Molecular Phylogenetics and Evolution xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Biogeography and systematics of *Aricia* butterflies (Lepidoptera, Lycaenidae)

Claudia P. Sañudo-Restrepo [a,b], Vlad Dincă [a,c], Gerard Talavera [a,d], Roger Vila [a,*]

[a] Institut de Biologia Evolutiva (CSIC-Universitat Pompeu Fabra), Passeig Marítim de la Barceloneta 37, 08003 Barcelona, Spain
[b] Facultat de Biologia, Universitat de Barcelona, Avinguda Diagonal 645, 08028 Barcelona, Spain
[c] Department of Zoology, Stockholm University, 106 91 Stockholm, Sweden
[d] Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, Bellaterra, 08193 Barcelona, Spain

ARTICLE INFO

Article history:
Received 11 November 2011
Revised 10 September 2012
Accepted 10 October 2012
Available online xxxx

Keywords:
Biogeography
Hybrids
Lepidoptera
Phylogeny
Systematics
Taxonomy

ABSTRACT

Butterflies of the *Aricia* species group represent a paradigm of unresolved taxonomy, both at the genus and species levels. We studied phylogenetic relationships, biogeography, and systematics based on genetic – nuclear and mitochondrial – and morphometric – external (wings) and internal (genitalia) – data. We show that *Aricia* is a monophyletic genus comprising the taxa *Pseudoaricia*, *Ultraaricia* and *Umpria*, which are here considered junior synonyms of *Aricia*. The taxa *allous*, *inhonora*, *issekutzi*, *mandzhuriana*, *myrmecias* and *transalaica*, which have often been raised to species rank, are shown to probably represent subspecies or synonyms. We show that *montensis* is likely a good species that is sister to all *A. artaxerxes* populations across the Palearctic region. The species *A. anteros* and *A. morronensis* are shown to display deep intraspecific divergences and they may harbor cryptic species. We also discovered that *A. cramera* and *A. agestis* exhibit a pattern of mutual exclusion on islands, and a parapatric distribution in mainland with a narrow contact zone where potential hybrids were detected. The lack of a prezygotic barrier that prevents their coexistence could explain this phenomenon. This study will hopefully contribute to the stability of the systematics of *Aricia*, a group with potential for the study of the link between speciation and biogeography.

1. Introduction

Butterflies are arguably the best studied group of invertebrates. The vast biological knowledge amassed for many species has resulted in their use as model group in a wide range of studies on ecology, evolution, population genetics, conservation and developmental biology (Boggs et al., 2003). This wealth of information, combined with recent advances in molecular tools, allows re-examinations of phylogenetic relationships at both higher and lower taxonomic levels for several groups that remain contentious, with competing hypotheses lacking strong empirical support (Ehrlich, 1958; de Jong et al., 1996; Scott, 1985; Vane-Wright, 2003).

The Lycaenidae butterfly genus *Aricia* Reichenbach, 1817 is one such group, where the systematics is largely unresolved at the generic and specific level. It comprises about 24 species of small-sized butterflies of brown or blue type (Supplementary Material, Fig. S1), distributed across the Palearctic region. In a comprehensive study dealing with the higher classification of the Lycaenidae, Eliot (1973) included the members of the genus *Aricia* in the subfamily Polyommatinae, tribe Polyommatini, and section *Polyommatus*. This higher classification has been widely accepted and used by most authors (e.g., Higgins, 1975; Higgins and Hargreaves, 1983; Karsholt and Razowski, 1996; Bálint and Johnson, 1997; Gorbunov, 2001; Min and Xiaoling, 2002). However, the systematics of the group is established in very few studies that address this issue at genus level. Six genera have been frequently subsumed within *Aricia*: *Eumedonia* Forster, 1938; *Icaricia* Nabokov, [1945]; *Plebulina* Nabokov, [1945]; *Pseudoaricia* Beuret, 1959; *Ultraaricia* Beuret, 1959; and *Umpria* Zhdanko, 1994. Alternatively, many of these taxa have often been treated as *Polyommatus* Kluk, 1801 or *Plebejus* Kluk, 1802.

The species composition of *Aricia* is also unclear, as many taxa have been granted specific status by some authors, while only being considered subspecies by others (Table 1). Relationships between several pairs of taxa such as *agestis/cramera*, *agestis/artaxerxes*, and *artaxerxes/montensis* have been subject to much debate among lepidopterists. Moreover, some taxa within *Aricia* display interesting distribution patterns with hybridization zones being reported, such as for *A. agestis* and *A. artaxerxes* in northern England (Aagaard et al., 2002; Mallet et al., 2011). Several *Aricia* taxa have been listed in red data lists of several countries and a resolved taxonomy is fundamental for their conservation.

Here we study the systematics and biogeography of the group by using molecular data from mitochondrial (cytochrome oxidase subunit I – COI) and nuclear (internal transcribed spacer 2 – ITS2) markers and morphometry of the genitalia and wings. We study

* Corresponding author.
E-mail address: roger.vila@csic.es, roger.vila@ibe.upf-csic.es (R. Vila).

1055-7903/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ympev.2012.10.010

Table 1
List of species that have been included within *Aricia* genus, along with alternative taxonomic position.

Taxon[a]	Alternative taxonomic position	Present in our data set
agestis (Denis and Schiffermüller, 1775)		x
allous (Hübner, 1819)	ssp. of *artaxerxes*	x
anteros (Freyer, 1839)		x
artaxerxes (Fabricius, 1793)		x
bassoni Larsen, 1974	ssp. of *anteros*	
chinensis (Murray, 1874)		x
cramera (Eschscholtz, 1821)	ssp. of *agestis*	x
crassipuncta (Christoph, 1893)	ssp. of *anteros*	x
dorsumstellae (Graves, 1923)	ssp. of *isaurica*	
hyacinthus (Herrich-Schäffer, 1847)		
inhonora (Jachontov, 1909)	ssp. of *artaxerxes*	x
isaurica (Staudinger, 1870)		x
issekutzi (Balogh, 1956)	ssp. of *artaxerxes*	x
mandschurica (Staudinger, 1892)	synonym of *chinensis*	
mandzhuriana (Obraztsov, 1935)	ssp. of *artaxerxes*	x
montensis Verity, 1928	ssp. of *artaxerxes*	x
morronensis (Ribbe, 1910)		x
myrmecias (Christoph, 1877)	ssp. of *chinensis*	x
nicias (Meigen, 1829)		x
scythissa Nekrutenko, 1985		
teberdina (Sheljuzhko, 1934)		
torulensis Hesselbarth and Siepe, 1993		x
transalaica (Obraztsov, 1935)	ssp. of *artaxerxes*	x
vandarbani (Pfeiffer, 1937)		x

[a] We excluded *Eumedonia*, *Icaricia* and *Plebulina* taxa (see text).

representative material of the genus *Aricia* and all other genera that have been included within it at least by some authors (see above). We excluded from our dataset taxa belonging to the genera *Icaricia*, *Plebulina* (New World) and *Eumedonia* (Old World), as it has recently been shown that these are not closely related to *Aricia* (Vila et al., 2011). We also discuss several continental scale biogeographical patterns displayed by the morphologically similar taxa *agestis*, *artaxerxes*, *cramera*, and *montensis*.

2. Material and methods

2.1. Taxon sampling and data collection

Our sampling contains representatives of the genera *Aricia, Ultraaricia, Pseudoaricia* and *Umpria*. The New World *Icaricia, Plebulina*, and the taxon *Plebejus saepiolus* Boisduval, 1852 as well as the genus *Eumedonia* from the Old World are not closely related to the genus *Aricia* (Vila et al., 2011). Thus, we excluded them from our analysis. Our dataset includes 15 taxa, plus *Polyommatus icarus, Polyommatus thersites, Lysandra coridon* and *Plebejus idas*, which were used as outgroup based on Vila et al. (2011). Six taxa that were considered as distinct species by at least some authors (Table 1) were not possible to obtain. We especially focused on the taxa *agestis, cramera, artaxerxes* and *montensis* for which the sampling covers most of their distributions and for which morphometric analyses were performed. Most of the samples are deposited in Roger Vila's DNA Collection at Institut de Biologia Evolutiva (CSIC-Universitat Pompeu Fabra), Barcelona, Spain, and are available upon request. In addition to these samples, we also included all *Aricia* sequences available in GenBank, most of them published by Wiemers and Fiedler (2007) and Lukhtanov et al. (2009). Specimens included in the analyses and associated information, are included in the Supplementary Material, Table S1.

2.2. DNA extraction and sequencing

Total genomic DNA was extracted using Chelex 100 resin, 100–200 mesh, sodium form (Bio-rad), under the following protocol: one leg was removed and introduced into 100 µl of Chelex 10% and 5 µl of Proteinase K (20 mg ml^{-1}) were added. The samples were incubated overnight at 55 °C and were subsequently incubated at 100 °C for 15 min. Afterwards they were centrifuged for 10 s at 3000 rpm. Primers LCO 1490 and Nancy were used for the amplification of the mitochondrial Cytochrome Oxidase subunit I (COI) (Folmer et al., 1994; Monteiro and Pierce, 2001; Simon et al., 1994), and ITS3/ITS4 for the nuclear internal transcribed spacer 2 (ITS2) (White et al., 1990). Double-stranded DNA was amplified in 25 µl volume reactions: 13.22 µl ultra pure (HPLC quality) water, 2.5 µl 10 × buffer, 4.5 µl 25 mM MgCl2, 0.25 µl 100 mM dNTP, 1.2 µl of each primer (10 mM), 0.13 µl Taq DNA Gold Polymerase (Qiagen) and 2 µl of extracted DNA. The typical thermal cycling profile for COI was 95 °C for 60 s, 44 °C for 60 s and 72 °C for 90 s, for 40 cycles. PCR products were purified and sequenced by Macrogen Inc. Sequences were deposited in the GenBank database under Accession Nos. JX678013–JX678216.

2.3. Phylogenetic analyses

Our study is based on the individual and combined analyses of the sequence data from 182 specimens, including representatives of 15 taxa, for the mitochondrial marker COI, and 57 specimens, including representatives of 14 taxa, for the nuclear marker ITS2. The incongruence length difference (ILD) test (Farris et al., 1994) was performed to study the homogeneity between our mitochondrial and nuclear datasets. The test was performed with PAUP* using heuristic searches with tree bisection-reconnection (TBR) branch swapping and 100 random taxon addition replicates, saving no more than ten equally parsimonious trees per replicate. Only parsimony informative sites were included. No significant conflict ($P = 0.69$) was detected by the ILD test between the mitochondrial (COI) and nuclear (ITS2) data. Thus, we combined mitochondrial and nuclear data to improve phylogenetic signal.

2.3.1. Alignment

All sequences were edited and aligned using Geneious 4.8.3 (Drummond et al., 2010). For COI, this resulted in an alignment of 672 bp for 182 specimens. This was obtained after removing regions where more than 50% of the sequences contained missing data using Gblocks 0.91 (Castresana, 2000). ITS2 sequences were aligned according to their secondary structure using the ITS2

Database Server (Koetschan et al., 2010), as described in Schultz and Wolf (2009). HMM-Annotator tool (Keller et al., 2009) was used to delimitate and crop the ITS2 margins (*E*-value < 0.001, metazoan HMMs), preserving the proximal stems (25 nucleotides of 5.8S and 28S rDNA). The secondary structure of ITS2 was predicted by custom homology modeling using the template structure of *Neolysandra coelestina* (MW99013) inferred by Wiemers et al. (2009), and at least 75% helix transfer was used (ITS2PAM50 matrix; gap costs: gap open 15, gap extension 2). For the few cases with incomplete proximal stem (3' end), the short missing sequence was completed using the equivalent fragment from the template. These additions were necessary to obtain a correct alignment, and were removed for the posterior phylogenetic analysis. Sequences and secondary structures were aligned synchronously with 4SALE 1.5 (Seibel et al., 2006, 2008) using an ITS2-specific 12×12 scoring matrix. For ITS2, the alignment resulted in 639 bp for 57 specimens.

2.3.2. Phylogenetic inference

Phylogenetic relationships were inferred using Maximum Likelihood (ML), Bayesian inference (BI) and Maximum Parsimony (MP) for COI and BI for ITS2 and a combined COI + ITS2 dataset with 56 specimens. jModeltest 0.118 (Posada, 2008) was used to determine substitution models according to AICc (Corrected Akaike Information Criterion), being GTR + I + G for COI and GTR + G for ITS2 datasets.

For ML trees, we used Phyml 2.4.4 (Guindon and Gascuel, 2003) with 100 bootstraps replicates to test the robustness of the tree clades. Maximum Parsimony (MP) analysis was conducted using PAUP 4.0b10 (Swofford, 2000). A heuristic search was performed with TBR branch swapping and ten thousand random taxon addition replicates, saving no more than ten equally parsimonious trees per replicate. To estimate branch support on the recovered topology, non-parametric bootstrap values (Felsenstein, 1985) were assessed with PAUP 4.0b10. One hundred bootstrap pseudoreplicates were obtained under a heuristic search with TBR branch swapping with 1000 random taxon addition replicates, saving no more than ten equally parsimonious trees per replicate. Bayesian analyses were conducted using BEAST 1.4.8 (Drummond and Rambaut, 2007). Base frequencies were estimated, 6 gamma rates categories were used and COI was partitioned by codon position. The parameters were estimated using two independent runs of 10 million generations each. Convergence was checked with the program Tracer v.1.4 and a burn-in of 1 million generations was applied to obtain the final tree.

BEAST 1.4.8 was also used to estimate node ages. The analysis was performed using the COI matrix of 182 specimens. Since no external calibration points, either in the form of a fossil or biogeographic event, are available for Lycaenidae, we applied a molecular clock using published substitution rates to our inferred branch lengths to convert them to absolute time. We used a published substitution rate of 1.5% uncorrected pairwise distance per million years, applied to the entire mitochondrial genome of various arthropods (Brower, 1994). *Plebejus idas* was set as a root and we used a uniform prior with an upper level of 8.0 Ma, according to previous age estimates for the divergence between *Plebejus* and *Aricia* (Vila et al., 2011). The parameters used for the Bayesian analysis were the same as those given above. COI uncorrected p distances were calculated with PAUP 4.0b10 excluding three specimens (two *A. agestis* and one *A. artaxerxes*) with sequences shorter than 426 bp.

2.4. Morphometric analyses

2.4.1. Data sources and measurements

All samples studied are male specimens of the taxa *agestis*, *cramera*, *artaxerxes* and *montensis*. Male genitalia are formed by different sclerotized pieces, and their morphology is widely used in taxonomy (Arnqvist, 1997). Genitalic preparations were made for 96 specimens and deposited in the same collections from which specimens were obtained.

Genitalia were processed by cutting the terminal part of the abdomen and heating it for fifteen minutes at 95 °C in a 10% KOH solution. Subsequently, the hard structures were cleaned in distilled water and were examined under a stereomicroscope. We took images of the male genitalia in two positions: lateral, with the phallus removed; and frontal, with the valvae spread to expose the labides and falces (Fig. 1). We used a DeltaPix camera attached to the stereomicroscope and its software to capture and digitize the images, using the same protocol for all specimens. For a few individuals certain measurements could not be performed because the involved structure was damaged.

The selection of variables took into account the characters traditionally used by specialists for the description and identification of *Aricia* taxa. Male genitalia terminology follows Higgins (1975). Other variables also included in the statistical analysis were forewing size (length and width) and number of lunules (orange marginal spots) of forewings upperside. In total, we chose ten variables that are shown in Fig. 1 and described below. Wings: maximum length of the forewing, measured between the wing base and the wing apex (LFW); forewing maximum width (WFW), measured between the wing apex and tornus; and number of lunules (orange marginal spots) on the upperside of the forewings (LUN), counting all visible traces as a full lunule. Genitalia: maximum length of the valva (LVA), maximum width of the valva (WVA), length from the valva apex to its distal end (LV1), length from the valva apex to its proximal end (LV2), length of the falces (FAL), length of the labides (LAB) and length of the phallus (LPH).

2.4.2. Statistical analyses

All statistical analyses were performed with the software SPSS version 18 (also known as PASW Statistics). All variables were measured with the same scale. First, we determined if the measured traits showed a normal distribution. Normality of each trait was tested independently with the Kolmogorov–Smirnov test. In order to test the null hypothesis, that the averages of two or more groups were not significantly different, we performed an ANOVA with post hoc test. This was done to identify the pairs of species that showed significant differences, applying Hochbergs GT2 test when assuming equal variances and Games-Howell test when the homogeneity of variance was not assumed. The similarity variance was verified through the Levene test. Subsequently, a Principal Component Analysis was made, retaining the factors with eigenvalues larger than one. Following Field (2005), we directly inspected the correlation matrix (*R*-matrix) and its *determinant* to test singularity and extreme multicollinearity. Moreover, we used the Keiser–Meyer–Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity to verify if the analysis can result in distinct and reliable factors and if the correlation matrix significantly differs from an identity matrix, respectively. We rotated the factors using the Varimax method to obtain the expected weight for each extracted factor.

A Discriminant Analysis with the stepwise method was then performed. The variables were selected with the Wilks' lambda statistic, which measures how each function separates cases into groups. Smaller values in Wilks' lambda indicate greater discriminatory ability of the function. In order to test the obtained classification a cross validation was carried out.

3. Results

3.1. Phylogenetic analyses

In all the phylogenetic analyses, based on COI, on ITS2 and secondary structure information, as well as on the combined

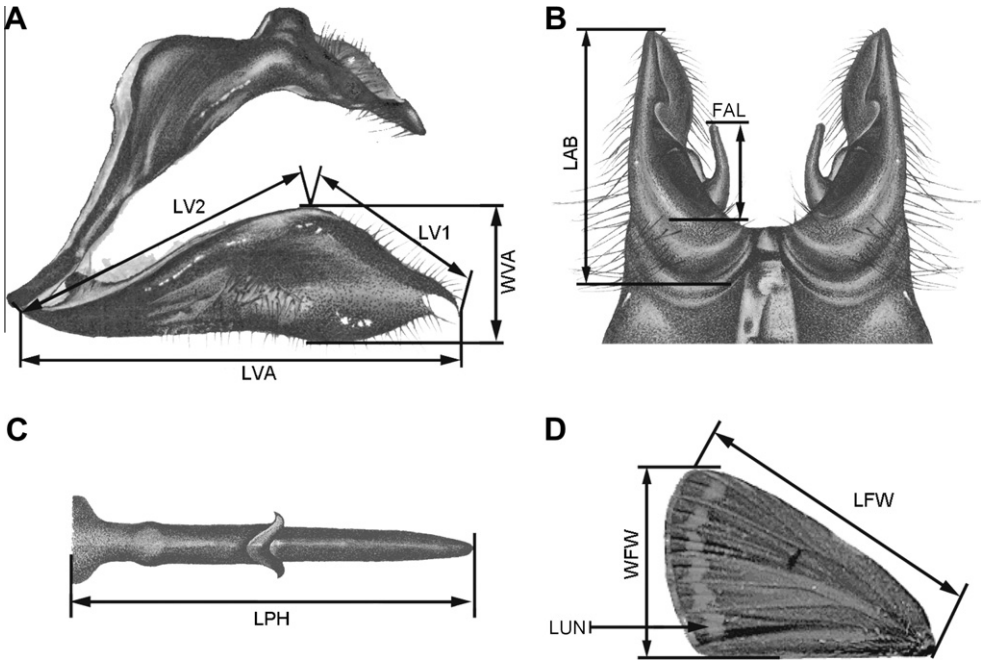

Fig. 1. Measured variables for the morphometric analysis of four taxa of the genus *Aricia* (*agestis*, *artaxerxes*, *cramera* and *montensis*). (LVA) valva length; (WVA) valva width; (LV1) length from the valva apex to its distal end; (LV2) length from the valva apex to its proximal end; (FAL) length of the falces; (LAB) length of the labides; (LPH) length of the phallus; (LFW) length of the forewing; (WFW) width of the forewing; (LUN) number of lunules.

COI + ITS2, the ingroup taxa formed a strongly supported monophyletic group (Fig. 2A–C). The estimated origin for the genus *Aricia* is ca. 5.69 Ma (Fig. 2A). Despite being a fast-evolving nuclear marker, ITS2 was not variable enough to study the relationships between recently diverged taxa (Fig. 2B). Nevertheless, it was informative for deeper relationships, and the tree based on the combined dataset of COI + ITS2 was well resolved and supported (Fig. 2C). Three major clades were recovered: (1) one clade including the taxa *artaxerxes*, *montensis, agestis*, *cramera*, *torulensis*, *isaurica, nicias* and *chinensis*; (2) the *anteros*, *crassipuncta* and *vandarbani* group; and (3) a clade comprising the Iberian endemic taxon *morronensis*.

According to COI and COI + ITS2 phylogenies, the first main group appeared divided into five well-supported clades: (1) the *chinensis* clade, (2) the *nicias* clade, (3) the *torulensis*/*isaurica* clade, (4) the *cramera* clade, and (5) the *agestis*/*artaxerxes*/*montensis* clade (Figs. 2A, C; 3A). The *chinensis* clade was well diverged from the other taxa (the minimum COI uncorrected p distance with another taxa was 2.1%) (Fig. 2A), but the taxon *myrmecias*, raised at species rank by some authors (e.g., Lukhtanov and Lukhtanov, 1994; Tshikolovets, 2000), displayed a COI sequence identical to *chinensis*. The taxon *nicias* formed another well diverged clade supporting its status as a species. The sister taxa *torulensis* and *isaurica* displayed a relatively low divergence level between them (COI uncorrected p distance of 0.8%) and it would be interesting to study their status using more specimens. The taxon *cramera* was recovered as a well diverged clade that is sister to the morphologically similar *agestis*, *artaxerxes* and *montensis* based on COI + ITS2 (Fig. 2C), a result that supports its status as a species. Despite morphological and ecological similarity, the taxa *cramera* and *agestis* are not sister species, since the taxon *agestis* was recovered as sister to *artaxerxes* plus *montensis*. Our comprehensive sampling of *cramera* clarified its general distribution (Fig. 3B). This species extends over North Africa (Morocco and Tunisia), Canary Islands, Iberian Peninsula, as well as the Balearics and Sardinia Islands. Interestingly, *cramera* seems to be absent from Corsica and Sicily, and the overall distribution is apparently restricted to the western Mediterranean, without penetrating east into Italy or the Balkans. Our results show that *cramera* and *agestis* display parapatric distributions, since *agestis* is widely distributed across the rest of the Palearctic, including Corsica and Sicily. The two species seem to come into contact in Catalonia, northeastern Spain (Fig. 3B).

Several taxa that have been considered species by some authors such as *allous, inhonora*, *issekutzi, mandzhuriana* and *transalaica* (Table 1) were recovered within the *artaxerxes* clade (Fig. 3A). These taxa did not display noticeable levels of genetic divergence correlated with geographical distribution patterns (the maximum COI uncorrected *p* distance within *artaxerxes* was 1.2%, despite sampling over a wide geographical area). By contrast, the taxon *montensis* (sometimes considered as a distinct species) was recovered as the sister clade of *artaxerxes* (the range of COI uncorrected p distance between both taxa was from 1.1% to 2.0%). Thus, based on our sampling and results, *artaxerxes* appears to be widely distributed across the Palearctic, except for North Africa and the entire Iberian Peninsula, where it is replaced by *montensis* (Fig. 3B).

Within the second main clade, the taxa *anteros* and *crassipuncta* formed a group divided in two clades that does not correspond to the current taxonomic arrangement. In one clade the *anteros* from the Balkans were closely related to one specimen of *anteros* from northeastern Turkey and to several samples of *crassipuncta* from Armenia and Iran. The sister clade included the samples of *anteros* from central Turkey and some *crassipuncta* from east Turkey and Armenia (Fig. 2A). *Aricia vandarbani* was recovered as the sister taxon to the *anteros* – *crassipuncta* group, from which it displayed a minimum COI uncorrected p distance of 1.4%.

The Iberian endemic taxon *morronensis* was recovered as a well supported and diverged clade sister to the *anteros*, *crassipuncta* and *vandarbani* group.

3.2. Morphological analyses

Ten variables of male genitalia and forewings were studied for the taxa *cramera*, *agestis*, *artaxerxes*, and *montensis*. The Kolmogorov–Smirnov test showed that eight variables had normal

Fig. 2. (A) Bayesian chronogram based on the mitochondrial marker COI (672 bp). Numbers at nodes indicate Bayesian posterior probability/Maximum likelihood bootstrap/Maximum Parsimony bootstrap, with non-matching clades among different analyses indicated by "–". Node bars represent the 95% highest posterior density for age estimations, according to the axis representing time in millions of years before present. (B) Bayesian tree based on ITS2 sequences aligned according to secondary structure information. Numbers at nodes indicate Bayesian posterior probability. (C) Bayesian tree based on a combined analysis of the mitochondrial COI (672 bp) and nuclear ITS2 (639 bp) markers. Numbers at nodes indicate Bayesian posterior probability.

Please cite this article in press as: Sañudo-Restrepo, C.P., et al. Biogeography and systematics of *Aricia* butterflies (Lepidoptera, Lycaenidae). Mol. Phylogenet. Evol. (2012), http://dx.doi.org/10.1016/j.ympev.2012.10.010

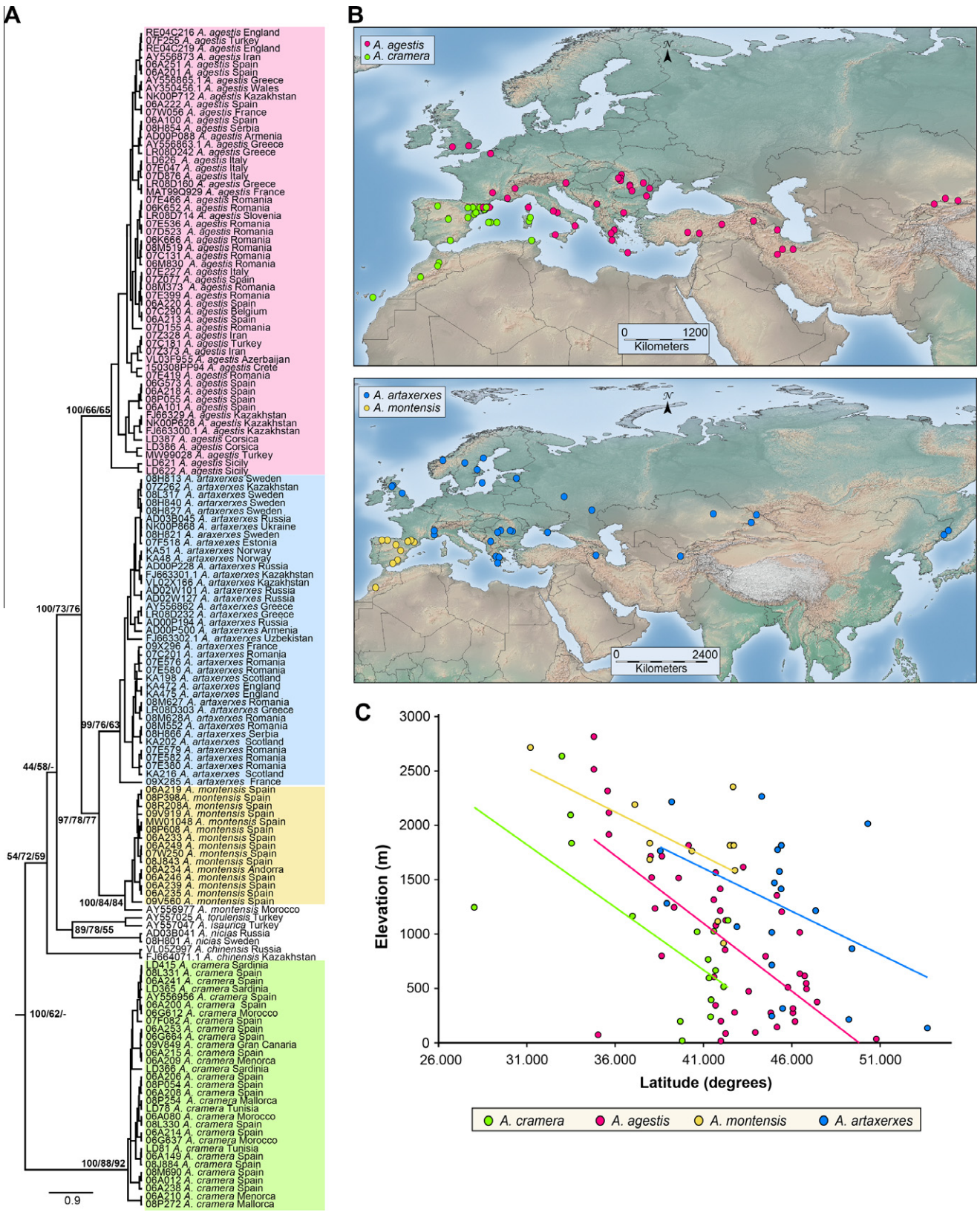

Fig. 3. (A) Part of the COI Bayesian inference tree including the *cramera*, *agestis*, *artaxerxes* and *montensis* clades. Numbers at nodes indicate Bayesian posterior probability/Maximum likelihood bootstrap/Maximum Parsimony bootstrap, with non-matching clades among different analyses indicated by "–". (B) Distribution maps of the taxa *cramera* and *agestis* (top), and *artaxerxes* and *montensis* (bottom). (C) Elevation versus latitude graph showing the four linear regression lines for the taxa *agestis*, *artaxerxes*, *cramera* and *montensis* based exclusively on sequenced specimens for which precise altitude was known.

distribution (LAB, LVA, WVA, LV1, LV2, LPH, LFW and WFW), but that FAL and LUN were not normally distributed. The Levene test confirmed the homogeneity of variances ($p > 0.05$) for six variables (LFW and LUN showed significantly heterogeneous variance). The

ANOVA test was significant for all variables ($p < 0.05$), so there were significant differences between the groups of species evaluated. The Post Hoc test (Games-Howell procedure) confirmed that there are significant differences among the taxa *cramera*, *agestis* and *montensis*. For the variables LFW and LUN, it showed significant differences among all groups.

3.2.1. Principal Component Analysis (PCA)

The *R*-matrix showed absolute values of correlation coefficients and levels of significance and not greater than 0. 9 and 0.05, respectively, in all pairs of variables. Therefore, singularity was not a problem for our data. According to Field (2005), the *determinant* of the correlation matrix must be greater than 0.00001 and in our dataset this value was 0.21, indicating that extreme multicollinearity was not present. Finally, the KMO measure of sampling adequacy was 0.822. This value is "good" according to the range defined by Hutcheson and Sofroniou (1999). In addition, the Bartlett Test of Sphericity showed that the correlation matrix significantly differ from an identity matrix ($p < 0.001$, $df = 21$). In the PCA analysis (Fig. 4B), the first two principal components accounted for over 73% of the total variability. The variables with a higher weight in the first component were LAB and FAL (average of the lengths of the two labides and of the two falces) and the WVA (width of the valva) thus accounting mostly for genitalia shape. In the second component, the most important variables were length and width of the forewing (LFW and WFW) thus accounting mostly for size. When the samples were projected onto the principal components (not shown) *cramera* specimens formed a differentiated cluster, demonstrating the utility of the proportion between length of the falces and length of the labides for taxonomic identification. We therefore confirmed that the falces in *cramera* are shorter (about a quarter the length of labides) than in *agestis*, *artaxerxes* and *montensis*, in agreement with previous studies (Higgins, 1975; Balletto et al., 1981).

3.2.2. Discriminant analyses (DA)

The homoscedasticity was assessed using the Box's M test ($p > 0.05$). The variables introduced in the prediction equation, using the stepwise method and Wilks' lambda, were the length of falces (FAL), the length of the labides (LAB) and the length of the phallus (LPH), in this order. The other variables were excluded from the analysis. Wilks' lambda and the Chi-square tests were significant, indicating the capacity of the function to discriminate between groups. The corresponding eigenvalue accounted for 92.3% of the variance and the canonical correlation coefficient had a value of 0.928, confirming the power of the discriminant function. FAL was the variable with most weight on function 1, reflecting its discriminatory power. The variables LPH and LAB were more important in function 2. The cross validation showed that these three variables correctly identified 72.5% of the individuals. This rather low value reflects the fact that only one of the four taxa (*A. cramera*) can be differentiated based on the morphological characters studied.

The first two functions accounted for 99% of the variance. The scatter plot (Fig. 4D) recovered *cramera* as a distinct group with respect to the other three taxa (*agestis*, *artaxerxes* and *montensis*), which were not well resolved. The canonical variable weights obtained from the structure matrix (not shown), confirmed that the length of the falces (FAL) is the best discriminant character between *cramera* and the other three taxa. Additionally, we carried out a discriminant analysis between *cramera* and *agestis*, based only on the length of falces and labides. FAL was the variable with greater weight in function 1, and LAB in function 2. These functions were able to largely discriminate between the two species (Fig. 4C). It is worth noting that five specimens displayed intermediate morphology between *cramera* and *agestis*. Interestingly, all these specimens were collected along the contact zone between these two species in Catalonia, which suggests that they are hybrids. Unfortunately, the hybrid nature of these samples could not be tested using ITS2, because this marker does not always differentiate *A. agestis* from *A. cramera* (Fig. 2B).

3.2.3. Marginal lunules on the upperside of the forewing

Fig. 4A shows the average and standard deviations for each taxon. The taxon *cramera* did not display variation in the specimens studied, with a constant number of six lunules for the specimens studied. The taxa *agestis*, *montensis* and *artaxerxes* showed a more pronounced variation ranging from three to six, four to six, and zero to five lunules, respectively. These results suggest that only *cramera* and *artaxerxes* may be safely distinguished from each other based on this character, while the rest of the taxa overlap in the number of lunules. Our findings are in accordance with other recent studies showing that the number of lunules on the forewings is not a reliable identification character between *agestis* and *artaxerxes*, for example in the United Kingdom (Aagaard et al., 2002) and Romania (Dincă et al., 2011). Moreover, it is to be expected that identification success based on this character is even lower if females are examined, since these usually have a higher number (and more developed) of orange lunules on the forewings upperside.

4. Discussion

According to the molecular phylogenies obtained in our study (Fig. 2) we define a strongly supported monophyletic genus *Aricia* that includes three other genera (*Pseudoaricia*, *Umpria* and *Ultraaricia*). In this study, we consider these taxa junior subjective synonyms of *Aricia*, but some of them could be eventually used as subgenera. The relationships between the species included in the analyses were largely resolved, although several cases require further studies to clarify their status (Table 2). *Aricia morronensis* is endemic to the Iberian Peninsula from where eight subspecies have been described (Munguira and Martín, 1988). Our sampling included subspecies *ramburi* Verity, 1929 from Sierra Nevada (Granada) and *elsae* Wyatt, 1952 from the Cantabrian Mountains, plus samples from Catalonia, Ávila and Soria. The genetic divergence between taxon *ramburi* and the rest was surprisingly high (COI uncorrected p distance from 1.5% to 2.1%), especially given the relatively limited range of *A. morronensis*. A deeper study including morphology and more specimens belonging to all subspecies would be necessary to ascertain the taxonomic status of these taxa.

The taxa *anteros* and *vandarbani* have usually been considered as species, but the status of *crassipuncta* is more controversial since it has often been treated as subspecies of *anteros* (Table 1). Our results support the specific distinctness of *A. vandarbani* (sister clade to *anteros* and *crassipuncta*, with moderate divergence), but reveal a rather puzzling situation for *anteros* and *crassipuncta* because the two clades formed by these taxa did not correspond to taxonomic assignments (Fig. 2A). The taxon *anteros* is supposedly distributed in the Balkans, Greece, Turkey and Iran, while *crassipuncta* has been reported from central and eastern Turkey, Armenia and Iran (Hesselbarth et al., 1995; Tuzov et al., 2000; Tolman and Lewington, 2008). Moreover, there are reports of potential hybrids in central Turkey, where their distributions overlap (Hesselbarth et al., 1995). The taxonomic mismatch between molecular data and current taxonomy, combined with the relatively high levels of divergence (the split between the two clades is dated at about 0.69 Ma), highlights the need for deeper studies on these taxa. Therefore, until more data are available, we follow the most widely accepted arrangement considering *vandarbani* and *anteros* as species and *crassipuncta* as a subspecies of *anteros*.

Please cite this article in press as: Sañudo-Restrepo, C.P., et al. Biogeography and systematics of *Aricia* butterflies (Lepidoptera, Lycaenidae). Mol. Phylogenet. Evol. (2012), http://dx.doi.org/10.1016/j.ympev.2012.10.010

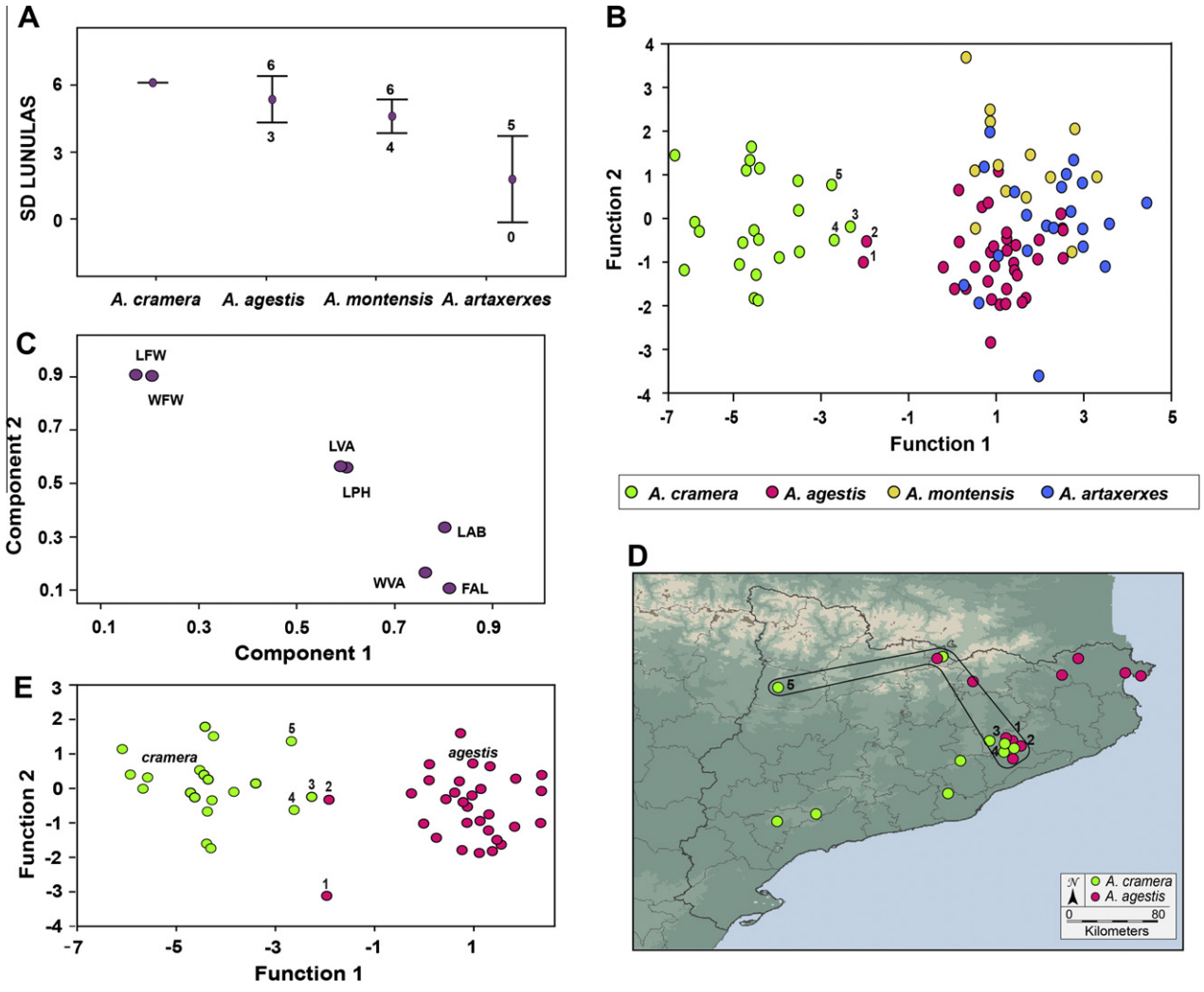

Fig. 4. Morphological analyses of the taxa *cramera*, *agestis*, *artaxerxes* and *montensis* ($N = 92$). (A) Average number of lunules of the forewings (LUN). Bars indicate standard deviations and numbers indicate minimum and maximum values. (B) Graph in rotated space of the two components that were extracted from the selected variables. (C) Scatter plot of the Discriminant Analysis between *A. cramera* and *A. agestis* using only the falces and the labides as variables. (D) Scatter plot of the Discriminant Analysis using all groups studied. (E) Map of Catalonia with contact zone between *A. cramera* and *A. agestis* indicated. Potential hybrid specimens with intermediate morphology between *A. cramera* and *A. agestis* are numbered in B, D and E.

Given the deep divergence of the taxon *chinensis* with respect to the rest of the taxa, it most probably represents a good species. However, the taxon *myrmecias*, considered a different species by some authors (e.g., Lukhtanov and Lukhtanov, 1994; Tshikolovets, 2000), had an identical COI sequence to *chinensis*. This suggests that *myrmecias* may be a synonym or subspecies of *chinensis*, but further research based on additional material are needed to fully clarify this point.

Aricia nicias was recovered as a well differentiated species, but the divergence between the taxa *torulensis* and *isaurica* is very small. *Aricia torulensis* was described quite recently (Hesselbarth and Siepe, 1993) and its biology has been partially studied (Siepe, 1995; Schurian, 2002), with its status remaining controversial. It is only known from a restricted area in northeastern Turkey, while the taxon *isaurica* occurs across Lebanon and Anatolia (Hesselbarth et al., 1995). Their low level of genetic divergence (COI uncorrected *p* distance 0.8%) proves their very close relationship. Based on the current data we cannot exclude that we are dealing with very young species, although additional studies are necessary to clarify their status.

The deep divergence between *A. cramera* and the externally and ecologically similar *A. agestis* strongly suggests that these represent different species. *Aricia cramera* appears as the most diverged taxon of the morphologically similar group formed by *cramera*, *agestis*, *artaxerxes*, and *montensis*, with an age of divergence of about 2.42 Ma. The distinctness of *A. cramera* is also supported by statistically significant differences found in male genitalia (length of the labides and falces). However, although molecular and morphological data were largely congruent for this species, there were some exceptions. Two specimens assigned to *A. agestis* based on their COI sequences, as well as three *A. cramera*, displayed male genitalia characters intermediate between the two species (Fig. 4C and D). Interestingly, all of them correspond to samples collected in the contact zone between *A. agestis* and *A. cramera* in Catalonia, northeastern Iberian Peninsula (Fig. 4E). This pattern suggests that hybridization may be occurring in this area. Unfortunately, the nuclear marker ITS2 was not variable enough to discriminate between both species. The phenomenon requires further research, and it is possible that we deal with a situation similar to the one reported by Mallet et al. (2011) involving *A. agestis* and *A. artaxerxes* in northern England. However, this should not be interpreted as proof of conspecificity. Together with the DNA-based results, which show that *A. agestis* and *A. cramera* are not sister taxa, the general distribution pattern here documented further

Please cite this article in press as: Sañudo-Restrepo, C.P., et al. Biogeography and systematics of *Aricia* butterflies (Lepidoptera, Lycaenidae). Mol. Phylogenet. Evol. (2012), http://dx.doi.org/10.1016/j.ympev.2012.10.010

Table 2
Summary of the updated taxonomic status of the studied *Aricia* taxa based on our data. Geographical distribution is mentioned for taxa that were sampled comprehensively.

Taxon	Comments	Confirmed distribution
agestis	Good species	Across the Palearctic, including Corsica, Sicily and Crete, but excluding N. Africa, Sardinia, and most of the Iberian Peninsula (contact zone with cramera in N.E. Spain)
artaxerxes	Good species	Across the Palearctic, excluding N. Africa and the Iberian Peninsula
allous	Synonym or subspecies of artaxerxes	
issekutzi	Synonym or subspecies of artaxerxes	
inhonora	Synonym or subspecies of artaxerxes	
mandzhuriana	Synonym or subspecies of artaxerxes	
transalaica	Synonym or subspecies of artaxerxes	
montensis	Likely a good species	N. Africa and the Iberian Peninsula (including the Pyrenees)
cramera	Good species	N. Africa, the Balearic Islands, the Canary Islands, Sardinia and the Iberian Peninsula (contact zone with agestis in N.E. Spain)
nicias	Good species	
torulensis	Relationship with isaurica needs further study	
isaurica	Relationship with torulensis needs further study	
chinensis	Good species	
myrmecias	Probably a synonym or subspecies of chinensis, but needs further study	
anteros	Good species, may include cryptic taxa	
crassipuncta	Polyphyletic according to our results, needs further study	
vandarbani	Good species	
morronensis	Good species, may include cryptic taxa	

supports their status as distinct species. With the exception of the discovered narrow contact zone, they appear to exclude each other both on continental areas and on islands. In fact, all studied islands seem to be inhabited exclusively by one species or the other: *A. cramera* in Sardinia and Balearics and *A. agestis* in Corsica and Sicily. This is surprising since dispersal events between Corsica and Sardinia should occur much easier than colonization of these islands from the mainland. Indeed, insufficient mobility can hardly be considered a plausible cause, since *Aricia* occurs on most Mediterranean islands. Therefore, the occurrence of multiple colonization events is very likely, at least for a good number of islands. The island mutual exclusion pattern observed, together with the discovery that *A. agestis* and *A. cramera* are parapatric species with a relatively narrow contact zone, leads us to conclude that *A. agestis* and *A. cramera* cannot coexist. Our hypothesis to explain this pattern is that the two species have not developed a prezygotic barrier and hybrids are not fertile or have reduced fertility. Therefore, the first species to colonize an island prevents the establishment of the other species. The narrow sympatry zone in northeastern Iberian Peninsula (Fig. 4D) would then represent a sink maintained by large populations of the two species coming into contact.

A. agestis is phylogenetically closely related to the taxa *artaxerxes* and *montensis*, in relation to which it displays an interesting biogeographical pattern (Fig. 3B). The three taxa have split during the Pleistocene, about 1.34 Ma. (Fig. 2A), and are very similar morphologically. Because of morphological similarity and relatively high level of intraspecific variation (especially in *A. artaxerxes*, for which a considerable number of subspecies have been described), the relationship between the two taxa has been subject to debate among lepidopterists (e.g., Høegh-Guldberg, 1979; Shreeve, 1993; Smyllie, 1995, 1996). The situation was clarified for northwest Europe by Aagaard et al. (2002). By employing an integrative approach of external morphology, mitochondrial and allozyme markers, they managed to prove the presence of both *A. agestis* and *A. artaxerxes* in the area. This study also reported possible introgression between the two taxa in northern England. This phenomenon was further documented by Mallet et al. (2011), who found evidence for introgression between *A. agestis* and *A. artaxerxes* along the contact zone in northern England and North Wales. Based on these results, at least for the area studied, the two taxa could again be considered conspecific under the traditional biological species concept and the genotypic cluster delimitation (Mallet et al., 2011). However, it has also been acknowledged that genotypic bimodality apparently occurs in some contact zones between *A. agestis* and *A. artaxerxes* (Aagaard et al., 2002; Mallet et al., 2011). The two species also display ecological differences, with *A. artaxerxes* usually flying at higher altitudes and latitudes than *A. agestis*, although overlaps occur (Fig. 3B, C). The fact that *A. artaxerxes* haplotypes found in separate mountainous regions across Europe are relatively homogeneous and distinct from those of the surrounding *A. agestis* populations suggests that some kind of barrier to gene flow exists that allows the presence of two distinct lineages.

Leaving apart the taxon *montensis*, *A. artaxerxes* turned out to be genetically fairly homogeneous across a wide geographical range. The maximum intraspecific COI uncorrected p distance was 1.2%, and the mean *p*-distance was 0.2%, despite the wide sampling ranging from United Kingdom to the Russian far east, with no markedly diverged clades. Thus, we conclude that the taxa *allous*, *inhonora*, *issekutzi*, *mandzhuriana* and *transalaica* (considered good species by certain authors) are probably either junior synonyms or subspecies of *A. artaxerxes* (Table 2).

The taxa *artaxerxes* and *montensis* were recovered as sister clades that, according to our estimations, diverged during the Pleistocene (about 1.10 Ma.), long before the last glacial maximum (Fig. 2A). This result, coupled with their allopatric distribution, raises the question whether *montensis* should be considered as a distinct species, or as a well-diverged subspecies of *artaxerxes*. Various authors have reported different ranges for *montensis*. For example Tolman and Lewington (1997, 2008) mentioned that the taxon ranges from northern Africa across Spain and south of France, Italy (including Sicily) and the Balkans, while Higgins (1991) reported it from North Africa, Spain and southern France, but also from Hungary, the Tatras and the Romanian Carpathians. Our findings show that *montensis* is restricted to North Africa and the Iberian Peninsula, while *artaxerxes* is found in the rest of the Palearctic region, including the Alps, as the nearest point to the Pyrenees we have studied (Fig. 3B). In southern Europe, the two taxa are restricted to mountains and occur at gradually lower altitudes with increasing latitudes (Fig. 3B and C). This pattern

suggests that, during glaciations, they were probably much more widespread at southern latitudes, not only restricted to mountains. Therefore, it is very likely that they have come into contact between the Pyrenees and the Alps, a region potentially suitable for these species, especially taking into account that *artaxerxes* has proven to be a good disperser and reached, for example, the British Isles. Assuming that they have been in contact, the fact that we did not find any haplotype of *artaxerxes* in the Pyrenees or further south, and none of *montensis* to the north of these mountains, suggests that there might be some barrier to gene flow between the two taxa. This is reinforced by the considerable genetic homogeneity of *artaxerxes* across a wide geographical area (with very similar haplotypes between Russia and the United Kingdom, for example). It is hard to imagine that the very different haplotypes of *montensis* have been preserved in the face of gene flow from nearby populations with typical *artaxerxes* haplotypes while there appears to be no interruption in gene flow across the rest of the Palearctic. Although further research is needed to clarify the status of *montensis*, including a more detailed study of southern France, it is likely that it represents a distinct species.

5. Conclusions

We define a monophyletic genus *Aricia*, which includes the taxa *Pseudoaricia*, *Ultraaricia*, and *Umpria* (considered here junior subjective synonyms), but excludes *Eumedonia*, *Icaricia* and *Plebulina*.

We confirm the morphological separation of *A. cramera* based on the proportion between the length of the falces and the length of the labides. This character performs best in discriminating *A. cramera* from the similar *A. agestis*, *A. artaxerxes* and *A. montensis*.

We reveal several cases of taxonomical oversplitting within the genus, especially concerning *A. artaxerxes*. We show that the taxa *allous*, *inhonora*, *issekutzi*, *mandzhuriana* and *transalaica* are not genetically differentiated from *artaxerxes* and conclude that they probably represent either subspecies or junior synonyms of *A. artaxerxes*. By contrast, the taxon *montensis* is sister to *artaxerxes* and could represent a good species. Moreover, we highlight the presence of potential cryptic species within *A. anteros* and *A. morronensis*.

We clarify the geographical distributions of the taxa *cramera*, *agestis*, *artaxerxes* and *montensis*. We confirm the presence of *A. montensis* in North Africa and the Iberian Peninsula including the Pyrenees, and of *A. artaxerxes* across the rest of the Palearctic region. *Aricia cramera* is distributed in North Africa, the Canary Islands, the Iberian Peninsula, the Balearic Islands and Sardinia, and *A. agestis* across the rest of the Palearctic region, including Corsica and Sicily.

Our findings indicate a strong link between speciation and biogeographical patterns in the genus *Aricia*. The island mutual exclusion pattern and the hybrid zone in northeastern Spain between *A. agestis* and *A. cramera* that we document suggest hybrid infertility coupled with the lack of a prezygotic barrier for these two species, and represent a phenomenon worth deeper studies.

Acknowledgments

We thank K. Aagaard, B. Acosta, P. Bina, D. Carreras, S. Cuvelier, J. Dantart, A.V. Dantchenko, L. Dapporto, M. Djuric, R. Eastwood, S. Estradé, O. Garcia, E. García-Barros, F. González, O. Gorbunov, M. Goya, J.A. García-Alamá, J. García, A. Heath, J. Hernández-Roldán, M.A. Ibáñez-Orrico, J. Jubany, N. Kandul, M. Marín, X. Merit, S. Montagud, M.S. Mølgaard, M.L. Munguira, V. Lukhtanov, N.E. Pierce, L. Rieppel, N. Ryrholm, A. Sendra, N. Shapoval, C. Stefanescu, T. Tammaru, M.A. Travassos, S. Viader, and the Catalan Butterfly Monitoring Scheme for their help in obtaining samples used in this study. We are grateful to H. Romo and J. Martínez-Vilalta for suggestions on statistical analyses, and to M.A. Arnedo for discussions during the preparation of this study. Support for this research was provided by the Spanish Ministerio de Ciencia e Innovación (Project CGL2010-21226/BOS and predoctoral fellowship BES-2008-002054 to GT), by Fundación BBVA (BIOCON08_021) and by the Wenner-Gren Foundation (postdoctoral fellowship to VD).

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.2012.10.010.

References

Aagaard, K., Hindar, K., Pullin, A.S., James, C.H., Hammarstedt, O., Balstad, T., Hanssen, O., 2002. Phylogenetic relationships in brown argus butterflies (Lepidoptera: Lycaenidae: *Aricia*) from north-western Europe. Biol. J. Linn. Soc. 75, 27–37.

Arnqvist, G., 1997. The evolution of animal genitalia: distinguishing between hypotheses by single species studies. Biol. J. Linn. Soc. 60, 365–379.

Bálint, Z., Johnson, K., 1997. Reformation of the *Polyommatus* section with a taxonomic and biogeographic overview (Lepidoptera, Lycaenidae, Polyommatini). Neue Entomol. Nachr. 40, 1–68.

Balletto, E., Toso, G., Troiano, G., 1981. *Aricia cramera* (Erschscholtz, 1821) in Sardinia (Lycaenidae, Plebejinae). Nota Lepid. 4, 81–92.

Boggs, C., Watt, W.B., Ehrlich, P.R. (Eds.), 2003. Butterflies: Ecology and Evolution Taking Flight. University of Chicago Press, Chicago.

Brower, A.V.Z., 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proc. Natl. Acad. Sci. U. S. A. 91, 6491–6495.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17, 540–552.

De Jong, R., Vane-Wright, R., Ackery, P., 1996. The higher classification of butterflies (Lepidoptera): problems and prospects. Entomol. Scand. 27, 65–101.

Dincă, V., Zakharov, E., Hebert, P., Vila, R., 2011. Complete DNA barcode reference library for a country's butterfly fauna reveals high performance for temperate Europe. Proc. R. Soc. B 278, 347–355.

Drummond, A.J., Rambaut, A., 2007. Beast: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Heled, J., Kearse, M., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T., Wilson, A., 2010. Geneious v.4.8.3. <http://www.geneious.com>.

Ehrlich, P.R., 1958. The comparative morphology, phylogeny and higher classification of the butterflies (Lepidoptera: Papilionoidea). Kansas Univ. Sci. Bull. 39, 305–370.

Eliot, J.N., 1973. The higher classification of the Lycaenidae (Lepidoptera): a tentative arrangement. Bull. Brit. Mus. (Nat. Hist.) Entomol. 6, 371–505.

Farris, J.S., Kallersjo, M., Kluge, A.G., Bult, C., 1994. Testing significance of congruence. Cladistics 10, 315–319.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Field, A.P., 2005. Discovering Statistics using SPSS, second ed.. Sage Publications, London.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R.C., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Marine Biol. Biotechnol. 3, 294–299.

Gorbunov, P.Y., 2001. The butterflies of Russia: classification, genitalia, keys for identification (Lepidoptera: Hesperioidea and Papilionoidea). Thesis, Ekaterinburg.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Hesselbarth, G., van Oorschot, H., Wagener, S., 1995. Die Tagfalter der Türkei unter Berücksichtigung der angrenzenden Länder, vols. 1–2. Selbstverlag Sigbert Wagener, Bocholt, Germany.

Hesselbarth, G., Siepe, W., 1993. *Polyommatus* (*Aricia*) *torulensis* -eine bisher nicht bekannte lycaenide aus anatolien (Lepidoptera: Lycaenidae). Phegea 21 (2), 47–53.

Higgins, L.G., 1975. The Classification of European Butterflies. Collins, London.

Higgins, L.G., Hargreaves, B., 1983. The Butterflies of Britain and Europe. Collins, London.

Høegh-Guldberg, O., 1979. The relationship of *Aricia agestis* (Lycaenidae) and its closest relatives in Europe. Nota Lepid. 2, 35–39.

Hutcheson, G., Sofroniou, N., 1999. The Multivariate Social Scientist: Introductory Statistics Using Generalized Linear Models. Sage Publications, London.

Karsholt, O., Razowski, J., 1996. The Lepidoptera of Europe. A Distributional Checklist. Apollo Books, Stenstrup.

Keller, A., Schleicher, T., Schultz, J., Müller, T., Dandekar, T., Wolf, M., 2009. 5.8S–28S rRNA interaction and HMM-based ITS2 annotation. Gene 430, 50–57.

Koetschan, C., Förster, F., Keller, A., Schleicher T., Ruderisch, B., Schwars, R., Müller, T., Wolf, M., Schultz, J., 2010. The ITS2 database III – sequences and structures for phylogeny. Nucleic Acids Res. 38 (Database issue), D275–279.

Lukhtanov, V., Lukhtanov, A., 1994. Die Tagfalter Nordwestasiens. Verlag Ulf Eitschberger, Marktleuthen.

Lukhtanov, V., Sourakov, A., Zakharov, E., Hebert, P.D.N., 2009. DNA barcoding Central Asian butterflies: increasing geographical dimension does not significantly reduce the success of species identification. Mol. Ecol. Resour. 9, 1302–1310.

Mallet, J., Wynne, I.R., Thomas, C.D., 2011. Hybridisation and climate change: brown argus butterflies in Britain (*Polyommatus* subgenus *Aricia*). Insect. Conserv. Diver. 4 (3), 192–199.

Min, W., Xiaoling, F., 2002. Butterflies Fauna Sinica: Lycaenidae. Henan Science and Technology Publishing House, Zhengzhou.

Monteiro, A., Pierce, N.E., 2001. Phylogeny of *Bicyclus* (Lepidoptera: Nymphalidae) inferred from COI, COII, and EF -1alpha gene sequences. Mol. Phylogenet. Evol. 18, 264–281.

Munguira, M., Martín, J., 1988. Variabilidad morfológica y biológica de *Aricia morronensis* (Ribbe), especie endémica de la Península Ibérica (Lepidoptera: Lycaenidae). Ecología 2, 343–358.

Posada, D., 2008. JModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.

Seibel, P.N., Müller, T., Dandekar, T., Wolf, M., 2008. Synchronous visual analysis and editing of RNA sequence and secondary structure alignments using 4SALE. BMC Res. Notes 1, 91.

Seibel, P.N., Müller, T., Dandekar, T., Schultz, J., Wolf, M., 2006. 4SALE – a tool for synchronous RNA sequence and secondary structure alignment and editing. BMC Bioinform. 7, 498.

Schultz, J., Wolf, M., 2009. ITS2 sequence-structure analysis in phylogenetics: a how-to manual for molecular systematics. Mol. Phylogenet. Evol. 52 (2), 520–523.

Schurian, K., 2002. Beobachtungen bei der zucht von *Polyommatus* (*Aricia*) *torulensis* (Lepidoptera: Lycaenidae). Phegea 30 (2), 55–60.

Scott, J., 1985. The phylogeny of butterflies (Papilionoidea and Hesperoidea). J. Res. Lepid. 23, 241–281.

Shreeve, T.G., 1993. Confusing the geographic variation within species of *Aricia* for hybridization. The Entomologist 112, 75–80.

Siepe, W., 1995. Die primaginalstadien und die aufzucht von *Polyommatus* (*Aricia*) *torulensis* hesselbarth & siepe, 1993 (Lepidoptera: Lycaenidae). Phegea 23, 167–172.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87, 651–701.

Smyllie, B., 1995. The brown argus butterfly in north-western Europe. Entomol. Rec. J. Var. 107, 15–24.

Smyllie, B., 1996. The brown argus butterfly: hybrids or no hybrids. Entomol. Rec. J. Var. 108, 19–23.

Swofford, D.L., 2000. PAUP*. Phylogenetic Analysis using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Tolman, T., Lewington, R., 1997. Butterflies of Britain and Europe. Harper Collins Publishers, London.

Tolman, T., Lewington, R., 2008. Collins Butterfly Guide. The Most Complete Guide to the Butterflies of Britain and Europe. Harper Collins Publishers, London.

Tshikolovets, V.V., 2000. The Butterflies of Uzbekistan. Vadim Tshikolovets, Kiev.

Tuzov, V., Bogdanov, P., Churkin, S., Dantchenko, A., Devyatkin, A., Murzin, V., Samodurov, G., Zhdanko, A., 2000. Guide to the butterflies of Russia and adjacent territories, vol. 2. Libytheidae, Danaidae, Nymphalidae, Riodinidae, Lycaenidae, Pensoft Publishers, Moscow.

Vane-Wright, R.I., 2003. Butterflies. Life Series. The Natural History Museum, London.

Vila, R., Bell, C.D., Macniven, R., Goldman-Huertas, B., Ree, R.H., Marshall, C.R., Bálint, Z., Johnson, K., Benyamini, D., Pierce, N.E., 2011. Phylogeny and palaeoecology of *Polyommatus* blue butterflies show Beringia was a climate-regulated gateway to the New World. Proc. R. Soc. B 278, 2737–2744.

White, T.J., Bruns, S., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M., Gelfandm, D.H., Misky, J.J., White, T. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, pp. 315–322.

Wiemers, M., Fiedler, K., 2007. Does the DNA barcoding gap exist? A case study in blue butterflies (Lepidoptera: Lycaenidae). Front. Zool. 4, 8.

Wiemers, M., Keller, A., Wolf, M., 2009. ITS2 secondary structure improves phylogeny estimation in a radiation of blue butterflies of the subgenus *Agrodiaetus* (Lepidoptera: Lycaenidae: *Polyommatus*). BMC Evol. Biol. 9, 300.

Please cite this article in press as: Sañudo-Restrepo, C.P., et al. Biogeography and systematics of *Aricia* butterflies (Lepidoptera, Lycaenidae). Mol. Phylogenet. Evol. (2012), http://dx.doi.org/10.1016/j.ympev.2012.10.010