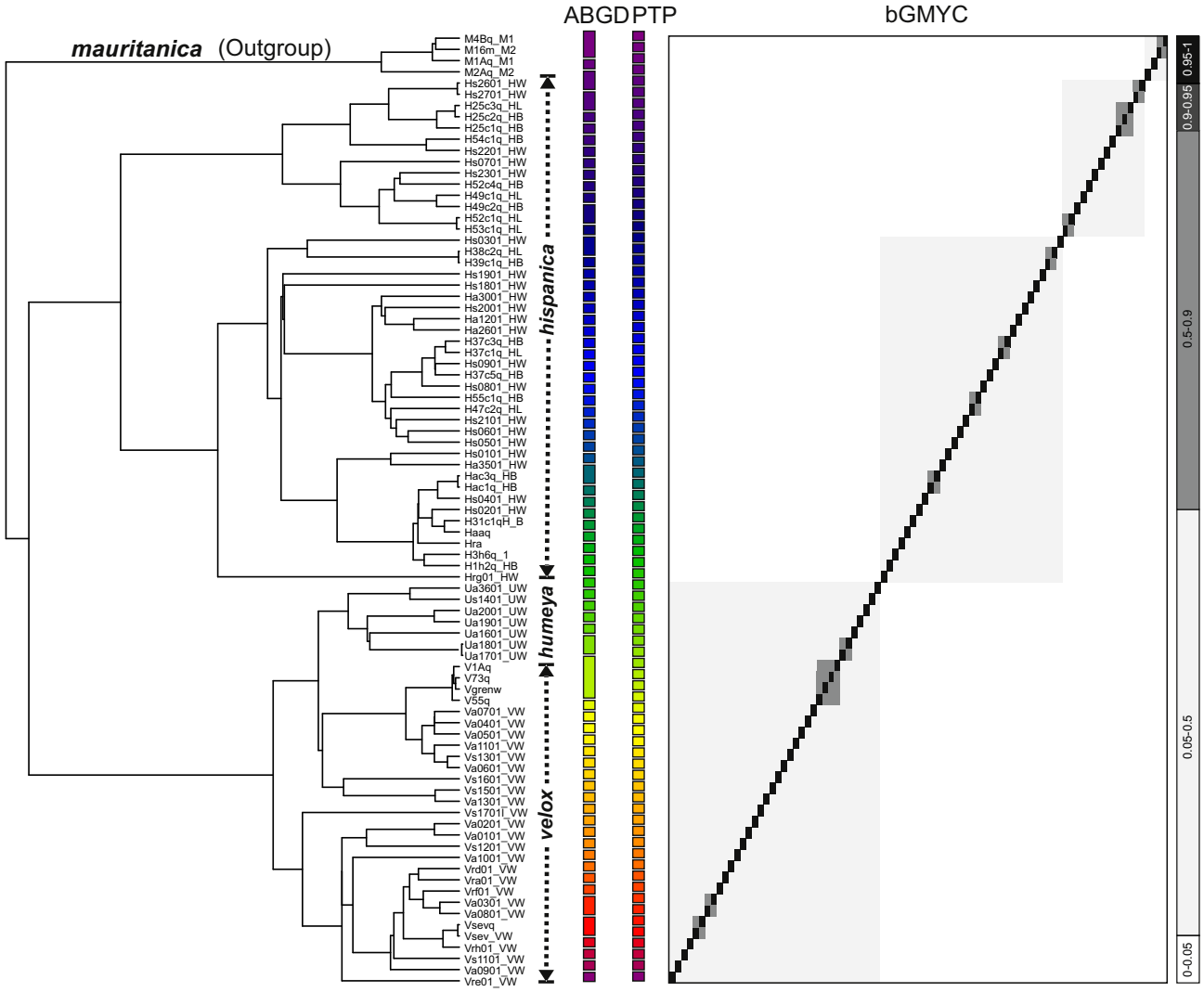

Fig. 5. Results of species delimitation analyses conducted on COI marker using ABGD, PTP and bGMYC methods. A ultrametric tree was obtained using BEAST based on a Bayesian analysis of 85 COI sequences of *Cataglyphis* ants. bGMYC results are given using a colored individual-by-individual matrix. Cells are colored according to the posterior probability that the corresponding individuals are conspecific. Black cells, indicate pairs of individuals that are considered conspecific with a high posterior probability (>0.95). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

a complete discrimination between distinct genetic lineages based on our set of nuclear genes. Due to mitochondrial introgression between lineages (Darras and Aron, 2015), the COI marker is not lineage specific, hence, unsuitable to further investigate the segregation of lineages in the three species studied. Second, we found that COI is hypervariable with higher diversity within species than between species. This marker reveals a strong geographic structure: different mitochondrial haplotypes occur in each locality sampled. This unusual mitochondrial pattern results in absurd species delimitation as every locality fractionates into a 'putative species', precluding the use of COI for delineating species in this group.

4.1. Nuclear haplowebs shed light on the distribution and evolution of hybridogenesis

Our three study species exhibit different patterns of heterozygosity (Fig. 3a). The 35 populations of *C. hispanica* sampled consist of a well-defined pair of genetic lineages, and all workers are heterozygous with one allele from each lineage. This supports that hybridogenesis occurs across the entire range of *C. hispanica*, with very little nuclear diversity within each lineage. In contrast with this nuclear uniformity, mtDNA analyses (both the phylogenetic reconstruction and the bGMYC analysis) reveal two major phylogroups that correspond to the northwestern and southern regions of the species' distribution (Fig. 3b). This suggests the possibility of two cryptic species, or the existence of an additional lineage pair in *C. hispanica*. In this system, the two members of a lineage pair must coevolve for the production of viable workers. Whether northern and southern lineage crosses produce functioning colonies awaits mating experiments between geographically distant partners belonging to alternative lineages. Formation of multiple lineage pairs through long-term isolation of geographically co-adapted lineages or population bottlenecks has been also reported in hybridogenetic populations of the harvester ant *Pogonomyrmex* (Schwander et al., 2007). However, it is important to note that this potential COI species boundary is not supported by any of the nuclear markers analyzed in the present study.

The subject for *C. velox* and *C. humeya* is less clear. First, the phylogenetic status of these two species remains ambiguous: their haplotypes belong to the same FFRs (Field For Recombination; Doyle, 1995) on the nuclear haplowebs and the same phylogroup on the COI network, suggesting that *C. velox* and *C. humeya* are morphotypes of a single species. Second, even though social hybridogenesis was previously documented in at least two populations of *C. velox*, the number and distribution of hybridogenetic lineages in *C. velox* and *C. humeya* remains uncertain. The nuclear haplowebs do not reveal any pair of genetic lineages connected by heterozygous workers within these species, unlike *C. hispanica* where this feature of hybridogenesis is clear. Another striking difference between these species is that only a small percentage of workers of *C. humeya* (14–39%) are heterozygous at nuclear markers (but 40–90% for *C. velox*), whereas workers of *C. hispanica* are nearly all heterozygous, as expected under hybridogenesis. At least three hypotheses may explain the absence of well-defined pairs of nuclear lineages connected through hybridogenesis in *C. velox* and *C. humeya*. (i) Hybridogenesis may be absent in some populations of *C. velox* and in all populations of *C. humeya*. (ii) Hybridogenesis may exist in both species, but allows occasional gene flow between genetic lineages. Such gene flow might stem from the production of rare hybrid queens, which could be derived from inter-lineage mating and/or asexual reproduction (parthenogenesis) by hybrid workers (Darras and Aron, 2015; Schwander and Keller, 2012). (iii) Hybridogenesis may be too recent to allow discriminating between distinct genetic lineages based on our set of nuclear genes because of insufficient sequence divergence between lineages and/or incomplete lineage sorting (ILS). Thus, the fact that we did not detect lineages segregation on nuclear genes in *C. velox* and *C. humeya* does not exclude the possibility that genetic lineages exist and comes to light using microsatellite markers, as found previously in two populations of *C. velox* (Eyer et al., 2013).

4.2. Evidence for mitochondrial introgression

For the three species studied, our data show that mitochondrial haplotypes do not cluster into hybridogenetic lineages. This is apparent in all populations of *C. humeya* (n = 7), and *C. velox* (n = 27) sampled, including the two populations of *C. velox* where hybridogenetic lineages were previously detected using microsatellite markers. This is also obvious for *C. hispanica* COI sequences, despite that fact that hybridogenetic lineages are well defined when using both microsatellite (Darras et al., 2014a; Darras and Aron, 2015) and nuclear sequence markers (this study). Discrepancies between nuclear and mitochondrial markers are usually explained by a difference in dispersal of sexes, incomplete lineage sorting (ILS) and introgression. In hybridogenetic species, neither differences in sex-specific dispersal nor ILS may account for such incongruences. This is because males and queens are clonally produced and harbor only the maternal genome. Nuclear and mitochondrial genes are therefore expected to follow the same pattern of inheritance. In the absence of mitochondrial gene flow, the two lineages are expected to accumulate significant genetic differences on mtDNA, especially if lineages have diverged from each other a long time ago (Darras et al., 2014a). Yet, our data show that mtDNA varies with geography in all 3 species studied; closely related mitochondrial haplotypes indeed come from adjacent localities, regardless of the hybridogenetic lineage they belong to. Thus, the most parsimonious explanation for the sharing of mitochondrial haplotypes between sympatric, but genetically divergent lineages is mitochondrial introgression (Darras and Aron, 2015).

4.3. Diversity and structure of the mitochondrial gene

A surprising finding of this study is the higher intraspecific diversity of mtDNA compared to interspecific: 55.1% of the mitochondrial variability was found within morphological species, whereas 41.8% of the variability was found between species. Within species, the mitochondrial diversity is geographically distributed, exhibiting different mitochondrial haplotypes for each locality sampled. This results in strong genetic differentiation among populations without clear genetic gap between species. The three species delimitation models (ABGD, bGMYC and PTP) failed to distinguish between the 3 morphospecies based on the mitochondrial COI marker. All analyses overestimated the number of species by assigning the specimens from each sampling locality to a different putative species. This shows that mitochondrial COI marker alone is not suitable to delineate species in this group. This result may also stem from models and assumptions of the species delimitation being inappropriate. Similar over-splitting results have been reported for other species where reduced levels of gene flow (Papadopoulou et al., 2008), high population-genetic structure (Lohse, 2009; but see Papadopoulou et al., 2009), the occurrence of singletons (Lim et al., 2012) or species-poor dataset (Dellicour and Flot, 2015) hampered the species delimitation (Dasmahapatra et al., 2010; DeSalle et al., 2005; Elias et al., 2007; Song et al., 2008; reviewed in Taylor and Harris, 2012). Neither bGMYC nor ABGD methods test whether the model fits the data significantly better than a pure coalescence one (*i.e.*, all samples are conspecific) (Dellicour and Flot, 2015; Reid and Carstens, 2012). For highly structured taxa, as found in the present study, the use of such species delimitation model can be confusing because virtually every locality potentially fractionates into a 'putative species' (Boyer et al., 2007; Hickerson et al., 2006; Meier et al., 2006).

The strong genetic differentiation across populations found in *C. hispanica*, *C. humeya* and *C. velox* using mitochondrial DNA is quite unusual in animals. High genetic structures at mtDNA have been documented at continental scale among populations separated by several million years (Ellsworth et al., 1994; Oremus et al., 2009). In rare cases, it results from high fidelity to a feeding ground or reproductive site (e.g., eels: Avise et al., 1986; turtles: Fitzsimmons et al., 1997; bats: Ramos Pereira et al., 2009; Killer Whales: Hoelzel et al., 2007; common warthogs: Muwanika et al., 2006; sea birds: Friesen et al., 2007; Welch et al., 2012; dolphins: Möller et al., 2007; Wiszniewski et al., 2010). In the 3 *Cataglyphis* species studied, population structuration most likely stems from the particular mode of colony foundation. These species found new colonies by dependent foundation (Eyer et al., 2013; Leniaud et al., 2012): young queens mate close to or in the natal nest and disperse by foot with a worker force to establish a new colony few meters away from the natal nest. Short-range dispersal typically results in a pattern of genetic isolation-by-distance within populations and enhances genetic differentiation between populations (Leppänen et al., 2013; Liautard and Keller, 2001; Seppä and Pamilo, 1995). High genetic viscosity of populations was reported in *Cataglyphis mauritanica* (Knaden and Wehner, 2006), another species of the *altisquamis* group where new colonies are also initiated by dependent foundation. In this species, Knaden and Wehner (2006) showed that all nests in a locality share a unique mtDNA haplotype. In contrast, no population differentiation was observed in *C. bicolor*, a desert ant belonging to another taxonomic group (*bicolor* group), where queens proceed in long-range dispersal through nuptial flights (Knaden and Wehner, 2006).

5. Conclusion

The present work shows how hybridization and mitochondrial introgression shape complex phylogeographic patterns in *Cataglyphis* desert ants. First, patterns on nuclear DNA greatly diverge between species. In all populations of *C. hispanica*, two genetic lineages co-exist and interbreed to produce workers. In contrast, nuclear genes do not segregate into hybridogenetic lineages in the study populations of *C. velox* and *C. humeya*. The fact that genetic lineages were previously uncovered with microsatellite markers in two populations of *C. velox* suggests that hybridogenesis may be less frequent than initially appreciated in this species or too recent to be evidenced with our nuclear markers. Second, in all three species, phylogeographic patterns differ between nuclear and mtDNA. Mitochondrial haplotypes do not separate into hybridogenetic lineage-specific clusters, even in *C. hispanica* where lineages are well defined at both nuclear microsatellite markers and nuclear sequences. Finally, our data show that mitochondrial gene is unreliable for species delimitation in *Cataglyphis* of the *altisquamis* group, due to its hypervariability and strong geographical structure. Overall, this study illustrates how unconventional reproductive systems may affect DNA inheritance, and how morphology as well as nuclear and mitochondrial DNA molecular analyses may lead to different estimates regarding evolutionary history and species delimitation.

Data accessibility

DNA sequences: GenBank accession numbers XXXXXX–XXXXXX.

Author contributions

PAE, LL and SA designed the research; field data collection was carried out by PAE and AT; PAE and SA performed the research; PAE analyzed the data with assistance from LL; PAE and SA wrote the paper.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.08.020.

References

Agosti, D., 1990. Review and reclassification of *Cataglyphis* (Hymenoptera, Formicidae). J. Nat. Hist. 24, 1457–1505.
Aron, S., Mardulyn, P., Leniuad, L., 2016. Evolution of reproductive traits in *Cataglyphis* desert ants: mating frequency, queen number and thelytoky. Behav. Ecol. Sociobiol. 70, 1367–1379.
Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Automat. Cont. 19, 716–723.
Avise, J.C., Helfman, G.S., Saunders, N.C., Stanton Hales, L., 1986. Mitochondrial DNA differentiation in North Atlantic eels: population genetic consequences of an unusual life history pattern. Proc. Nat. Acad. Sci. USA 83, 4350–4354.
Bandelt, H.J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48.
Benson, D.A., Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Sayers, E.W., 2015. GenBank. Nucl. Acid. Res. 43, D30–D35.
Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K., Das, I., 2007. Cryptic species as a window on diversity and conservation. Trends Ecol. Evol. 22, 148–155.
Boyer, S.L., Baker, J.M., Giribet, G., 2007. Deep genetic divergences in *Aoraki denticulata* (Arachnida, Opiliones, Cyphophthalmi): a widespread 'mite harvestman' defies DNA taxonomy. Mol. Ecol. 16, 4999–5016.
Brady, S.G., Schultz, T.R., Fisher, B.L., Ward, P.S., 2006. Evaluating alternative hypotheses for the early evolution and diversification of ants. Proc. Nat. Acad. Sci. USA 103, 18172–18177.
Castoe, T.A., de Koning, A.P.J., Kim, H.-M., Gu, W., Noonan, B.P., et al., 2009. Evidence for an ancient adaptive episode of convergent molecular evolution. Proc. Nat. Acad. Sci. USA 106, 8986–8991.
Dahbi, A., Lenoir, A., Tinaut, A., Taghizadeh, T., Franeke, W., Hefetzs, A., 1996. Chemistry of the postpharyngeal gland secretion and its implication for the phylogeny of Iberian *Cataglyphis* species (Hymenoptera: Formicidae). Chemoecology 7, 163–171.
Dahbi, A., Hefetz, A., Lenoir, A., 2008. Chemotaxonomy of some *Cataglyphis* ants from Morocco and Burkina Faso. Biochem. Syst. Ecol. 36, 564–572.
Darras, H., Leniaud, L., Aron, S., 2014a. Large-scale distribution of hybridogenetic lineages in a Spanish desert ant. Proc. R. Soc. Lond. B 281, 20132396.
Darras, H., Kuhn, A., Aron, S., 2014b. Genetic determination of female castes in a hybridogenetic desert ant. J. Evol. Biol. 27, 2265–2271.
Darras, H., Aron, S., 2015. Introgression of mitochondrial DNA among lineages in a hybridogenetic ant. Biol. Lett. 11, 20140971.
Dasmahapatra, K.K., Elias, M., Hill, R.I., Hoffman, J.I., Mallet, J., 2010. Mitochondrial DNA barcoding detects some species that are real, and some that are not. Mol. Ecol. Res. 10, 264–273.
Dávalos, L.M., Cirranello, A.L., Geisler, J.H., Simmons, N.B., 2012. Understanding phylogenetic incongruence: lessons from phyllostomid bats. Biol. Rev. 87, 991–1024.
Degnan, J.H., Rosenberg, N.A., 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. Trends Ecol. Evol. 24, 332–340.
Dellicour, S., Mardulyn, P., 2014. Spads 1.0: a toolbox to perform spatial analyses on DNA sequence data sets. Mol. Ecol. Res. 14, 647–651.
Dellicour, S., Flot, J.-F., 2015. Delimiting species-poor datasets using single molecular markers: a study of barcode gaps, haplowebs and GMYC. Syst. Biol. 64, 900–908.
Dettman, J.R., Jacobson, D.J., Taylor, J.W., 2003. A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote Neurospora. Evolution 57, 2703–2720.
DeSalle, R., Egan, M.G., Siddall, M., 2005. The unholy trinity: taxonomy, species delimitation and DNA barcoding. Philos. T. Roy. Soc. B 360, 1905–1916.
Doucet-Beaupré, H., Blier, P.U., Chapman, E.G., Piontkivska, H., Dufresne, F., Sietman, B.E., Mulcrone, R.S., Hoeh, W.R., 2012. Pyganodon (Bivalvia: Unionoida: Unionidae) phylogenetics: a male and female transmitted mitochondrial DNA perspective. Mol. Phylogenet. Evol. 63, 430–444.

Doyle, J.J., 1995. The irrelevance of allele tree topologies for species delimitation, and a non-topological alternative. Syst. Bot. 20, 574–588.
Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.
Dupanloup, I., Schneider, S., Excoffier, L., 2002. A simulated annealing approach to define the genetic structure of populations. Mol. Ecol. 11, 2571–2581.
Dupuis, J.R., Roe, A.D., Sperling, F.A.H., 2012. Multi-locus species delimitation in closely related animals and fungi: one marker is not enough. Mol. Ecol. 21, 4422–4436.
Edgar, R.C., 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5, 113–116.
Elias, M., Hill, R.I., Willmott, K.R., Dasmahapatra, K.K., Brower, A.V.Z., Mallet, J., Jiggins, C.D., 2007. Limited performance of DNA barcoding in a diverse community of tropical butterflies. Proc. R. Soc. Lond. B 274, 2881–2889.
Ellegren, H., Smeds, L., Burri, R., Olason, P.I., Backstrom, N., et al., 2012. The genomic landscape of species divergence in Ficedula flycatchers. Nature 491, 756–760.
Ellsworth, D.L., Honeycutt, R.L., Silvy, N.J., Bickham, J.W., Klimstra, W.D., 1994. Historical biogeography and contemporary patterns of mitochondrial DNA variation in white-tailed deer from the southeastern United States. Evolution 48, 122–136.
Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver.3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Res. 10, 564–567.
Eyer, P.A., Leniaud, L., Darras, H., Aron, S., 2013. Hybridogenesis through thelytokous parthenogenesis in two *Cataglyphis* desert ants. Mol. Ecol. 22, 947–955.
Fitzsimmons, N.N., Moritz, C., Limpus, C.J., Pope, L., Prince, R., 1997. Geographic structure of mitochondrial and nuclear gene polymorphisms in Australian green turtle populations and male-biased gene flow. Genetics 147, 1843–1854.
Flot, J.-F., 2010. SeqPHASE: a web tool for interconverting PHASE input/output files and FASTA sequence alignments. Mol. Ecol. Res. 10, 162–166.
Flot, J.-F., Couloux, A., Tillier, S., 2010. Haplowebs as a graphical tool for delimiting species: a revival of Doyle's "field for recombination" approach and its application to the coral genus *Pocillopora* in Clipperton. BMC Evol. Biol. 10, 372.
Flot, J.-F., 2015. Species delimitation's coming of age. Syst. Biol. 64, 897–899.
Fontaneto, D., Flot, J.-F., Tang, C.Q., 2015. Guidelines for DNA taxonomy, with a focus on the meiofauna. Mar. Biodivers. 45, 433–451.
Friesen, V.L., Burg, T.M., McCoy, D., 2007. Mechanisms of population differentiation in seabirds. Mol. Ecol. 16, 1765–1785.
Fujita, M.K., Leaché, A.D., Burbrink, F.T., McGuire, J.A., Moritz, C., 2012. Coalescent-based species delimitation in an integrative taxonomy. Trends Ecol. Evol. 27, 480–488.
Fujisawa, T., Barraclough, T.G., 2013. Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent approach: a revised method and evaluation on simulated data sets. Syst. Biol. 62, 707–724.
Ghiselli, F., Milani, L., Scali, V., Passamonti, M., 2007. The *Leptynia hispanica* species complex (Insecta Phasmida): polyploidy, parthenogenesis, hybridization and more. Mol. Ecol. 16, 4256–4268.
Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.
Hasegawa, M., Kishino, H., Yano, T., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22, 160–174.
Hedtke, S.M., Hillis, D.M., 2011. The potential role of androgenesis in cytoplasmic–nuclear phylogenetic discordance. Syst. Biol. 60, 87–96.
Hickerson, M.J., Meyer, C.P., Moritz, C., 2006. DNA barcoding will often fail to discover new animal species over broad parameter space. Syst. Biol. 55, 729–739.
Hoelzel, A.R., Hey, J., Dahlheim, M.E., Nicholson, C., Burkanov, V., Black, N., 2007. Evolution of population structure in a highly social top predator, the killer whale. Mol. Ecol. Evol. 24, 1407–1415.
Hudson, R.R., Coyne, J.A., 2002. Mathematical consequences of the genealogical species concept. Evolution 56, 1557–1565.
Hurvich, C.M., Tsai, C.-L., 1989. Regression and time series model selection in small samples. Biometrika 76, 297–307.
Keegans, S.J., Morgan, E.D., Agosti, D., Wehner, R., 1992. What do glands tell us about species? A chemical case study of *Cataglyphis* ants. Biochem. Syst. Ecol. 20, 559–572.
Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.
Knaden, M., Wehner, R., 2006. Fundamental difference in life history traits of two species of *Cataglyphis* ants. Front. Zool. 3, 21.
Knaden, M., Tinaut, A., Stökl, J., Cerdá, X., Wehner, R., 2012. Molecular phylogeny of the desert ant genus *Cataglyphis* (Hymenoptera: Formicidae). Myrmecol. News. 16, 123–132.
Lahaye, R., van der Bank, M., Bogarin, D., Warner, J., Pupulin, F., Gigot, G., Maurin, O., Duthoit, S., Barraclough, T.G., Savolainen, V., 2008. DNA barcoding the floras of biodiversity hotspots. Proc. Nat. Acad. Sci. USA 105, 2923–2928.
Lanave, C., Preparata, G., Sacone, C., Serio, G., 1984. A new method for calculating evolutionary substitution rates. J. Mol. Evol. 20, 86–93.
Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29, 1695–1701.
Lenoir, A., Aron, S., Cerdà, X., Hefetz, A., 2009. Cataglyphis desert ants: a good model for evolutionary biology in Darwin's anniversary year – a review. Isr. J. Entomol. 39, 1–32.
Leniaud, L., Darras, H., Boulay, R., Aron, S., 2012. Social hybridogenesis in the clonal ant *Cataglyphis hispanica*. Curr. Biol. 22, 1188–1193.
Leppänen, J., Vepsäläinen, K., Anthoni, H., Savolainen, R., 2013. Comparative phylogeography of the ants *Myrmica ruginodis* and *Myrmica rubra*. J. Biogeo. 40, 479–491.
Liautard, C., Keller, L., 2001. Restricted effective queen dispersal at a microgeographic scale in polygynous populations of the ant *Formica exsecta*. Evolution 55, 2484–2492.
Lim, G.S., Balke, M., Meier, R., 2012. Determining species boundaries in a world full of rarity: singletons, species delimitation methods. Syst. Biol. 61, 165–169.
Lohse, K., 2009. Can mtDNA barcodes be used to delimit species? A response to Pons et al. (2006). Syst. Biol. 58, 439–442.
Mallet, J., 2005. Hybridization as an invasion of the genome. Trends Ecol. Evol. 20, 229–237.
Mantel, N., 1967. The detection of disease clustering and generalized regression approach. Cancer Res. 27, 209–220.
Meier, R., Shiyang, K., Vaidya, G., Ng, P.K.L., 2006. DNA barcoding and taxonomy in diptera: a tale of high intraspecific variability and low identification success. Syst. Biol. 55, 715–728.
Möller, L.M., Wiszniewski, J., Allen, S.J., Beheregaray, L.B., 2007. Habitat type promotes rapid and extremely localized genetic differentiation in dolphins. Mar. Freshwat. Res. 58, 640–648.
Moreau, C.S., Bell, C.D., Vila, R., Archibald, S.B., Pierce, N.E., 2006. Phylogeny of the ants: diversification in the age of angiosperms. Science 312, 101–104.
Muñoz, J., Gómez, A., Green, A.J., Figuerola, J., Amat, F., Rico, C., 2010. Evolutionary origin and phylogeography of the diploid obligate parthenogen *Artemia parthenogenetica* (Branchiopoda: Anostraca). PLoS ONE 5, e11932.
Muwanika, V.B., Nyakaana, S., Siegismund, H.R., Arctander, P., 2006. Population genetic structure of the common warthog (*Phacochoerus africanus*) in Uganda: evidence for a strong philopatry among warthogs and social structure breakdown in a disturbed population. Afric. J. Ecol. 45, 22–30.
Oldham, N.J., Morgan, E.D., Agosti, D., Wehner, R., 1999. Species recognition from postpharyngeal gland contents of ants of the *Cataglyphis bicolor* group. J. Chem. Ecol. 25, 1383–1393.
Oremus, M., Gales, R., Dalebout, M.L., Funahashi, N., Endo, T., Kage, T., Steel, D., Baker, S.C., 2009. Worldwide mitochondrial DNA diversity and phylogeography of pilot whales (*Globicephala* spp.). Biol. J. Linn. Soc. 98, 729–744.
Papadopoulou, A., Bergsten, J., Fujisawa, T., Monaghan, M.T., Barraclough, T.G., Vogler, A.P., 2008. Speciation and DNA barcodes: testing the effects of dispersal on the formation of discrete sequence clusters. Philos. T. Roy. Soc. B 363, 2987–2996.
Papadopoulou, A., Monaghan, M.T., Barraclough, T.G., Vogler, A.P., 2009. Sampling error does not invalidate the Yule-coalescent model for species delimitation. A response to Lohse (2009). Syst. Biol. 58, 442–444.
Pamilo, P., Nei, M., 1988. Relationships between gene trees and species trees. Mol. Biol. Evol. 5, 568–583.
Passamonti, M., 2007. An unusual case of gender-associated mitochondrial DNA heteroplasmy: the mytilid *Musculista senhousia* (Mollusca *Bivalvia*). BMC Evol. Biol. 7, 1–11.
Payseur, B.A., 2010. Using differential introgression in hybrid zones to identify genomic regions involved in speciation. Mol. Ecol. Resour. 10, 806–820.
Pigneur, L.M., Marescaux, J., Roland, K., Etoundi, E., Descy, J.P., Van Doninck, K., 2011. Phylogeny and androgenesis in the invasive *Corbicula* clams (*Bivalvia, Corbiculidae*) in Western Europe. BMC Evol. Biol. 11, 147.
Pons, J., Barraclough, T.G., Gomez-Zurita, J., Cardoso, A., Duran, D.P., Hazell, S., Kamoun, S., Sumlin, W.D., Vogler, A.P., 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst. Biol. 55, 595–609.
Puillandre, N., Lambert, A., Brouillet, S., Achaz, G., 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Mol. Ecol. 21, 1864–1877.
R Development Core Team, 2010. *R:* A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Radchenko, A., 2001. The phylogeny and faunogenesis of the genus *Cataglyphis* Foerster (Hymenoptera, Formicidae). Entomologicheskoe Obozrenie 80, 885–895.
Ramos Pereira, M.J., Salgueiro, P., Rodrigues, L., Coelho, M.M., Palmeirim, J.M., 2009. Population structure of a cave-dwelling bat, *Miniopterus schreibersii*: Does it reflect history and social organization? J. Hered. 100, 533–544.
Reid, N., Carstens, B., 2012. Phylogenetic estimation error can decrease the accuracy of species delimitation: a Bayesian implementation of the general mixed Yule-coalescent model. BMC Evol. Biol. 12, 196.
Rokas, A., Williams, B.L., King, N., Carroll, S.B., 2003. Genome-scale approaches to resolving incongruence in molecular phylogenies. Nature 425, 798–804.
Ronquist, F., Teslenko, M., vanderMark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61 (3), 539–542.
Saitoh, K., Kim, I.S., Lee, E.H., 2004. Mitochondrial gene introgression between spined loaches via hybridogenesis. Zool. Sci. 21, 795–798.
Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, N.Y.. 1659 pp.
Schwander, T., Helms Cahan, S., Keller, L., 2007. Characterization and distribution of Pogonomyrmex harvester ant lineages with genetic caste determination. Mol. Ecol. 16, 367–387.

Schwander, T., Keller, L., 2012. Evolution: sociality as a driver of unorthodox reproduction. Curr. Biol. 22, R525–R527.
Seppä, P., Pamilo, P., 1995. Gene flow and population viscosity in *Myrmica* ants. Heredity 74, 200–209.
Song, H., Buhay, J.E., Whiting, M.F., Crandall, K.A., 2008. Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. Proc. Nat. Acad. Sci. USA 105, 13486–13491.
Stephens, M., Smith, N.J., Donnelly, P., 2001. A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. 68, 978–989.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.
Tang, C.Q., Humphreys, A.M., Fontaneto, D., Barraclough, T.G., 2014. Effects of phylogenetic reconstruction method on the robustness of species delimitation using single-locus data. Meth. Ecol. Evol. 5, 1086–1094.
Tavaré, S., 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. Am. Math. Soc. 17, 57–86.
Taylor, H.R., Harris, W.E., 2012. An emergent science on the brink of irrelevance: a review of the past 8 years of DNA barcoding. Mol. Ecol. Res. 12, 377–388.
Tinaut, A., 1990a. Situation taxonomica del genero *Cataglyphis* (Förster, 1850) en la peninsula iberica. III. El grupo de *C. velox* (Santchi, 1929) y description de *Cataglyphis humeya* sp. (Hymenoptera, *Formicidae*). Eos 66, 215–227.
Tinaut, A., 1990b. Taxonomic situation of the genus *Cataglyphis* (Förster, 1850) in the Iberian Peninsula. II. New position for *C. viatica* (Fabricius, 1787) and redescription of *C. velox* (Santchi, 1929) (Hymenoptera, Formicidae). Eos 66, 49–59.
Toews, D.P.L., Brelsford, A., 2012. The biogeography of mitochondrial and nuclear discordance in animals. Mol. Ecol. 21, 3907–3930.
Ward, P.S., Brady, S.G., Fisher, B.L., Schultz, T.R., 2010. Phylogeny and biogeography of Dolichoderine ants: effects of data partitioning and relict taxa on historical inference. Syst. Biol. 59, 342–362.
Ward, P.S., Downie, D.A., 2005. The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): phylogeny and evolution of big-eyed arboreal ants. Syst. Entomol. 30, 310–335.
Welch, A.J., Fleischer, R.C., James, H.F., Wiley, A.E., Ostrom, P.H., Adams, J., Duvall, F., Holmes, N., Hu, D., Penniman, J., Swindle, K.A., 2012. Population divergence and gene flow in an endangered and highly mobile seabird. Heredity 109, 19–28.
Wiszniewski, J., Beheregaray, L., Allen, S., Möller, L., 2010. Environmental and social influences on the genetic structure of bottlenose dolphins (*Tursiops aduncus*) in Southeastern Australia. Cons. Genet. 11, 1405–1419.
Zhang, J., Kapli, P., Pavlidis, P., Stamatakis, A., 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29, 2869–2876.