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A molecular phylogeny of *Polyommatus* s. str. and *Plebicula* based on mitochondrial *COI* and nuclear *ITS2* sequences (Lepidoptera: Lycaenidae)

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Abstract. The phylogenetic relationships of the subgenera *Polyommatus* and *Plebicula*, within the Palaearctic butterfly genus *Polyommatus*, were inferred from a combined analysis of the nuclear marker *ITS2* and the barcoding section of the mitochondrial gene *COI*. Eight major clades were recovered within *Polyommatus* s. l., which correspond closely to subgenera based on traditional systematics and are of late Pliocene to early Pleistocene origin. Extraordinary chromosomal evolution occurred independently in three of these clades. The disputed position of several species formerly placed in the subgenus *Plebicula* is clarified. A group of Central Asian species (*Bryna*) was recovered as a monophyletic clade within *Polyommatus* s. str. The Kurdistanian endemic *P. buzulmavi* appears as a sister species to *P. icarus*. *P. celina* replaces *P. icarus* in NW Africa and the Canary Islands, and split from the last common ancestor with *P. icarus* back in the early Pleistocene.

INTRODUCTION

Polyommatus Latreille, 1804 is a genus of blue butterflies, which is distributed throughout the Palaearctic region. Its type-species, *Polyommatus icarus* (Rottemburg, 1775), is the most widespread and common representative and was recently even introduced into Ontario in Canada (Hall, 2007). The delineation of *Polyommatus* has been contentious. Among the nomenclaturally available genus names listed in Eliot (1973) within his "*Polyommatus* section" several are currently often regarded as synonyms or subgenera of *Polyommatus* (e.g. Hesselbarth et al., 1995; Bálint & Johnson, 1997; see Table 1). These are *Cyaniris* Dalman, 1816, *Bryna* Evans, 1912, *Meleageria* de Sagarra, 1925, *Agrodiaetus* Hübner, 1822, *Lysandra* Hemming, 1933 and *Plebicula* Higgins, 1969. The same applies to three further (sub-)genera, which were described in 1977: *Neolysandra* Koçak, *Sublysandra* Koçak and *Paragrodiaetus* Rose & Schurian. *Kretania* Beuret, 1959 was included within *Polyommatus* by Bálint & Johnson (1997), whereas most other authors (e.g. Hesselbarth et al., 1995) treat *Kretania* as a subgenus of *Plebejus* Kluk, 1802. Bálint & Johnson (1997) also include the genera *Glabroculus* Lvovsky, 1993 (= *Elviria* Zhdanko, 1994) and *Rimisia* Zhdanko, 1994 within *Polyommatus*. Gorbunov (2001) synonymized *Polyommatus* with *Plebejus*, but this is not accepted by most current authors. It should be noted that no clear synapomorphic morphological features have been found that could be used to delineate the genus *Polyommatus*, although Bálint & Johnson (1997) tried to give a diagnosis. Also no phylogenetic analyses have been attempted based on morphological characters, and all currently available systematic treatments are mostly based on the intuition of the authors. Indicative of the state of the taxonomy within Polyommatini is the frustration expressed by Eliot (1973): "I have to admit complete failure in my efforts to find a satisfactory basis for subdividing this very large tribe into a few major natural groups. I have therefore fallen back on naming no less than thirty 'sections', many of them of no more than subsection or even generic worth."

Recent molecular genetic studies (Wiemers, 2003; Kandul et al., 2004; Lukhtanov et al., 2009; Wiemers et al., 2009) have largely confirmed a monophyletic genus *Polyommatus* sensu Hesselbarth et al. (1995) to the exclusion of *Glabroculus*, *Elviria* and *Kretania*. The latter appears to be closely related to *Plebejus* (*Plebejides*). The position of *Cyaniris* and *Lysandra*, however, differs depending on the molecular markers employed. In this paper, we mainly follow the systematic treatment of Hesselbarth et al. (1995), with the exception of *Lysandra.* This genus was synonymized with *Meleageria* by Hesselbarth et al. (1995), but none of the previous molecular studies have suggested a close relationship between these two genera. According to this delineation, the genus *Polyommatus* consists of approximately 200 species, of which more than half belong to the subgenus *Agrodiaetus* sensu Eckweiler & Häuser (1997).

Several molecular phylogenetic studies have been conducted on *Polyommatus* s. str., a group of taxa closely related to the type species *P. icarus* (Vodolazhsky & Stradomsky, 2008a, b; Vodolazhsky et al., 2009). Other studies focused on the subgenus *Agrodiaetus*, a large radiation of about 130 species, which is peculiar for its extraordinary variation in chromosome numbers (Lesse, 1960; Wiemers, 2003; Kandul et al., 2004; Lukhtanov et al., 2005; Kandul et al., 2007; Wiemers et al., 2009). Of these latter studies only Wiemers (2003) and Wiemers et

TABLE 1. Previous systematic arrangement of the genus *Polyommatus*.

Genus-level taxa	Hesselbarth et al. (1995)	Bálint & Johnson (1997)
Polyommatus Latreille, 1804	subgenus of *Polyommatus*	*eros*-group *icarus*-group
Cyaniris Dalman, 1816	subgenus of *Polyommatus*	*semiargus*-group
Bryna Evans, 1912	not treated	*stoliczkanus*-group
Meleageria Sagarra, 1925	subgenus of *Polyommatus*	*daphnis*-group
Agrodiaetus Hübner, 1822	subgenus of *Polyommatus*	*actinides*-group *actis*-group *admetus*-group *carmon*-group *dama*-group *damon*-group *damone*-group *dolus*-group *nadira*-group *poseidon*-group *poseidonides*-group *transcaspicus*-group
Lysandra Hemming, 1933	synonym of *Meleageria*	*coridon*-group
Plebicula Higgins, 1969	synonym of *Polyommatus*	*dorylas*-group *icarius*-group
Neolysandra Koçak, 1977	subgenus of *Polyommatus*	*coelestina*-group
Sublysandra Koçak, 1977	synonym of *Polyommatus*	*cornelia*-group *myrrha*-group
Paragrodiaetus Rose & Schurian, 1977	not treated	*erschoffii*-group *glaucias*-group
Kretania Beuret, 1959	subgenus of *Plebejus*	*eurypilus*-group
Glabroculus Lvovsky, 1993	not treated	*cyane*-group
Rimisia Zhdanko, 1994	not treated	*cyane*-group

al. (2009) include a near comprehensive selection of other *Polyommatus* taxa. Several species that might be important for understanding the phylogeny of *Polyommatus* were not included in these analyses, e.g. Central Asian taxa of the *stoliczkanus*-group (*Bryna*) and several species of the subgenus *Plebicula*, including the NW African *P. atlanticus* (Elwes, 1905), which has the highest chromosome number known in Metazoa (n = 221–223, De Lesse, 1970).

Such large deviations from the modal chromosome number of n = 24 are only known from three subgenera (*Agrodiaetus*, *Lysandra* and *Plebicula*) and therefore some authors assume them to be closely related (Lorković, 1990). Resolving the relationships between these subgenera might also increase the level of understanding of chromosomal evolution in *Polyommatus.*

Also of interest is the evolution of larval food plant associations in *Polyommatus*. Although the food plants of several Asian species are unknown, all *Polyommatus* species appear to be restricted to plants of the family Fabaceae. Only a Spanish population of *Cyaniris semiargus* is also known to utilize another family, Plumbaginaceae (Rodríguez et al., 1991, 1993). By contrast, closely related genera (like *Plebejus* s. l.) also use several other plant families (e.g. Geraniaceae, Cistaceae, Labiatae, Ericaceae, Primulaceae, Polygoniaceae and Chenopodiaceae). While larvae of the ubiquitous *P. icarus* accept many different genera, most other species appear to be oligophagous on a single or two closely related genera of Fabaceae (Hesselbarth et al., 1995; Tuzov et al., 2000; Tolman & Lewington, 2008).

This paper presents the first comprehensive study using both mitochondrial and nuclear DNA sequences to infer the phylogeny of *Polyommatus*.

In this paper, we also re-assess the taxonomic status and distribution of *P. celina* (Austaut, 1879). This taxon (type locality: Sidi-Bel-Abbès in Algeria) used to be regarded as a subspecies, synonym or form of *P. icarus* until molecular studies (Wiemers, 2003; Wiemers & Fiedler, 2007) discovered strong genetic differentiation (p-distance: 5.9–6.8% in COI and 1.5–2.5% in ITS2) between a population from the High Atlas mountains in Morocco and Eurasian populations. Vodolazhsky & Stradomsky (2008b) confirmed this result using another specimen from the Moroccan Anti-Atlas and raised *P. celina* to species level based not only on molecular differences but also on a morphological feature, the presence of a broad marginal darkening on the upper surface of the male forewing, which is always absent in *P. icarus* but typical of *P. eros*. However, the extent of the marginal darkening in Moroccan specimens appears to be quite variable and can be nearly absent in some cases. The question therefore arises whether *P. icarus* also occurs on the African continent, e.g. in coastal districts, or is completely replaced by *P. celina*. The identity of the *P. icarus* populations in the Canary Islands, which have also been

TABLE 2. Previous taxonomic assignments of *Polyommatus* species-level taxa.

Species	Hesselbarth et al. (1995)	Bálint & Johnson (1997)
abdelaziz	conspecific with *amandus*	species in the *icarius*-group
actinides	not treated	species in the *actinides*-group
admetus	species in the subgenus *Agrodiaetus*	species in the *admetus*-group
aedon	species in the subgenus *Polyommatus*	species in the *myrrha*-group
amandus	species in the subgenus *Polyommatus*	species in the *icarius*-group[1]
amor	not treated	species in the *eros*-group
amorata	not treated	not treated
andronicus	not treated	not treated
ariana	not treated	species in the *stoliczkanus*-group
armenus	subspecies of *dorylas*	species in the *dorylas*-group
atlanticus	not treated	species in the *dorylas*-group
boisduvalii	not treated	not treated
buzulmavi	species in the subgenus *Polyommatus*	species in the *daphnis*-group
carmon	species in the subgenus *Agrodiaetus*	species in the *carmon*-group
celina	not treated	not treated
ciloicus	species in the subgenus *Polyommatus*	species in the *stoliczkanus*-group
coelestina	species in the subgenus *Neolysandra*	species in the *coelestina*-group
coridon	species in the subgenus *Meleageria*	species in the *coridon*-group
cornelia	species in the subgenus *Polyommatus*	species in the *cornelia*-group
corona	species in the subgenus *Neolysandra*	species in the *coelestina*-group
damon	species in the subgenus *Agrodiaetus*	species in the *damon*-group
daphnis	species in the subgenus *Meleageria*	species in the *daphnis*-group
dolus	species in the subgenus *Agrodiaetus*	species in the *dolus*-group
dorylas	species in the subgenus *Polyommatus*	species in the *dorylas*-group
erigone	not treated	species in the *stoliczkanus*-group
eroides	species in the subgenus *Polyommatus*	species in the *eros*-group
eros	species in the subgenus *Polyommatus*	species in the *eros*-group
erotides	not treated	species in the *eros*-group
erschoffii	not treated	species in the *erschoffii*-group
escheri	species in the subgenus *Polyommatus*	species in the *icarius*-group
fatima	species in the subgenus *Neolysandra*	species in the *coelestina*-group
forsteri	subspecies of *eroides*	not treated
fuchsi	not treated	not treated
golgus	not treated	species in the *dorylas*-group
hunza	not treated	species in the *stoliczkanus*-group
icadius	not treated	species in the *icarus*-group
icarus	species in the subgenus *Polyommatus*	species in the *icarus*-group
iphidamon	not treated	species in the *damon*-group
iphigenia	species in the subgenus *Agrodiaetus*	species in the *damon*-group
iphigenides	not treated	species in the *poseidonides*-group
juno	subspecies of *icarus*	species in the *icarus*-group
kamtshadalis	not treated	not treated
marcida	not treated	species in the *daphnis*-group
menelaos	subspecies of *eros*	species in the *eros*-group
myrrha	species in the subgenus *Polyommatus*	species in the *myrrha*-group
myrrhinus	subspecies of *aedon*	species in the *myrrha*-group
nivescens	not treated	species in the *dorylas*-group
orientalis	not treated	not treated
poseidon	species in the subgenus *Agrodiaetus*	species in the *poseidon*-group
poseidonides	not treated	species in the *poseidonides*-group
semiargus	species in the subgenus *Cyaniris*	species in the *semiargus*-group
stoliczkanus	not treated	species in the *stoliczkanus*-group
tartarus	not treated	not treated
thersites	species in the subgenus *Polyommatus*	species in the *actinides*-group
tsvetajevi	not treated	not treated
venus	not treated	species in the *eros*-group
versicolor	subspecies of *daphnis*	not treated

[1] Bálint & Johnson (1997) treat *amandus* as a synonym of *icarius* (Esper, [1789]).

assigned to ssp. *celina* (Wiemers, 1995), likewise needs clarification.

MATERIAL AND METHODS

60 specimens of 23 species of the subgenus *Polyommatus* and outgroups from all other subgenera of the genus *Polyommatus* were included in this phylogenetic analysis. Included were taxa from all species groups of *Polyommatus* s. l. according to Bálint & Johnson (1997) apart from the *cyane-* and *eurypilus*-group (subgenera *Glabroculus*, *Elviria* & *Kretania*), which do not seem to belong to *Polyommatus* (see above). In *Agrodiaetus*, representatives of each of the clades found by Wiemers (2003) were chosen, plus *Agrodiaetus actinides* (Staudinger, 1886). Only a single representative of *Lysandra* was selected, because earlier studies indicate that this subgenus is monophyletic. Previous taxonomic assignments of taxa selected for this study are indicated in Table 2. *Chilades trochylus* (Freyer, 1845) was used as an outgroup to root the tree, because *Chilades* is the only Holarctic genus in the subtribe Polyommatina that does not belong to the genus *Polyommatus*, i.e. does not belong to *Polyommatus* s.l. or *Plebejus* s. l. No molecular data are currently available for the other three Polyommatina genera (*Pseudolucia* Nabokov, 1945; *Madeleinea* Bálint, 1993; *Paralycaeides* Nabokov, 1945), which are all restricted to the Neotropical region (mostly confined to the high Andes).

Selected specimens with voucher codes, locality data and GenBank accession numbers are listed in Table 3. The names of the subgenera used follow Hesselbarth et al. (1995) with the exception of *Lysandra*, which is listed as a distinct subgenus.

An additional 8 specimens of presumed *Polyommatus celina* from different areas in Morocco, Tunisia and the Canary Islands were used in a *COI* haplotype analysis (Table 3, Fig. 1).

A total of 122 sequences were used in these analyses. Of these, 29 were exclusively determined for this study. The remaining sequences were selected from previous studies (Wiemers & Fiedler, 2007; Vodolazhsky & Stradomsky, 2008a, b; Vodolazhsky et al., 2009; Wiemers et al., 2009).

DNA extraction, PCR and sequencing were carried out according to the protocols described in Vodolazhsky & Stradomsky (2008a) for the specimens with “ILL” voucher codes and in Wiemers (2003) for the remaining specimens.

The 5’ (barcode) section of the mitochondrial gene Cytochrome c Oxidase I (*COI*) and the (mostly complete) nuclear Internal Transcribed Spacer 2 (*ITS2*) were sequenced because these two sections have proved most successful for resolving the phylogeny of young radiations (Wiemers et al., 2009).

The following PCR primer pairs: PolF (forward, 5’-TAG CGA AAA TGA CTT TTT TCT A-3’) with PolR (reverse, 5’-AAG AAT GAG GTA TTG AGG TTT C-3’) or PolRR (reverse, 5’-TTG CTC CAG CTA ATA CAG GTA A-3’) were used to amplify *COI* in specimens with “ILL” voucher codes. K698 (forward, 5’-TAC AAT TTA TCG CCT AAA CTT CAG CC-3’ with Nancy (reverse, 5’-CCC GGT AAA ATT AAA ATA TA ACT TC-3’) were used for the remaining specimens. *ITS2* was amplified with PiF (forward, 5’- GGG CCG GCT GTA TAA AAT CAT A -3’) and PiR (reverse, 5’- AAA AAT TGA GGC AGA CGC GAT A -3’) in specimens with “ILL” voucher codes, and ITS3 (forward, 5’-GCA TCG ATG AAG AAC GCA GC-3’) with ITS4 (reverse, 5’-TCC TCC GCT TAT TGA TAT GC-3’) for the remaining specimens.

The following cycling protocols were used: For the primer pairs PolF/PolR, PolF/PolRR and PiF/PiR an initial 4 min denaturation at 94°C and 36 cycles of 40 s denaturation at 94°C, 40 s annealing at 58°C and 40 s extension at 72°C; for k698/Nancy an initial 4 min denaturation at 94°C and 35 cycles of 30 s denaturation at 94°C, 30 s annealing at 55°C, 1 min extension at 72°C and a final extension at 72°C for 7 min; for ITS3/ITS4 an initial 1 min denaturation at 94°C and 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 48°C, 1 minute extension at 72°C and a final extension at 72°C for 1 min.

The alignment of *ITS2* sequences was based on the alignment by Wiemers et al. (2009) taking secondary structure information into account. Additional *ITS2* sequences were subsequently aligned with ClustalW (Thompson et al., 1994) and some minor manual corrections were carried out. *COI* sequences were aligned manually.

A Bayesian approach for estimating phylogeny using MrBayes 3.1.1 was used (Ronquist & Huelsenbeck, 2003). The data set was divided into 4 partitions, one for each *COI* codon position and one for *ITS2*. Model parameters were estimated separately for each partition using MrModeltest 2.2 (Nylander, 2004) and unlinked across partitions. Standard model parameters were applied for the partition containing the 2nd *COI* codon position. For the other partitions, a General Time Reversible model was applied, with a proportion of invariable sites for the partitions containing the 1st and 3rd *COI* codon positions and a gamma-shaped distribution of rates across sites. The overall evolutionary rate was allowed to differ for the different partitions. The standard 4by4 substitution model and a flat Dirichlet prior were used for this analysis. Four completely independent analyses, each with four MCMC chains, were run for 10,000,000 generations and sampled every 100th generation. The heating temperature was set to 0.05 to improve chain mixing (compared to the standard setting of 0.20). The first 200 trees were discarded as burn in. PAUP 4.0 beta 10 (Swofford, 1998) was used to calculate Maximum Parsimony bootstrap values (1000 replicates). Incongruence between the mitochondrial and the nuclear data partitions was determined using the Incongruence Length Difference (ILD) test, implemented as “Partition Homogeneity Test” in PAUP (Farris et al., 1995; but see Barker & Lutzoni, 2002; Planet, 2006). Invariant characters were excluded from the data set (Cunningham, 1997). Separate analyses for the mitochondrial and nuclear partitions were also conducted with the same parameter settings. MEGA 4.1 (Tamura et al., 2007) was used to calculate sequence statistics and pairwise distances (Kimura 2 Parameter model). We used the same programme to date the age of several major nodes by calculating the mean pairwise uncorrected distance of the descendant species of this node. In order to avoid sampling bias, only one sequence per species of the nominate subspecies was used. Standard error estimates were obtained by a bootstrap procedure (1000 replicates). The mean distance was divided by a substitution rate of 1.5% per million years, which appears to be a conserved rate for the *COI* of arthropods (Quek et al., 2004).

Statistical parsimony networks of *COI* sequences were calculated with TCS 1.21 (Clement et al., 2000) using a statistical connection limit of 95%.

Mesquite 2.72 was used for ancestral character state reconstruction of karyological and food plant traits using unordered parsimony reconstruction (Maddison & Maddison, 2009).

RESULTS

Because there were no insertions or deletions in the dataset the alignment of *COI* sequences was straightforward. Of 690 positions, 219 were variable and 153 parsimony-informative. Variability was mainly confined to silent positions. The translation had only 35 (= 15.2%) variable amino acid positions of which 19 (= 8.3%) were parsimony-informative.

TABLE 3. List of material with voucher codes and GenBank accession number.

(Sub-)Genus	Species	Voucher	Country	Locality	COI acc	ITS2 acc
Agrodiaetus	*actinides*	WE94001	Kirgizia	Aram-Kungei valley, Alytyn Dara river, 3000 m, W Transalai	GU559748	AY556753
Agrodiaetus	*admetus*	JC01014	Greece	Mt. Taiyetos, 1200–1300 m, Peloponnisos	AY556867	AY556733
Agrodiaetus	*carmon*	MW98009	Turkey	Karabayir S Korkuteli, 1400 m, Antalya	AY556981	AY556622
Agrodiaetus	*damon*	MW99613	France	Col de Tende Tende, 1850 m, Alpes Maritimes	AY557131	AY556714
Agrodiaetus	*dolus*	MT06048	France	Auriol La Roussargue, Bouches-du-Rhône, 550 m	GU559741	GQ166173
Agrodiaetus	*erschoffii*	MW00393	Iran	Hajiabad, 25 km SSW Gorgan, 2150 m, Golestan	AY556925	AY556588
Agrodiaetus	*iphidamon*	MW00328	Iran	Shakuh, 2600 m, Golestan	AY556919	AY556584
Agrodiaetus	*iphigenia*	MW99009	Turkey	5 km SO Caglayan, 1500 m, Erzincan	AY557027	AY556656
Agrodiaetus	*iphigenides*	DS01001	Uzbekistan	Kitabsky national reserve, Zeravshansky Mts., 1500–2500 m	AY556853	AY556722
Agrodiaetus	*poseidon*	MW98180	Turkey	Gökpinar, Gürün, 1700 m, Sivas	AY557002	AY556636
Agrodiaetus	*poseidonides*	DS00001	Tajikistan	Safedou, Darvaz Mts, 2500 m	AY556851	AY556721
Chilades	*trochylus*	MW99425	Turkey	Dez Cay NE Hakkari, 1500 m, Hakkari	AY557100	GQ166186
Cyaniris	*semiargus persica*	MW00525	Iran	Takht-e Suleyman SW Marzanabad, 3500–3700 m	AY556937	AY556597
Cyaniris	*semiargus semiargus*	MW02034	Morocco	Oukaimeden, High Atlas, 2700 m, Marrakech	AY556978	AY556621
Lysandra	*coridon*	MW99612	Italy	Pondel, Val di Cogne, 900 m, Aosta	AY557130	AY556713
Meleageria	*daphnis versicolor*	MW98029	Turkey	Gülübeli Gecidi W Elmali, 1500 m, Fethiye	AY556983	AY556623
Meleageria	*daphnis marcida*	MW00290	Iran	S Veresk, 1800–1950 m, Mazandaran	AY556914	AY556580
Neolysandra	*coelestina*	MW99013	Turkey	5 km SO Caglayan, 1500 m, Erzincan	AY557028	AY556657
Neolysandra	*corona*	MW00504	Iran	Takht-e Suleyman, SW Marzanabad, 3000 m, Mazandaran	AY556935	AY556595
Neolysandra	*fatima*	MW99301	Turkey	5-18 km N Catak, 1600–1900 m, Van	AY557077	AY556682
Polyommatus	*aedon*	MW00326	Iran	Shakuh, 2600 m, Golestan	AY556917	AY556583
Polyommatus	*amandus abdelaziz*	MW02001	Morocco	Oukaimeden, High Atlas, 2300 m, Marrakech	AY556967	AY556617
Polyommatus	*amandus amandus*	MW99047	Turkey	Köskköy, 25 km N Erzurum, 1900 m, Erzurum	AY557035	AY556661
Polyommatus	*amor*	ILL067	Tadzhikistan	Anzob pass, 3200 m S. Gissar	GU354258	GU354252
Polyommatus	*amorata amorata*	ILL051	China	S.-W. Sichuan, 2500 m	GQ885168	GQ885149
Polyommatus	*amorata tartarus*	ILL076	China	Wudu distr., 2000 m, S. Gansu	GQ885169	GQ885150
Polyommatus	*amorata tsvetajevi*	ILL050	Russia	Suchodol riv, 30 m, Primorsky reg.	GQ885170	GQ885151
Polyommatus	*ariana*	ILL057	India	Kulu distr., 3000 m, Hymachal-Pradesh	GU354254	GU354248
Polyommatus	*atlanticus*	MT08006	Morocco	Agdz, Haute Vallée du Drâa, Anti-Atlas Oriental, 1200 m	GU559743	GU559749
Polyommatus	*buzulmavi*	KS09004	Turkey	Kirikdag, Dez valley, 2100–2200 m, Hakkari	GU559738	—
Polyommatus	*celina*	MW02006	Morocco	Oukaimeden, High Atlas, 2300 m, Marrakech	AY556968	AY556618
Polyommatus	*celina*	MW02027	Morocco	Tourchte, High Atlas, 1400 m, Marrakech	GU559745	—
Polyommatus	*celina*	MW02035	Morocco	Oukaimeden, High Atlas, 2700 m, Marrakech	GU559746	—
Polyommatus	*celina*	MT05019	Morocco	Col du Zad, S Timahdite, Middle Atlas, 1800–2100 m	GU559739	—
Polyommatus	*celina*	MT05021	Morocco	Oukaimeden, High Atlas, 2200–3300 m, Marrakech	GU559740	—
Polyommatus	*celina*	MT07012	Morocco	Tizi-n-Tarakatine, E Tafraoute, Antiatlas, 1400 m	GU559742	—
Polyommatus	*celina*	BA09010	Spain	Betancuria, 420 m, Fuerteventura, Canary Islands	GU559737	—
Polyommatus	*celina*	ILL044	Morocco	Tafraout, 1300 m, Antiatlas	FJ428805	GU377279
Polyommatus	*celina*	ILL069	Tunesia	Mahdia, 10 m	GU377278	—
Polyommatus	*ciloicus*	ILL039	Iran	S.Suleyman, 2300 m, Zanjan	FJ431282	GQ885153
Polyommatus	*cornelia*	MW98264	Turkey	Gezbeli Gecidi, 1800 m, Kayseri	AY557015	AY556647
Polyommatus	*dorylas dorylas*	MW01019	Spain	Ubierna, 20 km N Burgos, 900 m, Burgos	AY556946	AY556605
Polyommatus	*dorylas armenus*	MW99014	Turkey	5 km SO Caglayan, 1500 m, Erzincan	AY557029	AY556658
Polyommatus	*erigone erigone*	ILL065	Tadzhikistan	Chechekty riv., 4200 m, E. Pamir	GU354256	GU354250
Polyommatus	*erigone hunza*	ILL066	Tadzhikistan	Lake Dunkeldyk, 4200 m, S.E. Pamir	GU354257	GU354251
Polyommatus	*eros boisduvalii*	ILL040	Russia	Ilovlya, 40 m, Volgograd reg.	FJ428804	GQ885152
Polyommatus	*eros eroides*	JC00042	Greece	Rodopi Mts., 1200 m, Macedonia	AY556857	AY556726
Polyommatus	*eros eros*	ILL017	Italy	Taufers, 1500 m S.Tirol	EU597138	GQ885155
Polyommatus	*eros erotides*	ILL068	Russia	E. Sayan, Tissa riv., 1500 m. Burjatia	GQ885171	GQ885157
Polyommatus	*eros kamtshadalis*	RU02003	Russia	Sokol, NE Siberia, Magadan	GU244492	GQ166184
Polyommatus	*eros menelaos*	JC00029	Greece	Mt. Taiyetos, 1180–1200 m, Peloponnisos	AY556854	AY556723
Polyommatus	*eros orientalis*	ILL063	Russia	Fedyashevo vill., 220 m, Tula reg.	FJ435341	GQ885156
Polyommatus	*escheri*	JC00039	Greece	Mt. Falakro, 1650 m, Macedonia	AY556855	AY556724
Polyommatus	*forsteri*	MW00530	Iran	Takht-e Suleyman SW Marzanabad, 3500–3700 m	AY556938	AY556598
Polyommatus	*golgus*	MT08032	Spain	Pico Veleta, Sierra Nevada, 2700 m	GU559744	—
Polyommatus	*icadius*	ILL022	Tajikistan	Lake Dunkeldyk, 4100 m, S.-E. Pamir	EU597143	GQ885159
Polyommatus	*icarus*	JC00063	Greece	Mt. Falakro, 1650 m, Macedonia	AY556866	AY556732
Polyommatus	*icarus (= andronicus)*	JC00061	Greece	Mt. Falakro, 1650 m, Macedonia	AY556864	AY556731
Polyommatus	*icarus fuchsi*	ILL043	Russia	Nov. Chara, 750 m, Chita reg.	FJ428818	GQ885161
Polyommatus	*icarus juno*	DB08003	Israel	Mt. Hermon, 2050 m	GU244491	GQ166170
Polyommatus	*myrrhinus*	MW99550	Turkey	Kop Gecidi N Askale, 2200 m, Erzurum	AY557122	AY556706
Polyommatus	*nivescens*	RU08002	Spain	Malanquilla, Zaragoza	GU559747	—
Polyommatus	*stoliczkanus*	ILL077	India	Brandy riv., 5100 m, Ladakh	GU354259	GU354253
Polyommatus	*thersites*	MW00302	Iran	S Veresk, 1800–1950 m, Mazandaran	AY556915	AY556581
Polyommatus	*thersites*	MW01083	Spain	Triste, Embalse de la Pena, 600 m, Huesca	AY556959	AY556613
Polyommatus	*venus*	ILL058	Kirgizstan	Aram-Kungei riv., 3200 m, Transalai	GU354255	GU354249

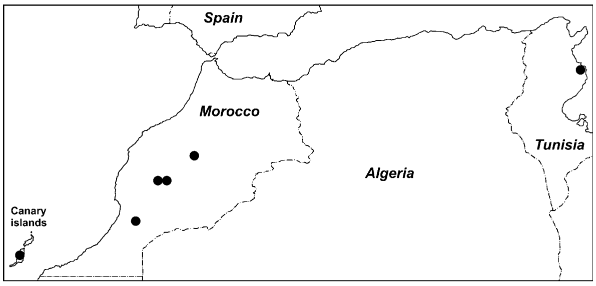

Fig. 1. Map showing the distribution of *Polyommatus celina*. Dots indicate populations that were subjected to DNA analysis.

Table 4. Estimates of the ages of the major clades (in millions of years ago). Node numbers refer to those used in Fig. 2.

Node Nr	Clade	Age
1	*icarus* species group	1.07–1.60
2	*stoliczkanus* species group	0.87–1.40
3	Subgenus *Polyommatus*	2.20–2.87
4	Subgenus *Plebicula*	2.07–2.73
5	Subgenus *Polyommatus* + *Plebicula*	2.53–3.07
6	Subgenus *Sublysandra*	1.87–2.53
7	Subgenus *Agrodiaetus*	2.87–3.40
8	Subgenus *Neolysandra* partim	1.93–2.60

The aligned *ITS2* dataset had 719 positions of which 199 were variable and 81 parsimony-informative (with gaps treated as missing data).

The resulting trees from the separate analyses of the mitochondrial and nuclear partitions (not shown) revealed that most of the phylogenetic signal was concentrated in the *ITS2* character set. Resolution of the *COI* tree was mainly confined to closely related species groups, e.g. among members of *Polyommatus* s. str. The ILD test indicated a significant difference between the mitochondrial and the nuclear partition ($p < 0.001$). However, the only major difference revealed by the two analyses is the position of *Lysandra*, which is sister to *Neolysandra coelestina* in the *COI* tree, with a Bayesian support of 0.99, whereas it has the most basal position in the trees inferred from the *ITS2* dataset. Another minor difference is in the Central Asian *Bryna* subclade. *COI* data suggest a sister relationship between *P. stoliczkanus* and *P. ariana*, whereas the *ITS2* data set favours *P. stoliczkanus* and *P. venus* as sister species. As the difference is small the two data sets were pooled and reanalysed.

Fig. 2 displays the phylogenetic tree obtained from the combined Bayesian analysis of *COI* and *ITS2*. *Polyommatus icarus* forms a cluster together with its sister species *P. buzulmavi* Carbonell [1992], as well as with *P. forsteri* (Pfeiffer, 1938), *P. ciloicus* de Freina & Witt, 1983, *P. icadius* (Grum-Grshimailo, 1890), *P. eros* (Ochsenheimer, 1808) and *P. amorata* (Alpheraky, 1897). Apart from *P. buzulmavi*, a local endemic of SE Anatolia, the phylogenetic postions of these taxa are covered and discussed by Vodolazhsky et al. (2009). This clade (① in Fig. 2) combines with *P. celina* (Austaut, 1879) and a clearly monophyletic group of Central Asian taxa (clade ②) into a monophyletic clade named here *Polyommatus* s. str. ③. The Central Asian subclade ②, includes *P. stoliczkanus* (Felder & Felder, 1865), the type species of *Bryna*, as well as *P. venus* (Staudinger, 1886), *P. ariana* Moore, 1865, *P. amor* (Lang, 1884) and *P. erigone* (Grum-Grshimailo, 1890). The subgenera *Agrodiaetus* sensu Eckweiler & Häuser (1997) ⑦ and *Sublysandra* ⑥ also appear to be monophyletic, whereas the subgenus *Neolysandra* does not. *Plebicula* does not seem to constitute a monophyletic unit in its original circumscription, but the reduced core genus sensu Higgins (1975) does and forms clade ④ together with its sister *P. thersites* (Cantener, 1834). *P. amandus* (Schneider, 1792) and *P. escheri* (Hübner, 1823), two further species, which were originally included with *Plebicula* by Higgins (1969), are located at other positions on the tree. *P. amandus* forms clade ⑧ together with *Neolysandra fatima* (Eckweiler & Schurian, 1980) and *N. corona* (Verity, 1936), whereas the position of *P. escheri* is not well resolved. The subgenus *Polyommatus* s. str. (clade ③) forms a monophyletic sister clade to clade ④. The resulting clade ⑤ clusters together with *Sublysandra, Agrodiaetus* and *Polyommatus escheri*, as a sister to clade ⑧. *Meleageria* and *Lysandra* form two clearly separate clusters at the base of the tree, and *Cyaniris* branches off outside *Polyommatus* (clade ⑩).

According to the dating estimates (Table 4), the genus *Polyommatus* originated in the Pliocene, 3.3–3.9 million years ago (MYA), and most subgenera (clades ③–⑧) originated in the Gelasian (early Quarternary according to the revised IUGS geological time scale) and the Piacenzian, about 1.9–3.4 MYA. The *icarus*- and *stoliczkanus*-species groups (clade ① and ②, respectively) evolved in the Calabrian period of the Pleistocene, 0.9–1.6 MYA.

Three clades contain species with high chromosome numbers, i.e. much higher than the modal value of 24: the clades containing the subgenera *Plebicula*, *Agrodiaetus* and *Lysandra* (Figs 2–3). None of these clades appear to be sister clades. Furthermore, the sister species to the *Plebicula* clade is a species with $n = 24$ (*P. thersites*). Therefore, marked chromosomal diversification appears to have taken place at least three times in the genus *Polyommatus.* These results are in agreement with Kandul et al. (2004).

Mapping the larval food plants onto the tree (Fig. 3) reveals at least eight food plant switches: the first one from *Trifolium* to either *Vicia* or *Coronilla (Securigera)/ Hippocrepis.* Two switches are required from feeders on *Vicia* to *Coronilla (Securigera)*/*Hippocrepis* or vice versa, because neither feeding group appears to be monophyletic. A fourth switch was either to *Astragalus/Oxytropis* or to *Onobrychis/Hedysarum.* In the first case two switches were required to *Onobrychis/Hedysarum* (in *Agrodiaetus* and *P. thersites*) and in the second case two switches to *Astragalus/Oxytropis* (in *P. escheri* and *Polyommatus* s. str.). A seventh switch was required to *Cicer* (in *Sublysandra*) and an eighth to *Anthyllis* (in *Plebicula*). Further switches might have been required in *Polyommatus* s. str., because several other genera are used in this group apart from *Astragalus/Oxytropis*, like

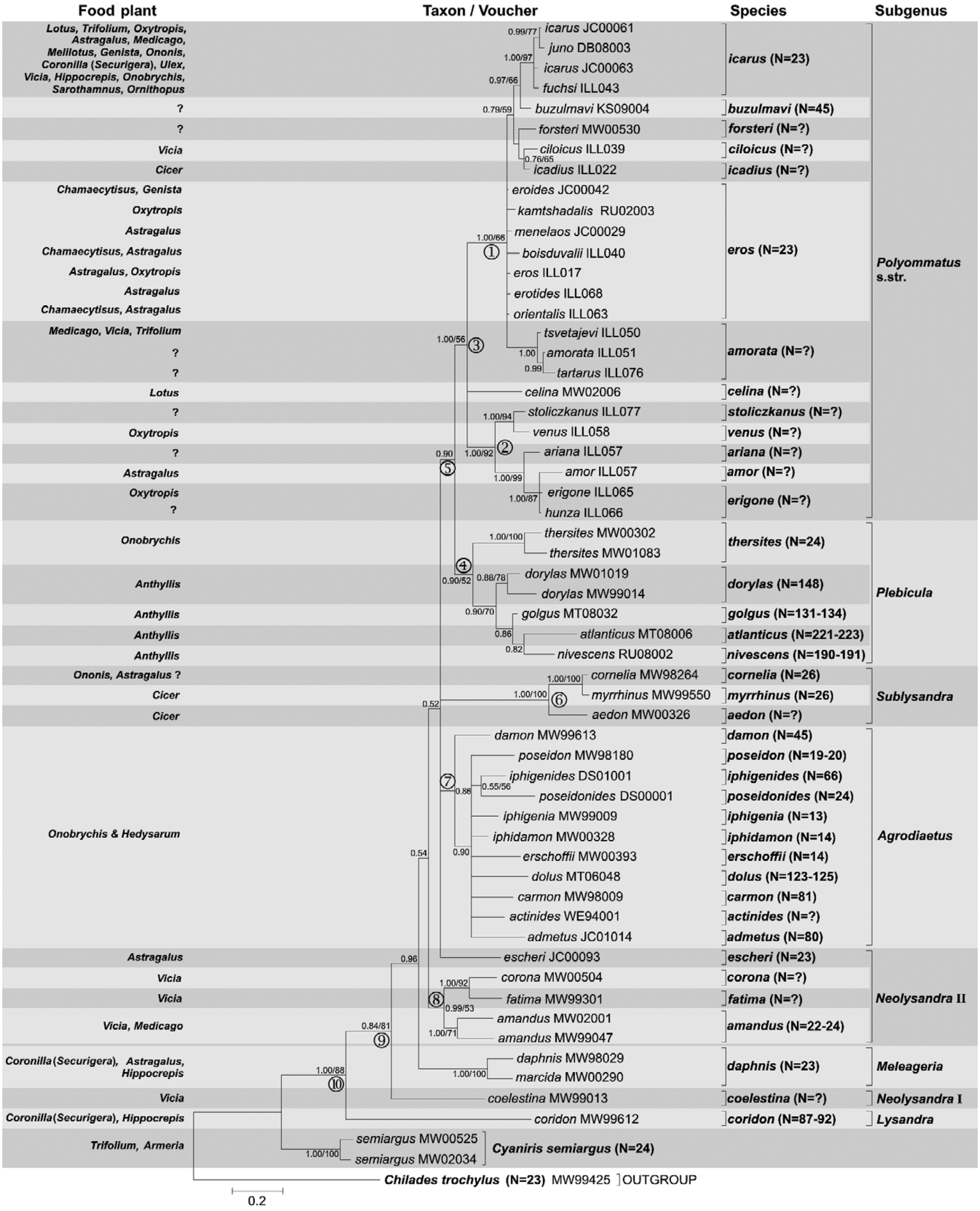

Fig. 2. Phylogenetic tree of the genus *Polyommatus* inferred from a combined Bayesian analysis of mitochondrial (*COI*) and nuclear (*ITS2*) sequences. The tree was rooted with *Chilades trochylus*. Support values (Bayesian / Maximum Parsimony Bootstrap) are shown at the nodes. Major clades are numbered ①-⑩ at nodes for reference purposes. Chromosome numbers are indicated after taxon names (taken from Lesse, 1960, 1962, 1970; Lorković, 1990; Hesselbarth et al., 1995; Puplesiene, 2000; Wiemers, 2003; Lukhtanov et al., 2005). Larval food plant genera are listed in the first column (according to Rodríguez et al., 1991; Owen & Wiemers, 1992; Rodríguez et al., 1993; Fiedler et al., 1994; Zhdanko, 1994; Hesselbarth et al., 1995; Korshunov & Gorbunov, 1995; Wiemers, 1995; Zhdanko, 1997; Tuzov et al., 2000; Klimczuk, 2005; Koshkin, 2005; Tarrier & Delacre, 2008; Tolman & Lewington, 2008; Toropov & Zhdanko, 2009; ten Hagen, pers. comm.). The following genera appear to be closely related according to recent molecular phylogenetic analyses (see Allan & Porter, 2000; Wojciechowski et al., 2004): *Astragalus/Oxytropis*, *Onobrychis/Hedysarum*, and *Coronilla (Securigera)/Hippocrepis*.

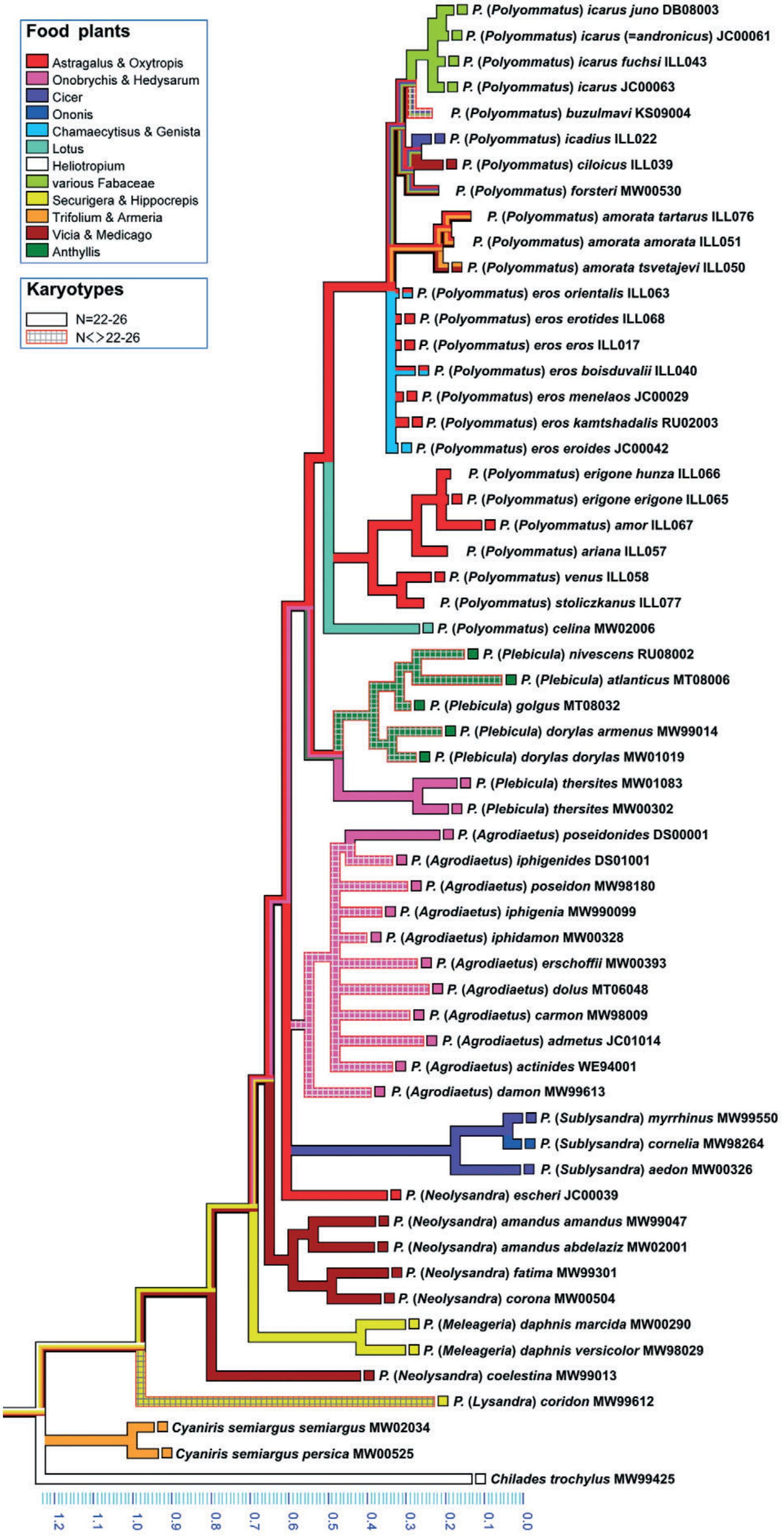

Fig. 3. Ancestral parsimony reconstruction of food plant use and evolution of chromosome number in *Polyommatus.* Actual and inferred larval host plant use and the incidence of chromosome numbers strongly deviating from the modal value are mapped onto the combined Bayesian phylogenetic tree (see Fig. 2 for further information).

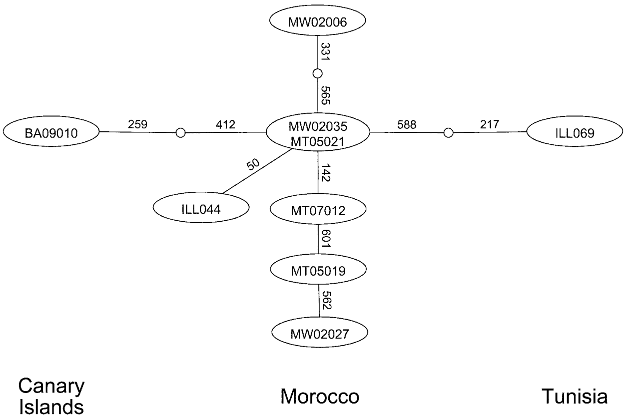

Fig. 4. Statistical Parsimony Network of *Polyommatus celina* *COI* haplotypes. Open nodes indicate unsampled haplotypes. Numbers along branches indicate the positions of nucleotide substitutions in the alignment.

Chamaecytisus, *Vicia* and *Cicer*. However, species of *Polyommatus* s.str. might be less specialized than other *Polyommatus*. An extreme case is *Polyommatus icarus*, which feeds on many Fabaceae genera some of which are not known to be used by other *Polyommatus* species, e.g. *Genista*, *Ulex*, *Melilotus* and *Ononis*.

The *COI* haplotype analysis (Fig. 4) recovers all specimens of *Polyommatus celina* from Africa and the Canary islands in a single network, which is not connected to the other *Polyommatus* networks. The lowest interspecific pairwise distance of 3.9% was found between the *celina* specimens BA09010 and MW02035 and *P. eros erotides* (ILL068).

With a minimum of only two nucleotide differences and a mean pairwise distance of 0.5% ± 0.002, the populations from Fuerteventura and Tunisia are hardly differentiated from the Moroccan populations. No geographical structure can be detected within the Moroccan populations, which are from different mountain ranges (High Atlas, Anti-Atlas and Middle Atlas).

DISCUSSION

Our molecular phylogeny produces monophyletic groupings, which are largely congruent with morphology-based taxonomic units. An exception appears to be the subgenus *Neolysandra*, which is not recovered by our analysis. However, this might be due to the strong differences among members of *Neolysandra* in the *COI* gene (Wiemers, 2003), while all *Neolysandra* appear to be closely related in the *ITS2* analysis (Wiemers et al., 2009). We also do not find evidence for synonymizing *Lysandra* under *Meleageria*, and therefore suggest that *Lysandra* is kept as a distinct subgenus.

Of special interest are those taxa whose taxonomic relationships have been debated for decades. These are *P. escheri*, *P. amandus* and *P. thersites*, which have been attributed to the (sub-)genera *Plebicula* (Higgins, 1969; Higgins & Riley, 1978), *Agrodiaetus* (Higgins, 1975; Higgins & Riley, 1983; Tolman & Lewington, 1997, 2008) or *Polyommatus* (Tolman & Lewington, 1998). Bálint & Johnson (1997) attached *P. thersites* to *Agrodiaetus* in their "*actinides*-group", whereas they combined *P. amandus* and *P. escheri* into a separate "*icarius*-group". This "*icarius*-group" (*P. icarius* Esper, [1793] is a synonym of *P. amandus*) was equated with "*Plebicula* s. str.", even though the authors did not include the type species of *Plebicula* in this group, which is *P. dorylas* ([Denis & Schiffermüller], 1775) (= *Papilio argester* Bergsträsser, [1779]). One reason for such shifts could simply be the inability of the Linnean taxonomic system to accurately represent highly nested phylogenetic relationships. However, the analysis of molecular data appears to result in ambiguous relationships between some of these taxa. While *ITS2* data suggest a sister relationship between *P. escheri* and *P. amandus* if information about secondary structure is taken into account (Wiemers et al., 2009), this is not supported by the *COI* data (Wiemers, 2003). This conflict might explain why the position of *P. escheri* is not resolved by the combined analysis. The sister relationship between *P. amandus* and (a part of) *Neolysandra* in our tree is surprising and has not previously been suggested. Although the considerable differences in wing pattern do not seem to support this result, the larval host plant associations do: *P. amandus* and *Neolysandra* (as far as currently known) share their specificity for *Vicia*, an otherwise unusual food plant of *Polyommatus*. On the other hand, a close relationship of *P. thersites* to *Agrodiaetus*, which is often suggested partly due to their shared use of *Onobrychis* as a host plant, is not confirmed by our analysis. The position of *P. thersites* as a sister to *Plebicula*, which is mainly based on *ITS2* data, appears well supported. It is therefore suggested that *P. thersites* be included in *Plebicula.* Although the subgenus *Neolysandra* does not appear as a monophyletic unit in this study, in the light of other evidence it is suggested this subgenus is retained pending further study, and that *P. amandus* and provisionally also *P. escheri* are attached to this subgenus. It should be mentioned that Bálint & Johnson (1997) split both *P. amandus* and *P. escheri* into several allopatric morphospecies. This has not been generally adopted and is not supported by our analysis of molecular data, even though there are large *COI* distances between African and Asian populations of *P. amandus* (Wiemers & Fiedler, 2007).

Within the subgenus *Polyommatus* the monophyly of *Polyommatus* s. str. sensu Vodolazhsky et al. (2009) is clearly established. This subclade ① includes the "*icarus*-group" sensu Bálint & Johnson (1997) and also *P. eros* (Ochsenheimer, 1808) from their "*eros*-group", but not the Central Asian taxa *P. amor* (Staudinger, 1886) and *P. venus* (Staudinger, 1886). *P. ciloicus* de Freina & Witt, 1983, which Bálint & Johnson (1997) placed in the "*stoliczkanus*-group", also belongs to the *Polyommatus* subclade ① and appears as a sister species to *P. icadius* (Grum-Grshimailo, 1890). The position of *P. buzulmavi* as a sister to *P. icarus* is also of interest, because the species status and relationship of *P. buzulmavi* is debatable. Carbonell (1991) suggested that *P. icarus*, *P. ciloicus* and *P. stoliczkanus* are close relatives, whereas Hesselbarth et al. (1995) mention the possibility of conspecifity with *P. icadius*. In contrast to *P. icarus* and *P. ciloicus*, which have sympatric distributions with *P. buzulmavi* in Hakkari Province (SE Turkey), *P. icadius* and *P. buzulmavi* have allopatric distributions and their genetic distance (*COI*) is only 1.2–1.7%. Bálint & Johnson (1997) attach *P. buzulmavi* to the *daphnis* species-group (*Meleageria*), but the molecular data clearly preclude such a treatment. A second monophyletic subclade ②, which consists of only Central Asian species (*Bryna*), is included by Bálint & Johnson (1997) either in the "*stoliczkanus*-group" [*P. stoliczkanus*, *P. ariana* Moore, 1865 and *P. erigone* (Grum-Grshimailo, 1890)] or in the "*eros*-group" (*P. amor*, *P. venus*). According to our analysis, the latter species is not especially closely related to *P. amor*, but a sister to *P. stoliczkanus*, while the former is closely related to *P. erigone*. In recent decades, *Bryna* is hardly ever recognized as a distinct (sub-)genus and usually synonymized with *Polyommatus*. The molecular results also confirm the close relationship of *Bryna* with the remaining species of *Polyommatus* s. str. including *P. celina*, a taxon which replaces the morphologically and ecologically similar *P. icarus* in North Africa and the Canary Islands. Its sister relationship remains unresolved and it forms a tritomy with the *Polyommatus* and *Bryna* subclades. The basal position of *P. celina* in the tree suggests an early divergence between African *P. celina* and its Eurasian sister taxon, which subsequently radiated on the Eurasian continent into the taxa now found in the *Polyommatus* subclade ①.

According to our dating estimates, this group is the result of a recent radiation, which occurred during the late Pliocene and Pleistocene, well after the last connection between Northwest Africa and Europe during the Messinian salinity crisis 5.3–5.6 MYA (Garcia-Castellanos et al., 2009). One of the oldest major clades is *Agrodiaetus*, which evolved 2.9–3.4 MYA. This time estimate matches well with that of Kandul et al. (2004) who postulated an age of 2.51–3.85 MYA for this subgenus, using a slightly different dating method and selection of genes (*COI* + *COII*). The fact that this species-rich radiation with ca 130 species is not represented on the African continent confirms its recent evolution occurred in the Palaearctic region (Wiemers et al., 2009). Most other major clades have representatives in Northwest Africa, but these are mainly species that are also found on the Eurasian continent. They must have reached Northwest Africa either via the Asian land bridge or via dispersal across the strait of Gibraltar. The latter colonization pathway seems most probable for the Moroccan endemic *P. atlanticus*, whose closest relatives are the Spanish endemics *P. nivescens* Keferstein, 1851 and *P. golgus* (Hübner, [1813]). Like almost all Northwest African *Polyommatus* species, *P. atlanticus* is confined to higher altitudes, which could explain its genetic differentiation. Nevertheless, the strait of Gibraltar, which is a mere 14 km wide at its narrowest point, appears to be a strong barrier to genetic exchange. This is supported by the strong genetic differentiation between the Eurasian *P. icarus* and the Northwest African *P. celina*. Both taxa are among the most ubiquitous butterflies within their range, occurring in almost all open habitats from sea level to high altitudes (up to 3200 m in the High Atlas Mountains). Because these two taxa hardly differ morphologically, their strong genetic differentiation, both in mitochondrial and nuclear DNA sequences, came as a big surprise (Wiemers, 2003). Apparently, *P. celina* is not even the sister species of *P. icarus*, and both taxa must have split from their common ancestor about 1.1–2.9 MYA, i.e. during the early Pleistocene. This is surprising because both species should have easily been able to cross the strait of Gibraltar. *P. icarus* is found on almost all Mediterranean islands, even those which have never been in contact with the continent (Dennis et al., 2000), and *P. celina* is the only *Polyommatus* species that has reached the Canary Islands. Their colonization involves crossing a minimum of almost 100 km of open sea. The low mean genetic distance between Canarian and Northwest African populations of *P. celina* indicates a relatively recent colonization, but not in historical times and thus not aided by man. On the Eurasian continent, the last common ancestor of *P. celina* and *P. icarus* gave rise to two distinct radiations. One of them, the *icarus*-subclade ①, includes the widespread Palaearctic species *P. icarus* and *P. eros,* a monophyletic Southeast Palaearctic radiation (*P. amorata*), the Central Asian *P. icadius,* as well as some local Kurdistanian and Iranian endemics, i.e. *P. buzulmavi*, *P. ciloicus* and *P. forsteri*. The low genetic divergences among species of this subclade, hybridization between *P. icarus* and *P. icadius* indicated by mtDNA introgression (Lukhtanov et al., 2009) and missing synapomorphic molecular characters for *P. eros* (Vodolazhsky et al., 2009), support its recent evolution. The other radiation, the *stoliczkanus*-group (subclade ②), is confined to the mountains of Central Asia, especially the Pamirs and Himalayas.

CONCLUSIONS

Based on our molecular analysis and additional evidence the following systematic treatment of the genus *Polyommatus* is suggested:

Genus *Polyommatus* Latreille, 1804
  Subgenus *Polyommatus* Latreille, 1804
    P. icarus (Rottemburg, 1775) = *P. andronicus* Coutsis & Ghavalas, 1995
    P. buzulmavi Carbonell, [1992]
    P. forsteri (Pfeiffer, 1938)
    P. ciloicus de Freina & Witt, 1983
    P. icadius (Grum-Grshimailo, 1890)
    P. eros (Ochsenheimer, 1808)
    P. amorata (Alpheraky, 1897)
    P. celina (Austaut, 1879)
    P. stoliczkanus (Felder & Felder, [1865])
    P. venus (Staudinger, 1886)
    P. ariana Moore, 1865
    P. amor (Lang, 1884)
    P. erigone (Grum-Grshimailo, 1890)
  Subgenus *Plebicula* Higgins, 1969
    P. dorylas ([Denis & Schiffermüller], 1775)
    P. golgus (Hübner, [1813])

  - *P. nivescens* Keferstein, 1851
  - *P. atlanticus* (Elwes, 1905)
  - *P. thersites* (Cantener, 1834)
- Subgenus *Sublysandra* Koçak, 1977
  - *P. cornelia* (Gerhard, 1851)
  - *P. myrrhinus* (Staudinger, 1901)
  - *P. aedon* (Christoph, 1887)
- Subgenus *Agrodiaetus* Hübner, 1822
  - *P. damon* ([Denis & Schiffermüller], 1775)
  - *P. poseidon* (Herrich-Schäffer, 1851)
  - *P. iphigenides* (Staudinger, 1886)
  - *P. poseidonides* (Staudinger, 1886)
  - *P. iphigenia* (Herrich-Schäffer, [1847])
  - *P. iphidamon* (Staudinger, 1899)
  - *P. erschoffii* (Lederer, 1869)
  - *P. dolus* (Hübner, [1823])
  - *P. carmon* (Herrich-Schäffer, [1851])
  - *P. actinides* (Staudinger, 1886)
  - *P. admetus* (Esper, 1783)
- Subgenus *Meleageria* de Sagarra, 1925
  - *P. daphnis* ([Denis & Schiffermüller], 1775)
- Subgenus *Neolysandra* Koçak, 1977
  - *P. coelestina* (Eversmann, 1843)
  - *P. corona* (Verity, 1936)
  - *P. fatima* (Eckweiler & Schurian, 1980)
  - *P. amandus* (Schneider, 1792)
  - *P. escheri* (Hübner, [1823])
- Subgenus *Lysandra* Hemming, 1933
  - *P. coridon* (Poda, 1761)

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